

Methods and Devices for Assessment of Fiprole Pesticides in Engineered Waterways

by

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ABSTRACT

This dissertation focused on the development and application of state-of-the-art monitoring tools and analysis methods for tracking the fate of trace level contaminants in the natural and built water environments, using fipronil as a model; fipronil and its primary degradates (known collectively as fiproles) are among a group of trace level emerging environmental contaminants that are extremely potent arthropodic neurotoxins. The work further aimed to fill in data gaps regarding the presence and fate of fipronil in engineered water systems, specifically in a wastewater treatment plant (WWTP), and in an engineered wetland. A review of manual and automated “active” water sampling technologies motivated the development of two new automated samplers capable of *in situ biphasic* extraction of water samples across the bulk water/sediment interface of surface water systems. Combined with an optimized method for the quantification of fiproles, the newly developed In Situ Sampler for Biphasic water monitoring (IS2B) was deployed along with conventional automated water samplers, to study the fate and occurrence of fiproles in engineered water environments; continuous sampling over two days and subsequent analysis yielded average total fiprole concentrations in wetland surface water (9.9 ± 4.6 to 18.1 ± 4.6 ng/L) and wetland sediment pore water (9.1 ± 3.0 to 12.6 ± 2.1 ng/L). A mass balance of the WWTP located immediately upstream demonstrated unattenuated breakthrough of total fiproles through the WWTP with 25 ± 3 % of fipronil conversion to degradates, and only limited removal of total fiproles in the wetland (47 ± 13 %). Extrapolation of local emissions (5–7 g/d) suggests nationwide annual fiprole loadings from WWTPs to U.S. surface waters on the order of about one half to three quarters of a metric tonne. The qualitative and quantitative data collected in

this work have regulatory implications, and the sampling tools and analysis strategies described in this thesis have broad applicability in the assessment of risks posed by trace level environmental contaminants.

DEDICATION

I would like to dedicate this work to my wife, Jen, who has supported me in good times and in bad, and without whose love and patience I could not have come so far. She is my guiding light.

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CHAPTER 1

INTRODUCTION

Sediment and water contamination is a pervasive problem in the United States, with the U.S. Environmental Protection Agency (EPA) estimating that 10% of all the nation's lakes and rivers have sediments that are impacted by chemical pollutants capable of harming ecosystems.¹ Among the most susceptible organisms to environmental contaminants are invertebrates, including aquatic and terrestrial insects.^{2,3} Some contaminants occur in the environment at trace levels (parts per trillion), yet still pose ecotoxicological risks due to their toxic potency toward sensitive organisms in the environment. Thus, the assessment of environmental contamination and exposure of sensitive species is integral to understanding and managing risks.

The sensitivity, accuracy, and precision with which the fate of trace compounds can be monitored in the environment hinges to a large degree on the tools and methods used for sampling and analysis. Tailoring sampling strategies for specific research goals is an important strategy for improving the data quality of environmental measurements.⁴ Sampling strategies that are being employed for site characterization, fate studies, and trend studies include passive sampling or active sampling, time-averaged sampling, flow-weighted sampling, and discrete grab sampling. The sampling strategy and technology employed should be suited to the goals of a given study. The most commonly used automatic water samplers collect large volumes of water (>50 mL) in either single composite bottles or an array of bottles. They are programmable, and are capable of being used for time-averaged sampling in continuous or pulse modes. They can also be connected to flow meters for flow-weighted sampling.^{5,6} Some automatic water samplers

perform solid phase extraction as they pump, and can therefore process large volumes of water and retain the analyte mass while storing none of the fluid.^{7,8} If the goal is to determine the fate of trace compounds in dynamic systems using mass balances, then automatic flow-weighted sampling is suitable.⁵

Two aims relevant to establishing exposure potential are contaminant fate determinations, and sediment characterization. Exposure assessment for biota can require sampling surface water and the pore water in the sediment spaces where benthic organisms live, rather than whole sediment extraction. Pore water concentrations are more relevant to biotic exposure assessment because sediment-bound contaminants frequently are less or not bioaccessible at all. In contrast, for biota dwelling above the sediment-water interface, the concentration of the water column is more relevant. For this reason, a sampling strategy involving the sampling of both pore water and the overlaying water would be most informative for exposure assessment studies. Contaminant fate studies in rivers, wetlands, or wastewater treatment facilities require sampling strategies suitable for monitoring concentrations and flow rates over time, because to determine mass loads into and out of aquatic systems, it is necessary to do a temporal integration of the product of time-discrete flow rate and concentration measurements. Strategically-placed automated active samplers programmed for flow-weighted sampling are therefore a good choice to conduct fate studies.

In order to detect and quantify trace chemicals, sensitive and analyte-selective instrumentation is required, such as a tandem mass spectrometer for unambiguous analyte detection and quantitation at low concentrations even in very complex matrices like sewage and sewage sludge. But considering that instrument detection limits for these

compounds may be on the order of 1 pg, a sample of water with a concentration of 10 ng/L or less analyzed by direct injection on a liquid chromatograph tandem mass spectrometer may not register a response on the instrument, while samples with concentrations close to the detection limit may not be quantifiable. Preconcentration is therefore necessary to amplify the signal from the sample, achieving lower limits of quantitation; this is typically done by solid phase extraction (SPE). In order to maintain high precision between sample preparations, SPE protocols can be automated, and quality control measures such as matrix spiking, isotope dilution, and standard addition should be employed.

Fiproles – a group of emerging contaminants serving as a model for a case study

Emerging contaminants such as phenylpyrazole pesticides are replacing legacy pollutants, such as dichlorodiphenyltrichloroethane (DDT). DDT is well-established as a chemical posing a great deal of risk to organisms, from arthropods to other organisms higher up the food chain, such as fish, reptiles, and birds of prey.⁹⁻¹² While the impacts of legacy pesticides like DDT are significant, newer, more powerful pesticides may be posing new adverse ecological impacts. The phenylpyrazole compound fipronil is one of the highest-production volume insecticides in the world, and like other modern pesticides, such as neonicotinoids, it is a potent neurotoxin. Several studies have indicated that it occurs in aquatic environments with high frequency at trace levels (typically less than 1 µg/L).¹³⁻¹⁵ Even at trace levels, fipronil causes sub-lethal toxic effects to a number of sensitive organisms, not the least of which is *Apis mellifera* (the honeybee). With a median lethal dose (LD₅₀) as low as 1-6 ng/bee,¹⁶⁻¹⁹ fipronil is about 6,500 times more toxic to bees than DDT.²⁰ Some studies suggest that sub-lethal doses of

fipronil can impact bees, thereby giving rise to erratic behavior and possibly contributing to the worldwide observed colony collapse disorder. Due to fipronil's potential harm, its use has been severely restricted in both Europe (banned for most agricultural uses in 2013) and China (banned for most uses in 2009).

Fipronil is used in turf treatments for the control of fire ants, mole crickets, nuisance ants, fleas, and ticks, and it is used in seed treatments for the control of seedcorn beetles and maggots, thrips, wireworms, corn rootworm larvae, European corn borers, stalk borers, chinchbugs, grape colaspis, grubs, and billbugs. Direct application of fipronil-containing products to agricultural fields and urban turfgrass (including golf courses, baseball fields, football fields, and more) is one potential source of pollinator exposure. Since fipronil and its transformation products can be transported through plant xylem, phenylpyrazole compounds can be deposited on plant leaves and pollen.²¹ Indeed, this is the very mechanism by which pesticide-laced seed treatments function. One study in France showed that fipronil congeners (fiproles) were present in pollen, alongside other pesticides like imidacloprid, coumaphos, and tau-fluvalinate.²² Direct application of fipronil to agricultural fields and turfs therefore likely causes exposure of pollinators. This mechanism of exposure, wherein pollinators ingest and carry pollen back to their hives, is analogous to the function of roach and ant baits, which also commonly contain fipronil and are designed such that the insects shuttle the poison from the site of application back to their nests to eliminate the entire colony. When non-target organisms like bees fall victim to this mechanism, the consequences can be catastrophic for an entire ecosystem. Co-occurrence of pesticides in pollen and parasitic hive infestation has further implications for synergistic toxic effects.²³

Fate determination

While intentional, direct agricultural application of fipronil can easily account for potential pollinator exposure, there are also inadvertent applications of pesticides that occur daily. Recycled, treated wastewater and biosolids represent one potential and likely source of fipronil dispersion into the environment,^{24,25} a source that has not yet been investigated in much detail. Discharge of recycled water or treated wastewater into surface waters, agricultural fields, and urban turf grass, and application of biosolids for inexpensive disposal of these abundant materials and as a soil fertilizer are a potential route for dispersion of fiproles. It is therefore plausible for pesticide residues to be taken up by angiosperms, deposited on pollen, and carried away by foragers and pollinators.

While treated wastewater and biosolids are potential sources of exposure for pollinators, there is little to no robust literature that focuses on tracking fiproles in wastewater streams, although there are studies that investigate the general potential for plants to accumulate pesticide residues as a result of being exposed to wastewater effluents.²⁶⁻²⁹

While a study by the United States Geological Survey (USGS) detected and quantified fipronil congeners in rivers impacted by wastewater streams, it did not endeavor to quantify them in wastewater streams with the precision and accuracy required for a full assessment of their fates in wastewater treatment plants or downstream discharge locations.³⁰ One study out of Johns Hopkins University in 2009 reported $18 \pm 22\%$ removal of fipronil from wastewater streams, and an overall persistence of $97 \pm 70\%$ in treatment plants.²⁴ The ambiguity highlighted by the error margins of these results is a reflection of the poor precision of the measurements, which in turn is a function of the sampling strategies employed, the extraction and cleanup methods used, and the

analytical method implemented. Narrowing the precision of these measurements can be challenging when analyzing complex matrices such as wastewater and sludge, particularly when the analytes of interest (e.g., fipronil) occur at concentrations of less than 1 µg/L. A better understanding of the fate of fipronil cannot be gained, however, without the development and application of better, more precise and accurate methods of sampling and analysis.

The ecological risk posed by contaminated water and sediments may not be reflected by assessing single parent compounds like aldrin, DDT, or fipronil. Many pesticides degrade readily into equally or more toxic byproducts: *p,p'*-DDT degrades into 4,4-dichlorodiphenyldichloroethylene (*p,p'*-DDE); aldrin degrades into dieldrin; fipronil degrades into several immediate byproducts, including sulfide, sulfone, desulfinyl, and amide derivatives of the parent compound. In each case, assessing the relative risk posed to the environment by these kinds of pollutants depends upon capturing their byproducts in risk assessment calculations. It is therefore important to include these compounds in screenings in order to provide the essential data inputs for risk analyses.

Quantitative risk assessments are determined using direct measurements and/or models that estimate environmental concentrations, which in turn then are used to estimate biotic exposures. It is therefore important to maximize the sensitivity, accuracy, and precision of measurement strategies. While this study cannot determine with confidence the entire mass loading and fate of wastewater-borne phenylpyrazole pesticides, a detailed study of this group of chemicals in the built water environment infrastructure was undertaken to aid in assessing the mass inputs of these compounds into the environment.

Primary goals

The general goal of my PhD thesis was to establish new, precise methods and tools for sampling, detecting, and quantifying emerging trace pollutants in complex environmental matrices, including municipal wastewater. These new approaches then were evaluated by case studies focusing on the emerging contaminant fipronil and its congeners, jointly known as fiproles. The approaches involved employing automated samplers to obtain time-weighted or flow-weighted composite samples that can be used to achieve the following goals: (1) assessment of the occurrence of emerging contaminants in sediment pore water and surface water at environmentally relevant concentrations (ng/L range); and (2) determination of fiprole fate in the built urban water environment.

Hypotheses

(1) Assessing fipronil *and* its byproducts in wastewater and surface water using automated, flow-weighted sampling combined with mass spectrometric analysis, isotope dilution, and standard addition quantitation will generate data precise enough to perform total fiprole mass balances in engineered water systems. (2) A new automatic sampling tool capable of time-weighted sampling and *in situ* solid phase extraction (SPE), when coupled to a method for detecting fiproles, can produce quantitative data comparable to those generated by conventional but more cumbersome methods. Specifically, the new sampling technology envisioned will allow me to simultaneously sample sediment pore water and surface water (a novel feature). As such, it can serve to demonstrate that fiprole concentrations in sediment pore water near the sediment/bulk water interface are equal to or higher than corresponding surface water concentrations, and increases with the

organic carbon content of the sediment. (3) Since fipronil is fairly resistant to degradation – and its immediate byproducts more so – I hypothesized that a mass balance conducted over a wastewater treatment train and engineered wetland will show fiproles as a group to be highly conserved, and that the fiprole-related toxic load conveyed in these water streams experiences only insignificant attenuation.

Specific aims

The first aim was to evaluate several sampling technologies and compare them in terms of their utility sampling and tracking trace level hydrophobic organic compounds like pesticides for various environmental assessment goals. The second aim was to develop a method for sampling, extracting, and analyzing wastewater and sludge for fiproles. The third aim was to develop a new automatic sampling tool, whose capabilities include sampling across the water-sediment interface for determining time-averaged concentrations in low-particulate aquatic systems, and to perform *in situ* SPE in order to mitigate sample handling issues. The fourth and final aim was to employ an appropriate sampling and analysis approach to perform a mass balance on a wastewater treatment train and a constructed wetland over a five-day sampling period.

TRANSITION 1

This dissertation is comprised of individual studies focused around the goal of accurately sampling and quantifying trace-level hydrophobic organic contaminants, with fipronil and its degradates serving as model compounds. In Chapter 2, I review various active sampling strategies and technologies with respect to their uses as HOC assessment tools, and I evaluate their applications, features, advantages, and disadvantages. I also contrasted these technologies and strategies with each other, thereby gaining insights toward the direction in which active sampling technologies need to evolve.

CHAPTER 2

REVIEW OF ACTIVE ENVIRONMENTAL WATER SAMPLING STRATEGIES FOR MONITORING ORGANIC POLLUTANTS

ABSTRACT

This review examines and compares means of actively sampling water: grab sampling, automatic sampling and storing of samples, and in situ extraction of analytes. The benefits and disadvantages of these various sampling strategies and technologies were compared by assessing their utility for assessing hydrophobic organic contaminants (HOCs) in various matrices and applications. A review of the literature showed that the most commonly used active sampling techniques for HOC monitoring are grab sampling (63%), and automated water collection using pumping devices such as ISCO or Sigma samplers (32%), while the least utilized technique was in situ solid phase extraction (5%). A few in situ extraction devices incorporate on-line extraction of contaminants, while a few also incorporate on-line, real-time instrumental analysis (3% of active samplers). Automated in situ extraction samplers were found to have comparable capital costs compared to automated water collectors, while their performance as monitoring tools for organic contaminants is comparable to that of automated water collectors and manual grab sampling. Since in situ extraction mitigates sample handling issues like adsorption and volatilization losses, as well as issues related to transporting large volumes of water, and has comparable cost, it is reasonable to utilize automated extraction devices in lieu of water collectors and grab samplers for monitoring organic contaminants.

Introduction

A sampling strategy is an integral part of site characterization and risk assessment in environments laden with hydrophobic organic contaminants (HOCs).^{31,32} Various technologies and strategies have been designed for specific purposes: grab sampling with a bottle is commonly used for screening lakes, rivers, wetlands, and other surface water locations; automated water collection is commonly used for water and wastewater compliance monitoring; sorptive passive sampling is one of the most typical methods of determining contaminant bioavailability; grab sampling via bailing is often used for ground water well monitoring, although sometimes other technologies like passive samplers or active pumping are used for more accurate depth-discrete and time-integrated or time-discrete sampling.³³⁻³⁵ This review will focus on the application of various active sampling technologies for screening trace-level HOCs in sediment-water matrices.

Sampling is integral to quality control

When determining the risk posed by environments contaminated by HOCs, or indeed any pollutant of consequence with respect to human and ecological health, the single most relevant piece of information necessary for risk assessment is concentration.^{36,37}

Determination of chemical concentrations in sediment, water, and air matrices hinges on sampling procedures. Sampling protocol is arguably at least as important as sample preparation and analysis. The value of analytical data from instruments is limited ultimately by the quality of the samples collected.³⁸ Sampling protocols for HOCs depend largely on the partitioning properties of the compounds in question. Hydrophobic, semi-volatile compounds like chlorinated pesticides and polychlorinated biphenyls (PCBs)

have a tendency to adsorb to glassware, tubing, suspended sediment, and humic acids.³⁹⁻⁴¹ Smaller molecules like perchloroethylene (PCE), trichloroethylene (TCE), and trichloroethane (TCA) also exhibit sorptive losses, and in addition, tend to volatilize out of solution when the samples in which they are collected have head space.⁴² In both cases, partitioning is a potential source for significant loss of analyte mass. These losses can occur during all stages of the sampling and analysis train, including sample collection, handling, preparation, and analysis.

Sampling technologies

The United States Environmental Protection Agency's (USEPA's) recommendations for surface water sampling methods can include using a sample container (dipping), scoops, peristaltic pumps, discrete depth samplers, bailers, buckets, submersible pumps, and automatic samplers. The EPA warns that "precautions should be taken to ensure that the sample collected is representative of the water body or conveyance," and that "there is no substitute for high quality sampling and field measurements."^{43,44} There are multiple varieties and applications of each of the types of samplers described in the EPA document SESDPROC-201-R3.

Sampling strategies can be divided into two basic categories: passive sampling, and active sampling. Passive sampling generally involves the passive accumulation of analytes into a container through a passive diffusion membrane, or onto a sorptive material like low density polyethylene (LDPE) or polydimethyl siloxane (PDMS). Passive samplers use the principle of partitioning equilibrium for the determination of sediment and water concentrations. Passive sampling is defined as "any sampling

technique based on free flow of analyte molecules from the sampling medium to the collecting medium, as a result of a difference in chemical potentials of the analyte between the two media.”⁴⁵ Sorptive sampling refers to the transfer of analytes from one phase to another (e.g. from the water-dissolved phase to the hexane-dissolved phase in semi-permeable membrane devices).

Calculating concentrations from masses collected on many passive sorptive samplers depends on the assumption that the sampler has reached equilibrium, which could take days to months, depending on the analyte, matrix, and sampler chemistry.⁴⁶

Concentrations are back-calculated using calibrated equilibrium data and fitting to one of several isotherm models.⁴⁷ One of the primary difficulties in using passive sorptive samplers is determining whether a sampler has reached equilibrium. Calibrations in the lab can be used to estimate the time necessary to reach equilibrium, but these time periods can either be very long (many months), or be complicated by matrix effects such as salinity. Other means of calibrating passive samplers for calculating contaminant concentrations include linear uptake modeling, and performance reference compound (PRC) calibration (not discussed here). These methods of passive sampling can allow for much shorter deployment times, although this is largely dependent upon the sampler uptake rates, which vary according to the target compound properties, and whether the contaminant load in the target environment is static or dynamic.⁴⁸

While passive sampling has some clear advantages over many active sampling techniques (e.g., cost), it does have some disadvantages (e.g., long deployment times, uncertainties in data quality). Active sampling involves the “active” collection of water by manual or automatic collection by means of a pump or other energy-use device.

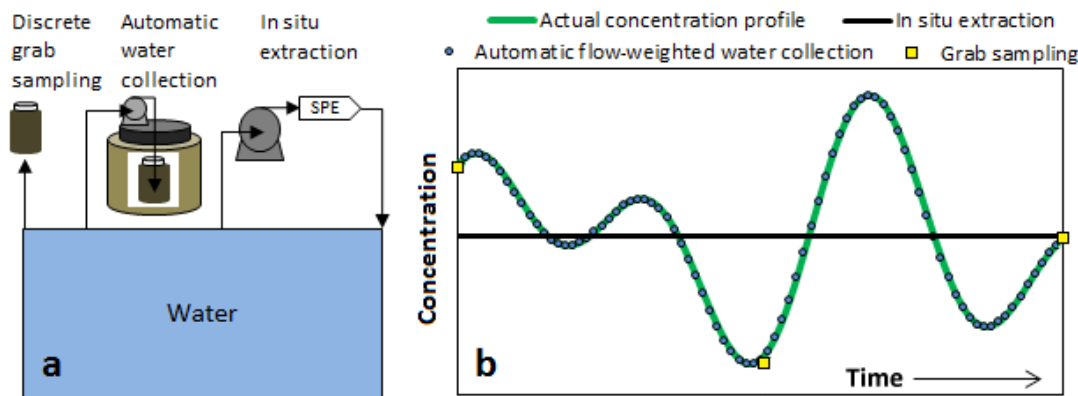


Figure 2-1. Active sampling strategies. (a) Methods of active sampling. Grab sampling methods are manual, while automatic water collection and active in situ extraction (with few exceptions) require pumps. SPE – solid phase extraction. (b) Illustration of the kind of data produced by various sampling strategies during a monitoring period of days to weeks. Automatic flow-weighted water collection generates an average concentration value for the sampling period that is dependent upon average mass loads through a flow stream over a given time interval. In situ extraction typically generates time-averaged concentrations, which are equal to the geometric mean concentration over the whole sampling period.

There are three general ways to actively sample water (illustrated in Figure 2-1): grab sampling directly with a bottle or other container, automatic water collection and storage, and *in situ* extraction of analytes from water.

Grab sampling methods include using bailers, bottles (e.g., Nalgene, or polytetrafluoroethylene/PTFE), snap samplers, split barrel messengers, LaMotte horizontal samplers, alpha samplers, and Kemmerer samplers (the latter five for depth-discrete sampling). All of these sampling tools may be employed as part of a composite (time-integrative) or discrete (space-integrative) sampling plan in order to give a spatial and temporal picture of the contamination at a given site. However, these tools are of limited use in the context of ultra-low-level contaminant monitoring. Sampling surface waters for very hydrophobic contaminants like dichlorodiphenyltrichloroethane (DDT), for example, to determine the bioavailable or bioaccessible concentrations often cannot

be easily done with any type of grab sampling method, because the concentrations, although still environmentally relevant, are likely to be below instrument detection limits, and analyte loss during sample transfers can be significant due to high partitioning coefficients.

The limitations of grab sampling, composite sampling, and discrete sampling are too significant for applicability to sediment pore-water sampling, or for bulk surface water sampling of lipophilic compounds in high-organic-carbon environments. There are several active sampling techniques like separation pumping, triple zone sampling, or horizontal dividing systems that are for groundwater well applications, and are not applicable to surface water sampling scenarios. For surface water, innovative uses of automatic large volume sampling devices (in hydrograph-based sampling, for example) have displayed impressive detection limits and help to generate useful time-integrated or flow-integrated data. Active sampling combined with *in situ* solid phase extraction (SPE) combines the benefits of metered pumping and sorptive sampling. This sampling approach sees limited field use, as there are few actively-pumping *in situ* preconcentrating samplers on the market. Even rarer is their application as pore water sampling devices, despite their promise as useful pore water and bulk surface water monitoring tools. This review will describe and weigh the advantages of active sampling technologies both extant and hypothetical against their disadvantages, and contrast them with passive and grab sampling techniques in the context of surface and pore water monitoring for hydrophobic organic contaminants HOCs.

Time discrete versus time-averaged/time-integrated sampling

As illustrated in Figure 2-1, time discrete grab sampling provides “snapshots” of chemical concentrations in time, and average concentrations, total maximum contaminant loads, and other risk assessment parameters are often calculated using these snapshots. Some contaminants display transient behavior, and are influenced by numerous environmental factors. For example, detection and quantitation of trace compounds can be complicated by the mixing of fresh and saline water in estuaries, high levels of natural organic matter (NOM), and dynamic hydrologic conditions of river, ocean, lake, and estuarine systems. This relationship illustrates the fact that organic compounds, including HOCs, preferentially partition onto sediments as the increased ionic strength causes increased flocculation of NOM. If sampling is done in an estuary at a location and period of high salinity, a non-representative concentration may result, and the assessment of mass transfer to the sea and the risk posed to aquatic ecosystems may be underestimated. Further complications in estuarine systems include longitudinal salinity profiles that are season-dependent.⁴⁹ This causes temporal fluctuations of organic contaminant concentrations. In lakes and rivers, there are also season-dependent DOC depth profiles,⁵⁰ which makes concentrations of HOCs fluctuate as well. Sampling under these conditions is complicated, and the quality of data depends heavily upon the sampling plan. Ideal sampling plans for some scenarios might require both spatial and temporal dimensions, as it may be important to have depth-discrete data as well as temporal data.

Surface water sampling

Concentrations of certain pollutants are typically very low in aquatic systems due to their very large organic carbon partitioning coefficients (K_{OC}). Sampling strategies employed for the purpose of quantifying nonpolar organics must have low enough detection limits to give useful data. Oftentimes, the partitioning properties of HOCs necessitate high-volume water sampling in order to obtain detectable or quantifiable masses of target compounds. The volume of water needed to obtain a detectable mass on column is dependent upon actual field concentrations, instrument sensitivity, and analyte recoverability. Equation 1 can be used to estimate the necessary sample volume (V_{field_sample}) for detection of an analyte based upon these parameters: the concentration of the contaminant (C_w), the instrument detection limit (IDL), the volume of sample after preparation ($V_{prepped_sample}$), the volume of prepped sample introduced to an analytical instrument ($V_{injected_column}$), and the total analyte mass recovery efficiency from sampling to analysis (R).

$$V_{field_sample} = \left(\frac{V_{prepped_sample}}{V_{injected_column}} \right) \times \frac{IDL}{C_w \cdot R} \quad (2-1)$$

Typical field concentrations of the pesticide fipronil, for example, range from less than 1 ng/L to about 100 ng/L.⁵¹ Modern triple quad mass spectrometry instruments are capable of achieving detection limits for some organic compounds of approximately 0.1 pg on column,⁵² which translates to sample concentrations in the range of 1–10 ng/L, depending on the injection volume. Most field samples are readily detectable with minimal sample preparation, although quantitation at those levels may necessitate preconcentration. For some compounds, like endosulfans, sample handling and preparation losses are usually

significant, and total recoveries may be as low as 2%.⁵³ At environmentally relevant concentrations of 1–10 µg/L, no preconcentration should be necessary to detect and quantify samples, even considering losses that occur in sample handling and preparation. However, quantifying the losses from sample handling is a challenge, since quality control measures like matrix spikes with isotope-labeled standards are done in the lab, after sample collection. Sometimes losses can be quantified by extracting an entire sample container with organic solvent. Without quantifying sample losses, it is not possible to assess actual field concentrations of a contaminant.

Active sampling of surface waters can be done in two ways: continuous collection and storage of or *in situ* extraction (e.g., solid phase extraction) of large volumes of water. Active sampling devices include the continuous flow integrative sampler (CFIS), hydrograph-based sampling (HBS), the system for the automated measurement of organic contaminants in surface water (SAMOS), the PDMS thin layer film active sampler (SPME agitated active sampler), the programmable field extraction system (PROFEXS), and other systems incorporating the Teledyne ISCO or Hach Sigma samplers. These devices, and others like them, have been evaluated and field tested, but aside from ISCO and Sigma samplers, evidence of the regular use of active samplers in surface water and wastewater monitoring plans is scant. A search for literature citing the use of given sampling technology for assessing HOCs reveals that passive methods are employed roughly three times as often as active methods, and the most common active sampling technology used for assessing HOCs is in fact large volume collection (ISCO or Sigma samplers). Results of this search are shown in Figure 2. It should be noted that grab sampling using bottles is probably a more common means of sampling than passive

sampling, even for HOC analysis; this fact is difficult to capture in a literature search, because the associated keywords may not appear (e.g., “grab sample”) in the article. Instead, the term “sample” might be used, and teasing out the articles that make this distinction is challenging.

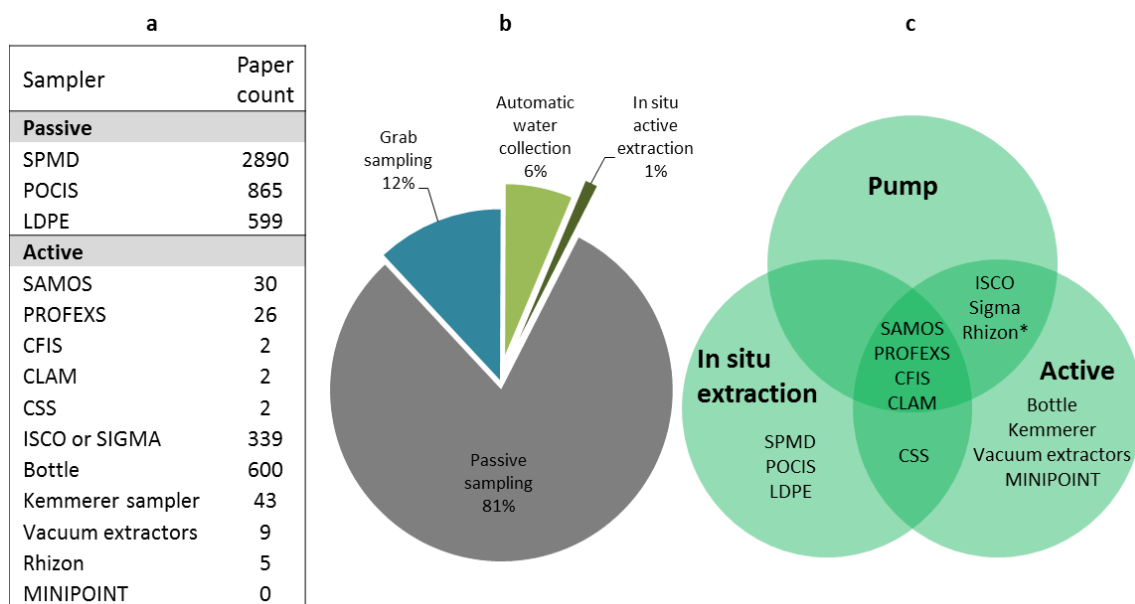


Figure 2-2. (a) Comparison of the number of studies in a literature search in which a particular water sampling technology was used for assessing hydrophobic organic compounds. SPMD, semi-permeable membrane device; POCIS, polar organic chemical integrative sampler; LDPE, low density polyethylene; SAMOS, system for the automated measurement of organic contaminants in surface water; PROFEXS, programmable field extraction system; CFIS, continuous flow integrative sampler; CSS, constantly stirred sorbent sampler; CLAM, continuous low-level aquatic monitoring sampler. (b) Relative percentage of studies in which given sampling technologies are used for monitoring HOCs. (c) Venn diagram showing basic characteristics of sampling technologies. *Rhizon samplers may be used with or without pumps.

Large volume collection and storage systems

There are two means of collecting large volumes of water: by intermittent or continuous pumping, or by direct grab sampling. Some grab sampling devices include bailers, and Kemmerer samplers. Of these, Kemmerer samplers are capable of depth-discrete

sampling. While these devices are relatively inexpensive (Kemmerer samplers are approximately \$500 each) and in common use, one thing to note about direct grab sampling devices is that they are time-discrete. For time-averaged, time-integrated, or flow-weighted data, pumping samplers are more appropriate.

The Teledyne ISCO and Hach Sigma automatic samplers utilize a peristaltic pump to collect large volumes of water, and they have a variety of capabilities. They are capable of collecting tens of liters of water, and can generate numerous discrete samples up to 1 L each, or composite samples of up to 9 L. The devices are programmable, and are capable of producing either time-weighted composites, or flow-weighted composites if equipped with bubble flow meters. It is also possible to program them to draw variable sample volumes at chosen time intervals. The devices may include a refrigerator and an optional onboard rechargeable battery and solar panels to power the pump and refrigeration unit as it samples for long periods of time on-site. The ISCO sampler is commonly used to sample effluents from wastewater treatment facilities and source water.^{54,55} The refrigeration capabilities are attractive because cooler temperatures inhibit microbial growth that can ultimately lead to degradation of analytes (this is particularly important when sampling effluents from wastewater streams). The drawback to refrigeration units is that they are not portable. Units without refrigeration can be moved easily from place to place. Some specialized surface water sampling devices make use of these large-volume automatic samplers, such as the hydrograph-based sampling method.⁵⁶ Hydrograph-based sampling makes use of continuous sampling via active pumping, spatially discrete data, and hydrographic data (water levels, flow rates, etc.). It has been employed for the purpose of monitoring pesticides in stream water, and due to the temporal and spatial

dimensions built into the sampling plan, the sampler detected pesticides 20%-30% more frequently than grab sampling, and had 1-3 orders of magnitude higher average sensitivity.⁵⁶ The large sample volumes collected had to be transported back to a lab for further processing, including filtration and extraction (SPE). The necessity of transport of large volumes of water is a significant limitation of these types of active sampling systems, particularly when analytical work is outsourced to commercial labs. The transport costs are not usually very high, but the sample handling and transport can significantly affect sample quality. When active sampling (using the ISCO 6712 portable sampler) in conjunction with lab-based SPE sample preparation was compared with passive sampling (using the POCIS sampler), the passive sampling method proved both easier (in terms of optimization and labor) and more effective at detecting contaminants in the Ruiné stream.⁵⁷ The fact that the POCIS performs SPE *in situ* simplifies the sample preparation procedure, and reduces the losses during collection and transport associated with grab and active sampling. If using continuous active samplers like the ISCO sampler in conjunction with *in situ* SPE, these difficulties and the difficulties associated with passive samplers (e.g. determination of time to equilibrium) might be mitigated.

In situ extraction

Various methods of in situ extraction may be employed which preclude the need to transport the large volumes of water generated by an ISCO sampler. Some methods incorporate solid-phase extraction cartridges or disks with continuous pumping, while others also incorporate an analytical system such as a liquid chromatography-mass spectrometry system (LC-MS).

One study employed a continuous peristaltic pump with polyethylene (PE) tubing to perform in situ SPE on shallow groundwater in Norway where agricultural runoff had penetrated the water table.⁵⁸ The method detection limit for various pesticides and herbicides was generally around 0.02 µg/L, and several herbicides (e.g. bentazon) were found in high concentrations (> 1.0 µg/L), sometimes as high as 33 µg/L (metribuzine); the MCL for individual pesticides in the study is 0.1 µg/L.⁵⁸ In this case, the PE tubing just had to be refrigerated, transported, and extracted in the lab, and the large volumes of water did not have to be transported.

Yet another SPE-based active sampling system, the continuous flow integrative sampler (CFIS), uses a peristaltic pump to pass water through a sorbent in a glass cell. This device is packed with PDMS (on a Twister™ bar) and one study compared it with another active sampling device, the constantly stirred sorbent sampler (CSS), assessing both samplers' ability to aid in the detection and quantification six polycyclic aromatic hydrocarbons (PAHs) and three organochlorine pesticides.⁵⁹ Both devices required about 0.5W of energy, so neither was technically a "passive" sampler, but only the CFIS used calibrated flow through a sorbent cartridge (see Figure A-3). The CSS used a motor that creates a turbulent zone around the sampling cell (see Figure A-4), and the uptake kinetics were dependent upon the mass transfer rate to the sorbent. It can be very difficult to time-integrate the masses collected onto the sorbent of the CSS to obtain time-integrated concentrations; to correct for turbulence variability, performance reference compounds (PRCs) could be spiked into the sorbent, and to be released at a rate proportional to the turbulence.⁶⁰ This concentration calculation method produces not time-weighted average concentrations, but flow-weighted averages. Since the mass transfer of analyte to the

sorbent is no longer diffusion-limited, the sorbent adsorbs analytes much more quickly. The CFIS on the other hand, uses the calibrated flow rate from the peristaltic pump for calculation of concentration if the mass transfer is governed primarily by the flow through the sorbent material. The CFIS and CSS were compared in one study, both using PRC calibration; the samplers were evaluated in wastewater treatment plant effluent, and the two methods obtained very similar results for *p,p'*-DDE, Benzo[a]pyrene, Chrysene, and Benzo[g,h,i]pyrene, although limits of detection (LODs) were about ten times lower for CSS than CFIS.⁵⁹ However, since these LODs were time-restricted (for a 5 day sampling period), and the rates of “flow” around the sampling cell in each case were not equivalent, it is hard to say how these methods really compare in terms of detection limits. The CSS and CFIS were validated for a sampling period of 48 hours, then deployed for 5 days, and produced data in agreement with data produced by grab samples that were extracted in the lab. These samplers show the potential for the use of in situ analyte extraction, which eliminates the need for water sample transport, as well as time-integration and flow-integration capability. They are also fairly small, and easy to deploy.

Another active sampler used either a solid phase microextraction (SPME) fiber or a polydimethyl siloxane (PDMS) thin layer film mounted to an electric drill. This approach aimed to gain some control over the exposure rate and sampling time not afforded by a passive sampling approach.⁶¹ Calibrated uptake curves for various PAHs were generated by extracting the chemicals from an exposed film at various time intervals (2-60 minutes) for up to 4 hours. After performing a linear regression (extracted mass vs. time), the uptake data was used to estimate analyte concentrations in the environment. The PDMS thin layer film sampler was employed in the field for rapid

extraction (5 minutes), and the data it produced was in agreement with while the fiber was used to extract water in the lab, and the results between the two methods were in agreement.

Other research groups have used other methods of *in situ* concentration, including Okumura et al at Shimane University in Japan.⁶² While their collector was not designed to sample HOCs, the principle of their design could be easily modified for that purpose. Okimura's group used a manually operated syringe to collect water and pass it through a functionalized C₁₈ Sep-Pak SPE cartridge packed additionally with an OH anion exchange resin. This method of collection was used on surface water at Lake Nakaumi, where the group determined the special distribution of manganese.⁶² Although the method was not automated, and samples collected were time-discrete, the mere inclusion of an enclosure and syringe pump would automate this bench-top-validated extraction method. A hypothetical design applying this modification is shown in Figure A-2. An automated syringe pump design is attractive due to its simplicity, and its potential for time-integrated or time-averaged sampling strategies.

A recent advent in the realm of automated water extraction is the Continuous Low-level Automatic Monitoring (CLAM) sampler, which is capable of extracting tens of liters of surface water through a solid-phase extraction disk over one to two days.⁷ It runs on four AA batteries, and has been shown to produce detection limits for numerous HOCs in the low pg/L range. However, it does not have a direct means of assessing the volume of water processed, and the flow rate through the diaphragm pump is not constant because of the deposition of particulates on the SPE disk; the volumetric load is calculated by averaging the flow rates before and after deployment. Low power use, time-integrated

data, low detection limits, ease of deployment, commercial availability, and relatively low cost (\$2500/unit) make the CLAM a strong choice for monitoring HOCs in surface water. However, while it is possible to stack SPE disks in series on the CLAM, the only way to get field replicates is to purchase multiple CLAMs, making a basic quality control measure very expensive.

In 1977, the Rhine Basin Program was initiated, the objective of which was the protection of waters from chemical pollutants and the focus of which was to design and implement automated monitoring systems. The monitoring system that arose from this international program was the System for the Automated Monitoring of Organic pollutants in Surface Water (SAMOS). Automated monitoring systems have the advantage of providing quick data, as sample preparation and analysis are all on-line. The SAMOS has been used to obtain pesticide data at several surface water locations in Europe.^{63,64} It has also been modified to include an ultra violet-visible (UV-Vis) diode array detector after a C₁₈ LC column and has been validated in this configuration on the bench as an effective detection and quantitation instrument for 27 different polar organics, including chloridazon, atrazine, and bromacil.⁶⁵ A SAMOS consists of a preconcentration unit that includes SPE columns (C₁₈, C₈, polystyrenedivinylbenzene, etc.), as well as an automated elution unit (see Figure A-1). The analysis module can incorporate either gas chromatography (GC), or liquid chromatography (LC).⁶⁵ The SAMOS preconcentration module is essentially a PROSPEKT (Programmable On-line Solid Phase EKstraction Technique) bench top automatic SPE preparation unit.⁶⁶ Detection units can include UV-Vis,⁶⁵ or mass spectrometry (MS).⁶⁷ The LC-MS configuration has shown good results for a number of polar organics like triazines and phenylurea, with detection limits in the range of 5-20

ng/L in river water,⁶⁸ while the UV-Vis method gives detection limits for 27 polar compounds in the range of 0.1-3 µg/L.⁶⁵ The cost and complexity of SAMOS is such that its regular use as a field monitoring device may be untenable, and an alternative on-site sampling/sample prep/analysis tool called PROFEXS was designed under the “On-line Waste Water Analysis” (OWWA) project. The PROFEXS performs SPE on up to sixteen small or large sample volumes, and the cartridges can then be shipped for further processing (e.g. elution/filtration via a PROSPEKT-2) and analysis. The PROFEXS by itself has been validated as a tool for measuring benzene- and naphthalene-sulfonates in environmental sewage.⁶⁹ López-Roldan et al tested the PROFEXS with an on-line LC-APCI-MS in a configuration analogous to that of SAMOS.⁶⁹ The bench-scale test identified 20-50 mL breakthrough volumes for 23 endocrine disrupting pesticides and herbicides and detection limits in spiked Milli-Q water were typically lower than 100 ng/L.⁶⁹ Recoveries with the PROFEXS-LC-MS system were compared with PROSPEKT bench-top SPE, and the results lined up well (typically within 10%).⁶⁹ The method was then applied to surface water, groundwater, and drinking water samples; surface and ground waters showed pesticides and phenols in ng/L and µg/L ranges, but no detection in treated drinking water.⁶⁹ López-Roldan et al imply that the PROFEXS SPE-LC-MS system is more viable as a field *in situ* preconcentration and analysis device than is SAMOS.⁶⁹ However, it is unclear what advantages the PROFEXS SPE-LC-MS holds over the analogous SAMOS configurations. It is also unclear if the advantage of on-line analysis offsets the disadvantages of lab analysis of field-loaded SPE cartridges. Field deployment of analytical instruments like these systems require is difficult and risky, and López-Roldan et al do note that the “[PROFEXS] design still lacks autonomy and easy

portability.”⁶⁹ Furthermore, an automated sampling system with an attached analytical instrument (e.g., the SAMOS) costs significantly more than other sampling technologies. The benefits offered by on-line in situ extraction and analysis may not outweigh the economic drawbacks. Additionally, if left to operate autonomously, the SAMOS or PROFEXS systems could become damaged, leading to very high repair costs.

Pore water sampling

Assessing HOCs can be complicated by the presence of organic carbon (OC). Water in equilibrium with contaminated OC-laden sediments ($C_{\text{sediment}} \sim 1$ ppm) can be lower than 1 ng/L. Estimation of pore water concentrations can be done by using equations 2-2 and 2-3 (derived from Schwarzenbach et al., 2005):⁷⁰

$$f_{H_2O} = \frac{1}{1 + \rho_{\text{sediment}} \left(\frac{1-\phi}{\phi} \right) K_{OC} f_{OC}} \quad (2-2)$$

$$C_{pw} = f_{H_2O} \left(\frac{C_s}{\phi} \right) \quad (2-3)$$

Where f_{H_2O} is the fraction of chemical in the aqueous phase, C_{pw} is the concentration of the analyte in the aqueous phase in pore water, C_s is the total sediment concentration, ρ_{sediment} is the dry sediment density, ϕ is the porosity, K_{OC} is the organic carbon partitioning coefficient, and f_{OC} is the fraction of organic carbon in the sediment. At a total sediment concentration of 1 mg/kg (1 ppm m/m), the aqueous concentrations of many HOCs will be less than 1 $\mu\text{g/L}$. One way to overcome the difficulty of detecting low-level contaminants using grab samplers is to collect large volumes of water and perform liquid-liquid extraction (LLE) or solid-phase extraction (SPE) in the lab. The drawbacks of LLE include the use of large volumes of solvents, and the potential for

contamination from dust in the lab. SPE uses relatively small volumes of solvents by comparison, but the potential for in-lab contamination still exists. Triclosan, for example, is a common sediment contaminant resulting from biosolids applications, and is also a common antimicrobial agent found in many antimicrobial soaps.⁷¹ Unintentional contamination of a triclosan-containing sample is reasonably likely to occur in a lab merely because researchers might use antimicrobial soaps to wash their hands.

Solid phase extraction is commonly used for preconcentration of HOCs, and it can be done either in the lab after collecting water samples, or it can be done directly in the field. Oftentimes, large volumes of water need to be extracted in order to enhance the signal produced by trace analytes. The volume of water needed to obtain detectable masses of analytes in sediment pore water, for example, can be calculated using equation 2-4.

$$V_{field_sample} = \left(\frac{V_{prepped_sample}}{V_{injected_column}} \right) \times \frac{IDL \cdot \left[1 + \rho_{sediment} \left(\frac{1-\varphi}{\varphi} \right) K_{OC} f_{OC} \right]}{\left(\frac{\rho_{sediment}}{\varphi} \right) C_{sediment} \cdot R} \quad (2-4)$$

Where $\rho_{sediment}$ is the density of the sediment, φ is the sediment porosity, K_{OC} is the organic carbon partitioning coefficient, f_{OC} is the fraction of organic carbon that makes up the sediment, $C_{sediment}$ is the total concentration of analyte in sediment, and R is absolute recovery. Figure 2 illustrates the fact that sample volume is a significant factor to consider when assessing HOCs in contaminated sediments. For example, in sediment contaminated with 1 mg/kg *p,p'*-DDE, assuming standard partitioning behavior and 100% analyte recovery, it could take a sample volume of 100 L, preconcentrated 10,000-fold to detect the aqueous phase compound using a tandem mass spectrometer with an IDL of 0.1 pg. Considering that loss of analyte during sample preparation can be significant, even larger sample volumes may be necessary. It is impractical to manually

collect enough sediment to extract tens or hundreds of liters of pore water, and quantifying sample losses would be challenging. In situ extraction is therefore a potentially useful means of determining pore water concentrations of HOCs, as it eliminates the need to transport and process large volumes of water and sediment, and reduces losses due to sampling handling. However, pore water samplers do not typically employ this strategy.

The relevance of the problem of high sample volume is particularly salient when the aim is to determine the chemical activity or labile concentration of trace level HOCs. The labile fraction of very hydrophobic compounds in waters with high amounts of organic carbon (OC) can be very low, yet this is the fraction that many researchers argue is representative of the risk posed to biota.⁷²⁻⁷⁵ Since the purpose of sampling contaminants is generally to determine either their bioavailability for microbial degradation, or the risk they pose to macrobiota such as fish, it is the mobile matrix that must be sampled, as it is well-known that the total sediment concentration is not representative of the bioaccumulation potential of HOCs, especially for aged sediments.^{76,77} For example, aged lindane-spiked sediments showed diminutive toxic effects on *Drosophila melanogaster* when compared with freshly-spiked sediment.⁷⁸ Other HOCs, such as volatile organic compounds (VOCs) present their own challenges, as VOCs tend to volatilize from the upper layers of surface water systems, and into the headspace of sampling containers. In both surface and groundwater systems, hydrophobic VOCs like perchloroethylene (PCE) also tend to partition preferentially to OC-laden sediments.^{79,80} All of these challenges must be met with adequate sampling plans to produce reliable and useful data.

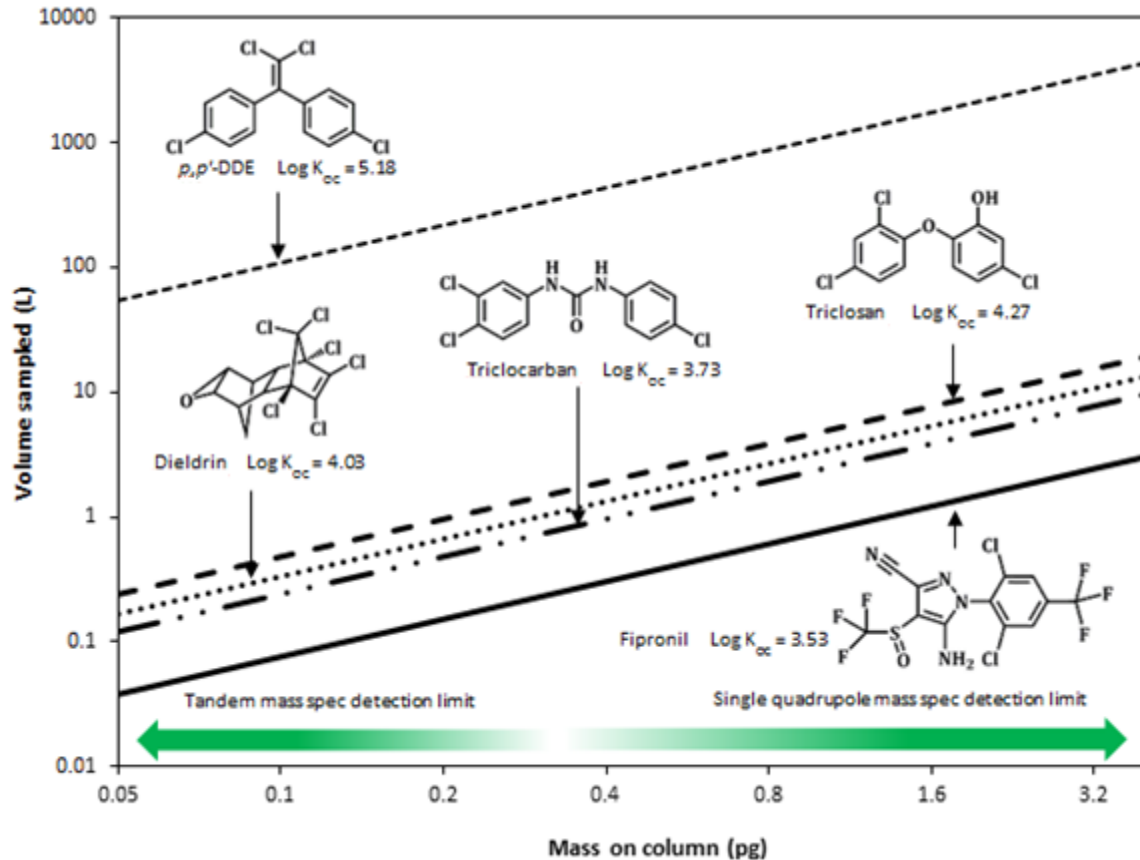


Figure 2-3. Volume of saturated pore water needed to acquire a given mass of five HOCs on a chromatography column, assuming 100% recoverability in a 10 mL extract, 30% OC, a dry sediment density of 1.6 kg/L, and 1 mg/kg total sediment concentration. Partitioning coefficients were estimated using EPI SuiteTM.⁸¹

Sediment pore water can be sampled using passive samplers, but due to the fact that some sediments have fairly stagnant pore water, advective transport of chemicals to the sampler surface can limit sampler uptake rates. Active sampling can sample comparable volumes of water in less time. One common way of sampling pore water is using a vacuum extraction technique, wherein a depressurized bottle is connected to a suction cup via plastic tubing; the suction cup is placed over the soil, and the pressure gradient drives the transfer of pore water to the bottle. According to some advocates, vacuum pore water extractors produce samples that retain the in situ characteristics of the pore water.⁸² The principles of vacuum pore water extraction have been incorporated into a device

called a Rhizon sampler. A Rhizon sampler is a narrow screened tube connected to a Luer fitting. The tube can be plunged into sediment, at which point the pore water can be drawn out. One study using Rhizon samplers used three different methods of drawing pore water: (1) vacuum tubes; (2) hand-operated syringes; (3) a peristaltic pump.⁸³ Another similar sampler called the MINIPOINT sampler is comprised of needles of varying lengths attached to a circular plate, and it is used for obtaining several simultaneous depth discrete samples.⁸⁴ To our knowledge, MINIPOINT samplers have not been used for monitoring HOCs. Nor have they been automated, but they can possibly be modified to automatically sample HOCs in pore water by using either a syringe pump or peristaltic pump. Further modification could involve the incorporation of SPE resin or other sorptive materials in order to enable *in situ* preconcentration. The advantage of pore water samplers is that they can be used to assess an environmental compartment that is relevant for benthic aquatic organisms, and to elucidate how contaminants traverse the sediment-water interface.

Comparisons

There are a variety of active sampling options, some of which are more robust or more flexible than others. Passive sampling is used much more commonly than active sampling for HOC monitoring. Active in situ extraction devices like the thin layer film sampler or SAMOS systems are used much less commonly still; thin layer film sampling as described by Qin et al is designed for rapid (5 min) sampling, and is therefore not capable of time-integrated sampling, while the SAMOS is extremely expensive to operate and maintain. While the precision of data produced by active sampling devices is comparable to that produced using passive sampling devices (see Table 2-1), there are drawbacks to

applying these particular technologies in scenarios where time-integrated concentrations are desired. Indeed, some active sampling devices like the CSS, or an automated in situ SPE device (a pump attached to an SPE column) are capable of producing time-integrated or time averaged data with high precision, and without the need for the laborious calibration that passive samplers require.

Table 2-1. Comparison of the precision resulting from field replicates generated by various sampling technologies used for organic pollutant assessment.

Sampler type	Compound(s)	Water type	# of analytes	<i>n</i>	%RSD or reproducibility	Source
Active samplers						
ISCO	PCBs	Storm	40	NR	≤16	(Gilbreath et al, 2012) ⁸⁵
HBS	Pesticides	Stream	5	NR	3-37	(Xing et al, 2013) ⁵⁶
IS2B	Fiproles	Surface & pore water	4 ^b	3	8-60	(Supowit et al, 2015) ⁸⁶
PROFEXS	HOCS	Milli-Q (spiked)	24	3	0.4-60.6	(Lopez-Roldan et al, 2004) ⁶⁹
Pump + PUF (active) ^a	PCCD/Fs	Bay	19	4	21-133	(Cornelissen et al, 2010) ⁸⁷
SAMOS-GC	Pesticides	Tap (spiked)	6	7	NR	(Pittertschatscher et al, 1999) ⁸⁸
SAMOS-GC	Pesticides	River (spiked)	6	NR	2-4	(Brinkman et al, 1994) ⁶⁶
SAMOS-LC	Pesticides	River (spiked)	9	5	2-49	(Lacorte et al, 1998) ⁸⁹
SAMOS-LC	Pesticides	River (spiked)	27	8	1-9	(Brinkman et al, 1994) ⁶⁶
Thin film sampler	PAHs	River	6	3	6-11	(Qin et al, 2009) ⁶¹
Passive samplers						
Chemcatcher	Organotins	Harbor	3	6	14-29	(Aguilar-Martínez et al, 2008) ⁹⁰
PE	Triclosan	River, bay, harbor	1	NR	6-8	(Perron et al, 2013) ⁹¹
PE	PBDEs	River, bay, harbor	5	NR	4-35	(Perron et al, 2013) ⁹¹
POM	PCCD/Fs	Bay	19	4	30-100	(Cornelissen et al, 2010) ⁸⁷
POM	Triclosan	River, bay, harbor	1	NR	2-29	(Perron et al, 2013) ⁹¹
POM	PBDEs	River, bay, harbor	5	NR	0.4-13	(Perron et al, 2013) ⁹¹
SPMD	PAHs	Wastewater influent	9	2	6-31	(Stuer-Lauridsen & Kjølholt, 2000) ⁹²
SPMD	NPEs/DEHPs	Wastewater influent	3	2	14-56	(Stuer-Lauridsen & Kjølholt, 2000) ⁹²
SPME	PAHs	Pore water	3	20	20-77	(Stringer et al, 2014) ⁹³

a – Concentrations are at the pg/m³ level (all other concentrations are typically reported between 1 and 10⁴ pg/mL)

b – A fifth analyte was omitted, due to the fact that reported concentrations were estimated near the detection limit

n – field replicates

NR – Not reported

As mentioned before, it is important to tailor a sampling strategy for a given application. Table 2-2 shows a variety of active sampling technologies and ranks their various capabilities and applications in terms of their utility for a given application or ease of operation. While SPE-based active sampling techniques have been designed and validated by a number of researchers, their use in the field is still limited. Devices like the SAMOS and PROFEXS on-site sample prep and analysis tools streamline the process of water monitoring, have low detection limits, and can be used for low-level surface water monitoring of POPs like pesticides. But they are still expensive, and burdensome to deploy. These all-in-one tools might one day prove to be mainstream monitoring devices if they can be scaled down. Furthermore, transporting expensive analytical instruments like mass spectrometers to field locations may not be ideal. On the other side of the spectrum, in situ extraction devices that require very little power and are small and easy to deploy offer the same sample preparation benefits as a SAMOS, without the bulk and expense. The up-front expense of machinery is instead diverted to laboratory analysis costs.

Most of the active sampling devices discussed are not commercially available, but were developed as prototype proofs of concept for means of extracting analytes from water in situ. The CLAM, however, is one of the first commercially available devices of its kind. It is able to generate a time-integrated composite sample on an SPE disk, without actually collecting any water. It is, however, not programmable, and the means of determining the total volumetric load through the SPE disk are not precise.

Options for sampling pore water are few, with the most common means of sampling pore water being grab sampling of wet sediment, followed by separation of the water from the sediment. Rhizon samplers are apparently scarcely used to monitor HOCs as far as we can tell, and the MINIPPOINT sampler does not appear to be used for this purpose at all. Furthermore, these devices are not automated. Rhizon samplers can potentially be automated with the incorporation of a pump, controller, and software.

Table 2-2. Comparison of active sampler features, capabilities, and applications (including potential applications). Cost bins are as follows: \$ = \$1–\$100; \$\$ = \$101–\$1,000; \$\$\$ = \$1,001–\$10,000; \$\$\$\$ = \$10,000+. Large numbers in cells refer to the magnitude of evidence of use for the given criterion in terms of relative number of publications. Superscript numbers are references to examples in literature.

Feature	Grab						Water collection				In situ extraction					
	Bottle/ bailer	Kemmerer sampler	MINI- POINT	Rhizon	Thin layer film sampler	Syringes <i>In situ</i> SPE	Active GFF/PUF sampling	ISCO/ Sigma	Gulper samplers	Osmo- Sampler	CFIS	CSS	Automatic SPE units*	CLAM	PROFEXS	SAMOS
Sampling specification																
Time integration	0	0	0 ^a	0 ^a	0	0 ^a	0	0	0	2 ^{94,95}	1 ⁵⁹	1 ⁵⁹	1 ⁹⁶	1 ⁷	0	0
Flow-weighting	0	0	0	0	0	0	130 ^{97,99}	0	0	0	0	0	0	0	0	0
Time discrete	12 ^{100,101}	70 ¹⁰²	32 ⁸⁴	70 ¹⁰²	39 ^{89,103}	2 ^{62,104}	1 ⁸⁷	1 ¹⁰⁵	1 ¹⁰⁶	0	0	30 ^{107,108}	0	0	47 ⁶⁹	39 ^{89,103}
Sample handling																
Volatile loss reduction	0	0	0	0	0	0	0	0	0	0	0	0	0	1 ⁷	0	0
Adsorption loss reduction (HOCs)	0	0	0	0	0	0	0	0	0	0	0	0	0	1 ⁷	0	0
Applications																
Mass balances	3900 ¹⁰⁹⁻¹¹¹	0	N/A	N/A	N/A	0	0	139 ^{97,112}	N/A	0	0	0	0	0	0	0
Spacial characterization	50 ^{113,114}	12 ¹¹⁵	0	12 ¹¹⁵	Potential 0	0	10 ¹¹⁶	1 ¹¹⁷	0	0	0	0	0	2 ¹¹⁸	0	0
Matrices																
Sediment pore water	860 ^{119,120}	0	32 ^{84,121}	130 ^{102,122}	0	0	0	0	0	0	0	0	0	0	0	0
Wastewater	1990 ^{123,124}	50 ^{125,126}	0	0	0	0	273 ^{127,128}	0	0	0	0	0	0	0	0	0
Cost range	\$	\$	\$	\$	\$	\$	\$	\$\$\$	\$\$\$	\$\$\$	\$\$	\$\$\$	\$\$\$	\$\$\$	\$\$\$	\$\$\$\$
Automation	N/A	Potential ^a	Potential ^a	Potential ^a	None	Potential ^a	None	High	High	Moderate	Moderate	Moderate	Moderate	Moderate	High	High
Commercially available	Y	N	N	Y	N	Y	Y	N	N	Y	N	N	N	Y	N	N

a – If an automatic pump were to be integrated (not present in current design)

*Any device aside from the CLAM that automatically extracts water using a pump and SPE resin.

Least suitable  Most suitable

Conclusions

While most cost-effective active sampling techniques like bailing or Kemmerer sampling provide useful data, this data is of limited importance when applied for some purposes (e.g., mass transport calculations). The alternative active sampling methods involving collection or extraction of large volumes of water can produce time-integrated data.

While large volume water collectors are useful for some applications requiring time-integrated or flow-weighted sampling, analyte loss due to sample handling issues may still be a concern. In situ preconcentration methods are already an accepted and practiced method for sampling surface waters, and they are proven effective for a wide range of compounds, including some of the most challenging compounds. Very nonpolar compounds like halogenated pesticides that are found at very low concentrations typically require method detection limits afforded by preconcentration techniques. In situ preconcentration may have potential benefits over lab preconcentration options, the most important of which is sample loss mitigation. It should be noted, however, that this assessment is primarily theoretical, as studies evaluating the sample losses attributed to a given sampling technique are limited. The drawbacks to passive sorptive sampling (e.g., lack of uptake rate control) can be overcome by active sorptive sampling. Indications are that active sampling techniques may allow for detection of certain contaminants that grab sampling and passive sampling techniques may miss. Further studies may lead toward a paradigm shift with regard to the “standard” approaches for monitoring of HOCs in surface waters, where active, automated techniques might be mainstream methods of sampling. Given that the cost of automated in situ extraction devices like the CLAM are comparable to the cost of basic water collectors, this shift should occur with the

incorporation of programmable options for extraction devices in order to match the capabilities of ISCO or Sigma samplers. For bioaccessibility assessment, modification of active samplers to include large particulate filters may allow for more rapid sampling of pore waters for pesticides, herbicides, or PCBs by percolation rather than slow diffusion onto membranes. As it is necessary to ensure the human and ecological safety of our surface waters, so it is also necessary to continue to develop and employ the best possible tools for environmental assessment.

Literature search methods

Literature searches were done using Google Scholar. In order to determine the number of publications referencing a given sampler's utilization for the purpose of monitoring or sampling for HOCs, the following search strategy was employed: [sampler name] AND HOCs OR VOC OR pesticide OR PAH OR OCP OR PCB OR POPs OR "persistent organic pollutant" OR "hydrophobic organic contaminant" "pore water" OR porewater NOT metals NOT arsenic NOT groundwater. The results were screened for irrelevant results, which were discarded when found. In order to determine the relative number of publications in which a given sampler was used for a particular matrix, utilized for a particular application, or has a particular feature (e.g., time-integrated sampling capability), the following strategy was employed: the matrix, application, or feature was defined as one variable parameter, while the sampler type (e.g., Rhizon sampler) was defined as the other; using Google Scholar, the search terms [sampler] AND [matrix/application/feature] AND water were applied. The results were screened and irrelevant results were omitted when the number of results was low (<100).

TRANSITION 2

Accurate assessment of contaminants in the environment hinges on several factors, one of which is the selection of sampling strategies and technologies. Another equally important factor is the use of an analytical method which is both sensitive and precise enough to generate data that can be used to assess various outcomes, like temporal and spatial trends, or fate and transport. Chapter 2 provided an overview of available active sampling technologies. The next section focuses on analytical tools necessary to measure emerging contaminants such as broadly used pesticides. In Chapter 3, I describe the development and application of a liquid chromatography tandem mass spectrometry method for the determination of phenylpyrazole pesticides belonging to the fiprole family. I further applied this analytical tool to study the occurrence of fiproles in water systems (e.g., wastewater) with the goal of establishing the utility of this tool as a robust, sensitive, and precise analytical method. Considering that environmental concentrations of fiprole compounds can be in the 0.01-1000 ng/L range, tracking the fate of fiproles in complex matrices at low concentrations (<100 ng/L) is challenging. While these compounds have been studied in surface water matrices, and to a small extent in wastewater matrices, it is clear that the methods used at the time for quantification were insufficient to enable accurate fate assessments.

Chapter 3 introduces a strategy for precisely quantifying fiproles at low ng/L levels in complex wastewater and sludge matrices. The method, in conjunction with an appropriate *sampling strategy* using ISCO samplers, is then applied to assess fiproles in various wastewater streams. The method detection limits, analyte recoveries, and precision are reported as measures of method performance.

CHAPTER 3

DETERMINATION OF THE PHENYLPYRAZOLE PESTICIDE FIPRONIL AND ITS MAJOR DEGRADATES IN SEWAGE, SLUDGE, AND SURFACE WATER

ABSTRACT

Tracking the fate of phenylpyrazole pesticides in wastewater treatment has been shown to be challenging due to low concentrations of fipronil and its degradates (fiproles).

Previously existing methods did not feature the precision and accuracy required to determine their fate during wastewater treatment with confidence. Here we introduce and apply a sensitive method for the detection and quantification of five fiproles (fipronil, as well as the sulfide, sulfone, amide, and desulfinyl byproducts) in wastewater matrices.

Method detection limits for the various analytes ranged from of 50-770 pg/L and 20-240 ng/g for surrogate wastewater and dewatered sludge, respectively. Average absolute recoveries in those respective matrices ranged from $60 \pm 14\%$ to $101 \pm 19\%$ and 48 ± 18 to $90 \pm 21\%$, while relative recoveries of fipronil using a labeled standard surrogate were 116 ± 14 and $120 \pm 13\%$. The method was used to assess fiproles in a wastewater treatment plant and downstream wetland by analyzing plant influent, effluent, wetland effluent, and dewatered sludge generated by anaerobic digestion. Concentrations of total fiproles (as fipronil) in those respective streams were 29 ± 5 ng/L, 28 ± 6 ng/L, 21 ± 4 ng/L, and 14 ± 7 ng/g. Application of the method to various waste streams of a full-scale wastewater treatment plant demonstrated persistence of many of these compounds and limited conversion of fipronil to fipronil sulfide during anaerobic digestion.

Introduction

Fipronil and its degradates (known collectively as “fiproles”) are phenylpyrazole pesticides that disrupt the central nervous system of insects.¹²⁹ The parent compound, fipronil, is the active ingredient in a number of insecticidal products, including flea treatments for pets, roach and ant bait, grass and turf treatments, and agricultural products such as seed coatings. Fiproles are extremely toxic to many arthropods, and to some vertebrate aquatic organisms.

Fipronil and the neonicotinoid imidacloprid have been detected in depopulated honeycombs at levels known to cause disorientation in bees.¹³⁰ Considering that the total estimated economic value of pollinators worldwide amounts to about \$170 billion (€153 billion),¹³¹ likely sources of pesticide discharge into the environment where non-target organisms such as bees may be exposed is a relevant concern.

Fipronil is applied to urban turfgrass, agricultural fields, as seed coatings for general pest control, and to household foundations in the form of commercial termiticides. While fipronil is only moderately persistent in the environment (half-life = 21 days in silt loam),¹³² its degradation byproducts are highly persistent (195–589 days), even under facultative conditions.^{132,133} It is therefore reasonable to expect that fiproles will build up in soils treated by pesticide products using fipronil as the active ingredient, and then be transferred to urban and rural waterways.¹³⁴⁻¹³⁷ Fiproles may also end up in sewersheds as a result of agricultural or urban runoff; the parent compound fipronil has been detected in wastewater streams,^{24,25} and in aquatic systems impacted by wastewater.^{25,138,139}

There are several examples in the literature where fiproles are assessed. A method for HLB extraction of fipronil, along with the sulfide, sulfone, and desulfinyl degradates in river water matrices using gas chromatography/mass spectrometry (GC/MS) analysis yielded method detection limits (MDLs) ranging from 1.6 to 7.9 ng/L, with average recoveries ranging from $73 \pm 15\%$ to $110 \pm 3\%$; this method did not use isotopically labeled internal standards or standard addition for quality control.¹⁴⁰ A United States Geological Survey (USGS) method for assessing five fiproles via C-18 extraction and GC/MS analysis yielded MDLs averaging 2.9 ng/L, and average recoveries of 98%; extraction-specific performance was monitored using two analog surrogate compounds (Diazinon-*d*₁₀ and *alpha*-HCH-*d*₆); Phenanthrene-*d*₁₀ was used as an internal standard to monitor instrument performance.¹⁴¹ Thus far, fipronil and its degradates have been assessed in wastewater matrices in a few studies. Heidler and Halden achieved MDLs of 20 ng/L, 10 ng/L, and 0.4 ng/g, with recoveries of $112 \pm 4\%$, $165 \pm 22\%$, and $53 \pm 10\%$ for fipronil in wastewater influent, effluent, and sludge, respectively.²⁴ Furthermore, the precision of the measurements reported therein was 10 ng/L, which is large compared to the averaged measured concentration of 30 ng/L.²⁴ Weston and Lydy determined a fipronil concentration range of 39–119 ng/L and fipronil-desulfinyl concentrations of <6 ng/L in wastewater influent using filtration followed by liquid-liquid extraction and GC/MS analysis; the sulfide and sulfone degradates were not detected in untreated wastewater.²⁵

The goal of this study was to precisely measure fipronil and four of its degradation byproducts such that accurate fate assessments in wastewater streams is possible. The extraction and analysis methods for wastewater and dewatered sludge (biosolids) were

designed to yield sub-ppt and sub-ppb (ng/L and ng/g) detection limits in order to enable precise assessment of fiproles in wastewater and sludge, respectively.

Results and discussion

Instrument results

Liquid chromatographic separation was performed using both C-8 and IBD columns and with both MeOH/H₂O and ACN/H₂O mobile phases with varied gradients. MeOH was too weak a solvent to achieve peak separation of fiproles on the C-8 column, while ACN was able to achieve adequate peak separation at a flow rate of 1 mL/min and a gradient of 10% ACN/min. MeOH achieved adequate peak separation of fiproles on the IBD column at a flow rate of 0.4 mL/min and a gradient of 10% MeOH/min to 90% MeOH. Run times using these two eluent/column combinations were similar, so the MeOH/IBD eluent/column combination was chosen, due to the high cost of acetonitrile. After optimization, fipronil amide, fipronil, fipronil sulfide, and fipronil sulfone eluted from the column at 6.50, 6.57, 6.72, and 6.86 minutes, respectively. The GC temperature program began at 70°C, and ramped at 20°C/min to 300°C. Fipronil-desulfinyl eluted from the column at 7.70 minutes. Optimized mass spectrometer instrument parameters are given in Table B-1.

Analytical method performance

Fipronil and the sulfide, sulfone, and amide degradates displayed good sensitivity when using LC-ESI-MS/MS, while fipronil-desulfinyl had an instrument detection limit above 1 µg/L. Only fipronil-desulfinyl displayed good sensitivity when using GC-EI-MS/MS.

Method detection limits determined using surrogate matrices are shown in Table 3-1.

Absolute recoveries at low spike levels (≤ 1 ng/L in water or ≤ 0.5 ng/g in solids) ranged from $48 \pm 18\%$ to $101 \pm 19\%$. Relative recovery of fipronil is $116 \pm 14\%$ in surrogate wastewater, and $120 \pm 13\%$ in surrogate dewatered sludge. Detection limits are over ten times lower than concentrations detected for most analytes. Fipronil-desulfinyl concentrations are near or below the detection limit, and are therefore estimated.

Real biosolids samples were spiked to a nominal concentration of 20 ng/g, and then extracted and analyzed as described. Between 50 and 90% of the recoverable mass was detected in the first eluate (DCM), and the signal-to-noise ratios were very favorable ($>100:1$), as were recoveries ($> 60\%$). All fiproles but one (fipronil amide) were detected in unspiked biosolids in pre-screening.

Table 3-1. Spike levels, detection limits, and recoveries of fiproles extracted from surrogate wastewater and sludge matrices ($n = 7$).

Chemical	Wastewater				Solids			
	Spiking level (pg/L)	MDL (pg/L)	Relative recovery (%)	Absolute recovery (%)	Spiking level (pg/g)	MDL (pg/g)	Relative recovery (%)	Absolute recovery (%)
Fipronil	100	45	116 ± 14	60 ± 14	50	20	120 ± 13	55 ± 18
-Sulfide	300	160	N/A	67 ± 13	150	140	N/A	48 ± 18
-Sulfone	200	70	N/A	101 ± 19	100	100	N/A	89 ± 32
-Amide	500	300	N/A	87 ± 22	250	90	N/A	90 ± 21
-Desulfinyl	1000	770	N/A	78 ± 15	500	240	N/A	85 ± 15

N/A \equiv Not applicable

The selected ion transitions used for analyte quantitation and qualification are shown in Figure 3-1. Since the first eluate produced favorable results, but did not elute 100% of the recoverable mass from the Florisil cartridges, subsequent sludge extractions involved commingling equal volumes of the serial eluates (DCM and acetone) and reducing the final solvent volume to half, using either 1:1 MeOH/water (v/v) or 100% hexane for LC-MS/MS analysis and GC-MS/MS analysis, respectively.

Fipronil was quantified using isotope dilution and a 7-point calibration curve with an R^2 value of 1.000 (see Figure B-1). Fipronil-desulfinyl was quantified using external calibration and a 6-point calibration curve with an R^2 value of 1.000. Fipronil sulfide, sulfone, and amide were all quantified using standard addition; sludge extract standard addition calibration curves yielded R^2 values of 0.977 to 0.987, while various wastewater stream standard addition calibration curves yielded R^2 values ranging from 0.901 to 0.999.

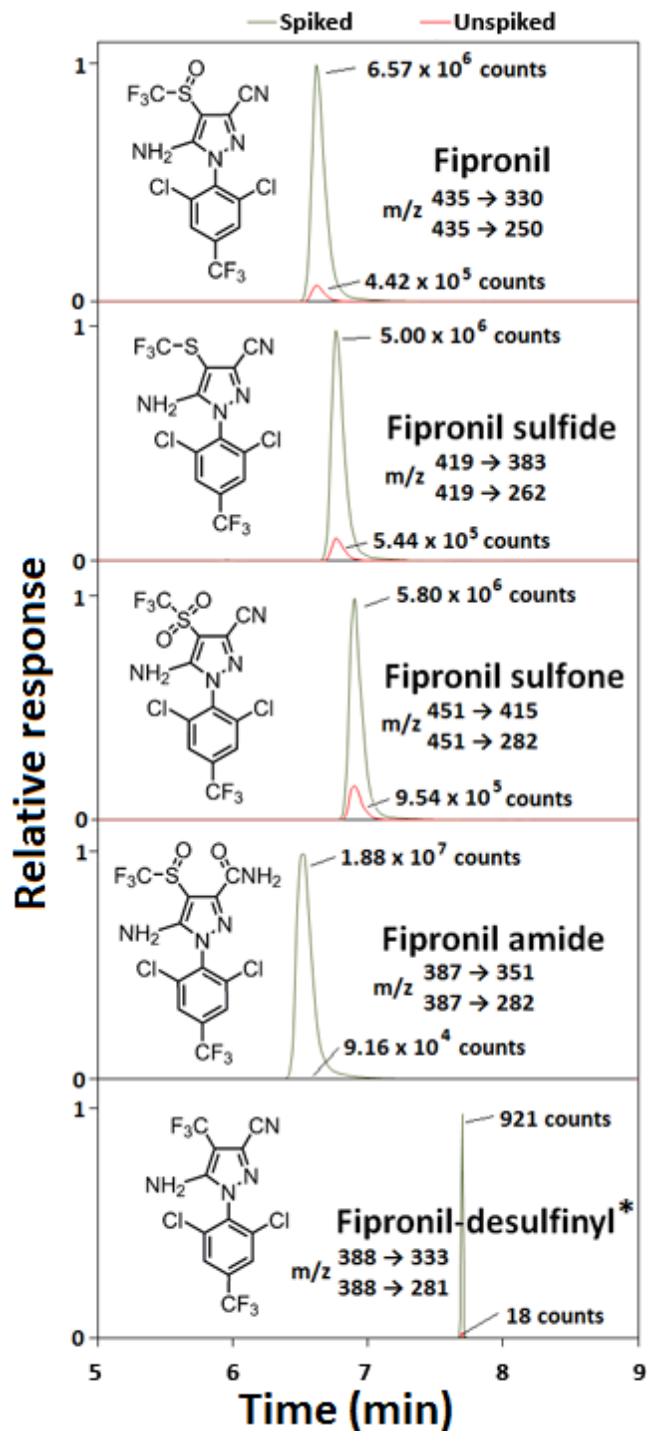


Figure 3-1. Chromatograms of five fiproles extracted from spiked (20 ng/g nominal) and unspiked dewatered sludge, after cleanup on Florisil and elution with 4 mL DCM. Quantitative (top) and qualitative (bottom) ion transitions are displayed beneath the analyte name. Ion counts for the spiked and unspiked biosolids samples are shown at top and bottom, respectively. *Fipronil-desulfinyl was analyzed by GC-MS/MS.

Occurrence in wastewater and biosolids

Concentrations of fiproles in the WWTP influent ranged from 17.4–30.8 ng/L for fipronil, and 4.3–11.0 ng/L for fipronil sulfone. Fipronil-desulfinyl was tentatively detected on days 1–4, but not day 5, and all but one detection was below the MDL. The sulfide and amide degradates were not detected in the influent stream. Concentrations in the WWTP effluent ranged from 16.2–28.2 ng/L for fipronil, 1.8–15.0 ng/L for fipronil sulfone, 0.3–1.1 ng/L for fipronil sulfide, and 0.3–3.8 ng/L for fipronil amide. Peaks for fipronil-desulfinyl were detected on all days, but were below the detection limit. Fipronil sulfide was detected once, and fipronil amide was not detected. Concentrations in the wetland effluent ranged from 11.6–20.0 ng/L for fipronil, 0.5–8.2 ng/L for fipronil sulfone, 0.3–1.4 ng/L for fipronil sulfide, 0.3–2.1 ng/L for fipronil amide, and fipronil-desulfinyl peaks were detected 4 out of 5 days, always below the detection limit.

Concentrations in dewatered sludge ranged from 1.00–2.73 ng/g for fipronil, 0.50–3.78 ng/g for fipronil sulfone, 4.38–18.25 ng/g for fipronil sulfide, 0.11–0.21 ng/g for fipronil amide, and 0.17 (estimated)–6.50 ng/g for fipronil-desulfinyl. Daily average concentrations in the various streams are shown in Table 3-2. Concentrations were relatively consistent for all analytes in all streams, although the concentration of fipronil-desulfinyl on the first day of biosolids sampling was considerable higher than on the other four days. In all three water streams, the concentration of the parent compound fipronil was highest, but in biosolids, the most abundant congener was the sulfide byproduct.

Table 3-2. Average daily fiprole concentrations for various wastewater and wetland streams (expressed in ng/L), and dewatered sludge (expressed in ng/g). Error values are standard deviations ($n = 10$ for water, $n = 15$ for dewatered sludge).

Stream	Fipronil	-sulfide	-sulfone	-amide	-desulfinyl ¹	Total fiproles (as fipronil)
Influent	22.5 ± 4.5	NP	6.7 ± 1.8	NP	0.5 ± 0.8	29.5 ± 4.8
Primary effluent	21.4 ± 3.4	0.9 ± 2.9	5.2 ± 2.6	NP	0.2 ± 0.2	27.6 ± 5.8
Primary sludge	99.7 ± 53.0	3.0 ± 6.7	13.8 ± 9.3	NP	NP	107.0 ± 54.5
Return activated sludge	33.7 ± 8.7	7.8 ± 0.8	25.0 ± 3.8	3.0 ± 0.3	0.01 ± 0.02	76.9 ± 25.6
Secondary effluent	16.4 ± 2.6	2.0 ± 1.4	12.4 ± 11.9	NP	0.1 ± 0.1	30.5 ± 12.9
Chlorination basin effluent	16.2 ± 2.3	0.8 ± 0.6	7.3 ± 5.1	0.7 ± 0.9	0.1 ± 0.1	24.9 ± 5.6
Plant effluent	20.1 ± 3.7	0.6 ± 0.3	5.9 ± 3.9	1.1 ± 1.0	0.1 ± 0.2	27.6 ± 5.6
Wetland effluent	14.7 ± 2.7	0.8 ± 0.4	4.4 ± 2.9	1.1 ± 0.6	0.1 ± 0.2	21.0 ± 4.2
Dewatered sludge*	2.0 ± 0.6	8.4 ± 4.2	1.3 ± 0.9	0.1 ± 0.0	1.2 ± 1.8	13.1 ± 3.0

NP, no peaks detected

* concentrations expressed as ng/g dry weight sludge

¹ detected concentrations near the MDL, estimated

Method utility

The detection limits achieved here are considerably lower than those achieved by other methods, due in part to the use of tandem mass spectrometry (see Table 3-3). Most published methods do not include fipronil amide in their analyses. The method developed herein was more sensitive to fipronil compared to the degradates, while in other studies that use other methods of detection (e.g., electron capture detection), method sensitivity is relatively consistent for all fiproles. Furthermore, this study utilized GC-MS/MS to quantify fipronil-desulfinyl, due to unfavorable ionization using electrospray ionization in the LC-MS interface. However, the other four fiproles displayed less favorable ionization under electron impact conditions. For these reasons, four of the fiproles were analyzed by LC-ESI-MS/MS.

Table 3-3. Method detection limits for fiproles from various studies in water and sludge matrices.

Source	Water (ng/L)					Sludge (pg/g)	
	Schlenck et al, 2001	Heidler & Halden, 2009	Hladik et al, 2008	Weston & Lydy, 2014 ^b	This study	Heidler & Halden, 2009	This study
Matrix	Surface water	Wastewater inf/eff	River water	Wastewater influent	Surrogate wastewater	Sludge	Surrogate sludge
Extraction	LLE (pentane) + normal phase SPE (Florisil)	Reversed phase SPE (HLB)	Reversed phase SPE (HLB)	LLE (DCM) + filtration + GPC	Reversed phase SPE (Strata TM -X)	SLE (MeOH/acetone)	SLE (acetone) + normal phase SPE (Florisil)
Analysis	GC-ECD	LC-MS/MS	GC-ion trap MS	GC-MS	LC-MS/MS & GC-MS/MS	LC-MS/MS	LC-MS/MS & GC-MS/MS
Fipronil	500	10-20	2-2.9	0.88-1.49	0.045	400	20
-sulfide	1000	N/A	1.8-2.2	0.88-1.49	0.16	N/A	140
-sulfone	2000	N/A	3.5-7.0	0.88-1.49	0.07	N/A	100
-amide	N/A	N/A	N/A	0.88-1.49	0.3	N/A	90
-desulfinyl	500	N/A	1.6-2.7	0.88-1.49	0.77	N/A	240

^a The range shown (0.88-1.49) was given for all fiproles, and no individual MDLs are published
^b HLB, hydrophilic-lipophilic balance; DCM, dichloromethane; MeOH, methanol; GPC, gel permeation chromatography; SPE, solid phase extraction; LLE, liquid-liquid extraction; SLE, solid-liquid extraction

Considering that the desulfinyl degradate was considerably less abundant than fipronil, fipronil sulfide, and fipronil sulfone, use of only LC-MS/MS for analysis of the other four congeners should be sufficient to adequately characterize the hazard posed by fiproles in wastewater and surface water streams.

The methods developed herein are sensitive enough to quantify fiproles in wastewater and biosolids to a resolution of 1–5 ng/L and 1–10 ng/g, respectively. The concentration profiles of fiproles in the wastewater streams in Figure B-2 indicate that there is a fairly consistent source of fipronil into the wastewater treatment plant. It is not yet certain what the source of fipronil into the urban sewershed is. Nor is it clear whether the input into the sewershed is consistent, seasonal, or fluctuating, as there have not been longitudinal studies to determine this. The most abundant fiproles in all water streams examined are

the parent compound and the sulfone byproduct, which is consistent with prior studies on the fate of fipronil in aerobic aquatic systems.¹⁴²⁻¹⁴⁴ The most abundant fiprole in biosolids was the sulfide byproduct, which is consistent with studies of the fate of fipronil in anaerobic systems.^{14,132,133}

Methods

Solvents and standards

LC-MS grade solvents (water, acetonitrile) were purchased from Thermo Fisher Scientific (Waltham, MA USA) and EMD Millipore (Billerica, MA USA). Neat analytical standards of fipronil and fipronil-desulfinyl were obtained through Sigma Aldrich (St. Louis, MO), while neat standards of fipronil sulfide, sulfone, and amide produced by Bayer and BASF (Ludwigshafen, Germany) were supplied by the Environmental Protection Agency. Isotopically labeled fipronil ($^{13}\text{C}_2\text{ }^{15}\text{N}_2$ -fipronil) was purchased from Toronto Research Chemicals, Incorporated (Toronto, Ontario Canada).

Instruments and analysis

Fiproles were separated by liquid chromatography, and quantified by electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) running multiple reaction monitoring (MRM) and operating in negative mode. Gas chromatography electron impact tandem mass spectrometry (GC-EI-MS/MS) was much more sensitive to fipronil-desulfinyl than LC-MS/MS, and was therefore used for the quantitation of this compound. LC-MS/MS was performed using a Shimadzu Prominence HPLC (Shimadzu Scientific, Kyoto, Japan) coupled to an ABSciex API-4000 MS/MS (Applied Biosystems,

Framingham, MA) controlled by Analyst 1.5 software (Applied Biosystems, Framingham, MA). Liquid chromatography was performed using both a Waters XBridge C-8 column (3.5 μm particle size, 2.1 mm \times 100 mm; Waters Corporation Milford, MA, USA) and an Ultra IBD column (5 μm particle size, 2.1 \times 150 mm; Restek Corporation, Bellefonte, PA). The mobile phase was tested at organic/aqueous ratios of 20/80 and 40/60 using methanol (MeOH) as the organic solvent, and also at 50/50 using acetonitrile (ACN) as the organic solvent. Flow rates were tested incrementally from 0.4–1 mL/min for the various mobile phases, always with a 50 μL injection volume. Solvent gradient profiles were optimized to achieve peak separation and short run times. Quantitation of fipronil was done using isotope dilution and a 7-point calibration curve, with matrix spikes using $^{13}\text{C}_2^{15}\text{N}_2$ -fipronil. Quantitation of other fiproles was done using the standard addition method with four analysis sample spike levels (see Figure B-1). GC-MS/MS analyses were performed using an Agilent 7890 GC coupled to an Agilent 7000 triple quad MS (Agilent Technologies, Santa Clara, CA) using electron impact ionization, and running MRM in negative mode. LC-MS/MS source optimization was performed using the automated incremental optimization routine in the ABSciex Analyst software; optimized parameters included collision energy, entrance potential, declustering potential, ion source gas, and temperature. GC-MS/MS optimization was done manually, and optimized parameters included inlet temperature, temperature ramp, ion source temperature, and collision energy, always with an injection volume of 1.5 μL . All compounds were identified using the two most abundant ion transitions, with the most abundant transition being used for quantitation, and the second transition for qualification.

Water sampling

Automatic samplers were deployed for five consecutive days (from 12 pm Thursday through 12 pm the following Tuesday) at the wastewater influent, wastewater effluent/wetland influent and wetland effluent locations. The ISCO 6712 samplers (Teledyne Technologies, Thousand Oaks, CA USA) were programmed for flow-weighted composite sampling in 20 mL intervals. Three weeks of hourly flow data were obtained from the wastewater facility in order to determine how to program the samplers. The number of 20 mL samples taken in a given hour was proportional the hourly flow rate deviations from the daily average. The total composite sample yield was approximately 2.5 L per day. Aliquots of flow-weighted composites of wastewater influent were obtained for 5 days. Biosolids were taken as grab samples in 40-mL glass vials, starting 21 days after the first day of the water sampling campaign, in order to approximately account for the solids retention time in the anaerobic digesters. Sample bottles were certified as resistant to labile pesticide adsorption. Samples were spiked with 500 ppm Kathon ICP-CG biocide and stored in a 4°C walk-in refrigerator for 1-2 weeks before extraction.

Solids collection and analysis

Dewatered sludge samples were dried, and weighed to 1.00 g aliquots. Solid samples were extracted using a modified version of EPA method 1694. Samples were dried, weighed, spiked with 20 ng labeled fipronil, extracted with 10 mL of acetone, and set on a rotary shaker at 60 rpm for 24 hours. The extraction mixture was centrifuged again, and the solvent was collected in a glass vial. After a second extraction with 10 mL of acetone,

the two extracts were combined, evaporated under nitrogen to near dryness, and reconstituted with 6 mL of hexane. Sample cleanup was done using 1g/6 mL Sep-Pak® (Waters Corporation, Milford, MA) cartridges containing Florisil. The cartridges were conditioned with 6 mL dichloromethane, 6 mL acetone, and 6 mL of hexane before the samples were loaded. Once loaded, the cartridges were washed with 6 mL hexane, then dried for 10 minutes under vacuum and eluted serially with 5 mL dichloromethane (DCM), followed by 5 mL acetone. The final volume of each eluate was reduced to 4 mL by evaporation.

For quality control, aliquots of dewatered sludge were spiked with native standards and the labeled surrogate at a concentration of 20 ng/g in order to determine extraction efficiency and signal-to-noise ratios resulting from the cleanup method. The final eluates were initially prepped separately: each eluate was split into two 1 mL aliquots, then one aliquot was solvent-switched to 50% methanol in water for LC-MS/MS analysis, and the other to 100% hexane for GC-MS/MS analysis. The absolute recoveries of individual fiproles using each eluent (DCM or acetone) were compared. The sum of the absolute recoveries resulting from each elution step were also compared to those obtained by commingling the DCM and acetone eluates (1:1 v/v).

Method detection limits were determined by spiking 1 g of a “clean” surrogate matrix with native standards and labeled fipronil in septuplicate. Per the recommendation in EPA method 1694, peat moss was selected as a surrogate matrix for the acidic fraction of biosolids. MDLs were calculated using the algorithm described by the United States Environmental Protection Agency (USEPA).¹⁴⁵

Water extraction and analysis

500 mL aliquots (in duplicate for all streams except primary sludge) were preconcentrated using automated, high-volume solid phase extraction (SPE). Extraction was performed using 500 mg SPE cartridges containing pyrrolidone-activated (poly)styrene-divinylbenzene (SDB) resin (500 mg/3mL Strata X and Strata XL, Phenomenex, Torrance, CA USA) installed on an Autotrace 280 (Thermo Scientific Dionex, Sunnydale, CA USA). The resin was eluted with 8 mL of 5% formic acid in methanol, and then aliquots of these extracts were evaporated under nitrogen and reconstituted to half the volume in either 1:1 methanol/water v/v (for LC analysis) or 100% hexane (for GC analysis). Matrix spikes using 20 ng $^{13}\text{C}_2^{15}\text{N}_2$ -fipronil were used to enable isotope dilution quantitation for fipronil, and one sample from each water matrix (WWTP influent, WWTP effluent/wetland influent, and wetland effluent) was split for standard addition-based quantitation of the other fiproles (excluding fipronil-desulfinyl).

MDLs were determined by spiking seven 500 mL samples of a “clean” surrogate matrix with native standards and labeled fipronil in septuplicate. Surrogate wastewater free of fiproles was generated by shaking 10 g peat moss in 5 L demineralized water for 10 minutes, allowing the particulates to settle above and below according to density, and decanting the water from between the two layers of solid matter. MDLs were calculated using the algorithm described by the United States Environmental Protection Agency (USEPA).¹⁴⁵

TRANSITION 3

Chapter 3 focused on the development of an optimized sample preparation and analytical method for assessing the occurrence and fate of fiproles in wastewater, sludge, and wetland water. Chapter 3 involved the use of one type of active sampler: I used an array of automated, water-storing commercial samplers that could be programmed for flow-weighted collection of fluids. In Chapter 4, I introduce a newly conceived, designed and manufactured device for automated, time-integrative sampling that extracts analytes in situ. The sampling strategy was coupled with a modified version of the analytical methodology described in Chapter 3, and the data generated by the automatic sampler was compared with data generated using a more laborious, but conventional grab sampling and ex situ extraction method.

The sampling device introduced in the following is not only a time-integrative sampler, it is capable of “biphasic” sampling, meaning that it can sample across the sediment-water interface to provide two types of samples from two distinct environmental compartments: bulk water overlaying surface water sediment, and pore water present in the interstitial space of saturated sediments. In addition to comparing grab sampling/ex situ extraction with in situ extraction, I also provide in Chapter 4 a case study illustrating the biphasic sampling capability of the newly developed sampling device.

CHAPTER 4

ACTIVE SAMPLING DEVICE FOR DETERMINING POLLUTANTS IN SURFACE AND PORE WATER – THE IN SITU SAMPLER FOR BIPHASIC WATER MONITORING

ABSTRACT

Accurate determination of hydrophobic organic chemicals (HOCs) in bulk surface water and sediment pore water is essential for environmental risk and exposure assessment. We designed and evaluated an active sampling device, using as analytical targets a family of pesticides purported to contribute to honeybee colony collapse disorder. Simultaneous sampling of bulk water and pore water was accomplished using a low-flow, multi-channel pump to deliver water to an array of solid-phase extraction cartridges. Achieved recoveries of fipronil and degradates in water spiked to nominal concentrations of 0.1, 1, and 10 ng/L ranged from 77 ± 12 to $110 \pm 18\%$. Method detection limits (MDLs) were as low as 40 picograms/L. Extraction and quantitation of total fiproles at a wastewater-receiving wetland yielded concentrations in surface water and pore water ranging from 9.9 ± 4.6 to 18.1 ± 4.6 ng/L and 9.1 ± 3.0 to 12.6 ± 2.1 ng/L, respectively. Detected concentrations were statistically indistinguishable from those determined by conventional, more laborious techniques ($p > 0.2$ for the three most abundant fiproles). Aside from offering time-averaged sampling capabilities for two phases simultaneously with picogram-per-liter MDLs, the novel methodology eliminates the need for water and sediment transport via in situ solid phase extraction.

Introduction

The United States Environmental Protection Agency (USEPA) estimates that approximately 10% of all domestic lakes, rivers, and bays harbor sediments contaminated by chemicals that threaten aquatic wildlife and human health.^{146,147} Accurate and efficient environmental sampling is therefore integral to evaluating the inherent risks associated with environmental contamination. Measured concentrations of environmental contaminants are used in compliance reporting, modeling, and risk assessment for biota and humans.^{148,149} Some contaminants, such as persistent organic pollutants, pose a long-term threat to ecosystems because they can remain in the environment for decades.¹⁵⁰ This issue is complicated by the fact that many persistent pollutants are hydrophobic, with *n*-octanol-water partitioning coefficient (K_{OW}) values on the order of 10^4 or greater. These hydrophobic organic contaminants (HOCs) are mostly sequestered by organic carbon (OC) in sediments, often irreversibly.^{151,152} As a consequence, the total sediment concentration may not provide a good representation of the labile or bioaccessible concentration of hydrophobic chemicals, particularly in the quiescent phase inherent to sediment pore spaces.^{151,153} Aggressive sediment extraction using organic solvents in conjunction with the standard Soxhlet extraction apparatus can facilitate determination of the total sediment contaminant burden. Sediment concentrations then may be used to calculate presumed pore water concentrations by normalizing against the sediment distribution coefficient K_D (equation 4-1), which is related to sediment organic carbon content:

$$C_{porewater} = \frac{C_{sediment}}{K_D} = \frac{C_{sediment}}{K_{OC} f_{OC}} \quad (4-1)$$

This method of estimation seeks to account for sorption resulting from the organic carbon fraction (f_{OC}) in the sediment. The organic carbon partitioning coefficient (K_{OC}) can be directly measured, or estimated from the corresponding K_{OW} value.¹⁵⁴ This method of assessing chemical activity in pore water is in wide use for estimating bioavailability, and by extension the ecological risk posed by “truly dissolved” chemicals; however, it does not account for additional, potentially mobile chemical mass associated with colloids and dissolved organic matter.^{153,155}

Passive sampling is a popular method of *in situ* pre-concentration used for determining chemical activity, frequently as a proxy for assessing bioavailability of sediment-borne pollutants.¹⁵⁶⁻¹⁵⁸ Calibration of passive samplers requires either equilibrium or linear uptake isotherms, often supplemented by the use of performance reference compounds (PRCs) for quality control. The time required by HOCs to reach equilibrium may be on the order of weeks to months, as exemplified by studies using solid phase microextraction (SPME) for the pesticide dichlorodiphenyltrichloroethane or DDT (18 d), or low density polyethylene (LDPE) strips for field sampling of large polycyclic aromatic hydrocarbons (>119 d).^{159,160,161} Passive samplers are relatively inexpensive, reliable, and well-suited to estimate the chemical activity of truly dissolved compounds.^{159,160,161} In some configurations, they also can enable the determination of time-averaged concentrations.¹⁶¹

Active samplers offer an alternate function, in that they can capture the mass of analytes associated with colloidal dissolved organic matter (DOM)¹⁶² and suspended fine particulates in addition to truly dissolved species. Automatic active sampling offers the

benefit of short sampling durations. Deployment times achieved by automatic active samplers may be considerably shorter than those required by samplers relying on equilibrium approaches or the use of PRC calibrants. The Continuous Low Level Aquatic Monitoring (C.L.A.M.) device is one such active sampler; it automatically extracts tens of liters of water in one to two days, and utilizes a low-energy-consumption diaphragm pump to pull water through a solid phase extraction disk.¹⁶³ It can achieve detection limits in the parts-per-quadrillion range for several hydrophobic organic compounds, by extracting a single composite sample of bulk water per deployment,¹⁶³ but it has not been configured to sample pore water.

The present work focused on the production of an active sampler that can simultaneously determine bulk water and pore water contaminant levels over long durations to yield time-averaged concentrations of chemical mass in water, whether fully dissolved, or partitioned onto DOM, colloids, and suspended particulates (<30 μm). To illustrate the utility of the sampler described herein, we deployed it in an engineered wetland to monitor fipronil and its transformation products. These compounds are collectively referred to as fiproles, and have been hypothesized to play a role in the ongoing worldwide honeybee colony collapse disorder.^{164,165} Fipronil is a halogenated pesticide and emerging contaminant recently banned for most agricultural uses in the European Union.¹⁶⁶ Used in common urban and agricultural applications, it is the active ingredient in many termite treatments, turf treatments, and in agricultural pesticide formulations, commonly in the form of seed treatments. Fiproles are known to occur in urban surface waters, and have been observed in at least one study to exceed aquatic benchmarks in over 70% of samples ($n=94$) from Orange County, CA, for both fipronil and fipronil

sulfone.¹⁶⁷ Fipronil also has been quantified in conventional wastewater treatment plants, wherein removal by activated sludge was limited to $18 \pm 22\%$.¹⁶⁸ Fipronil is bioaccumulative (log K_{OW} values for fiproles range from 4.0 to 5.43),^{81,169} and toxic to a number of aquatic benthic invertebrates at part-per-trillion (ppt) levels.^{170,171}

The objectives of this study were to (1) design a device that actively samples both the bulk water and sediment pore water of surface water environments, and (2) to demonstrate its utility for *in situ* pre-concentration of environmentally relevant hydrophobic targets, namely fipronil and four of its immediate degradates, at environmentally relevant concentrations in the parts-per-trillion range. The study further was designed to (3) provide data on a group of emerging contaminants speculated to play a role in the ongoing, worldwide honeybee collapse disorder. The three-part validation study included recovery tests, determination of method detection limits (MDLs), and a quantitative analysis of surface water and pore water using the innovative sampler introduced herein.

Results and Discussion

Sampler design, fabrication, and optimization

A functional IS2B sampler was designed in the Center for Environmental Security at Arizona State University (ASU) using SolidWorks® design software, and was fabricated by the ASU machine shop (Figure 4-1). The external parts, including the shell, inlets, and fittings are made of stainless steel, and the inlet tubing is made from polytetrafluoroethylene (PTFE). The materials were chosen to minimize chemical

interaction with water matrices and analytes. Internal tubing materials chosen were PharMed, PTFE, and Viton for the 2-stop pump cartridges, influent manifold connections, and effluent tubing, respectively.

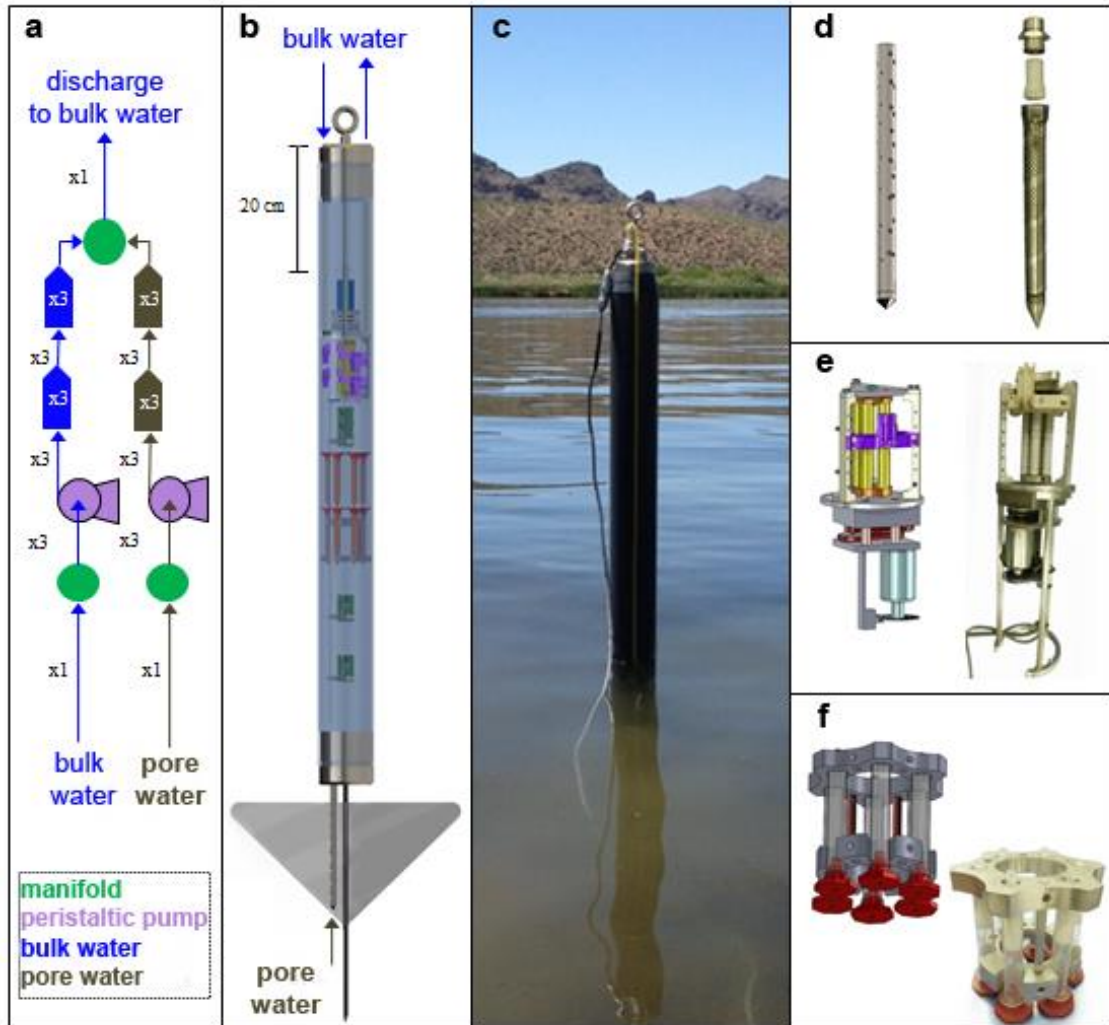


Figure 4-1. Overview of the IS2B dual-phase sampling methodology and hardware showing: a flow diagram illustrating the extraction process for simultaneous sampling and extraction of bulk and pore water (a); computer-aided design drawing of an assembled IS2B unit (b); photo of an IS2B deployed in surface water in Arizona, USA (c); detailed drawings of (d) the sediment pore water inlet spike (right) harboring the perforated inlet screen (left), and (e) the pump assembly with mounting frame (right) securing the modified ISMATEC pump (left); also shown are (f) the caddy with solid phase extraction cartridges (right) fabricated using the computer-generated blueprint (left).

The pore water inlet design includes a perforated steel spike with 1 mm holes to screen out large particulates; the pore water inlet tube within the spike is fitted with a stainless steel mesh. This 2-stage screening system was found to be effective in excluding particulates larger than 30 μm in diameter and producing minimal filter cake on the SPE frits, even after pumping several hundreds of mL of water featuring a high dissolved organic carbon (DOC) content. In this configuration, clogging of the unit was not observed even when it was challenged by placement in fine, high OC soils ($f_{\text{OC}} = 0.3$) with metering pumps set to flow rates of 150 $\mu\text{L}/\text{min}$. More information on the efficacy of the pore water filtration device can be found in Appendix B.

Method performance

The average volume of water delivered (flow rate = 70 $\mu\text{L}/\text{min}/\text{channel}$) to a given channel was 203.3 ± 13.9 mL ($\pm 6\%$ RSD). Detailed data regarding the volumes delivered can be found in Appendix C. The relative error of $\pm 6\%$ for the volume delivered to each cartridge was low and acceptable for inferring the precision of subsequent concentration calculations using this sampler.

Method detection limits of 0.04 to 0.8 ng/L were observed and are presented along with analyte recovery rates in Table 4-1. Average absolute recovery rates in water spiked to 10 ng/L (1 ng/L for fipronil-desulfinyl) were between $82 \pm 14\%$ and $110 \pm 18\%$, as determined from 8 replicates. At the lower analyte levels presented in Table 4-1 (0.1 and 1 ng/L for fipronil-desulfinyl and the other fiproles, respectively), absolute recoveries ranged from $77 \pm 12\%$ to $95 \pm 13\%$. These detection limits and performance data are comparable to prior work using off-line extraction and analytical methods for fiproles.¹⁷²

One study team used SPE columns to concentrate water samples and analyzed for four fiprole residues (excluding fipronil amide) via gas chromatography tandem mass spectrometry (GC-MS/MS), yielding MDLs from 1.6 to 7.0 ng/L, while absolute recoveries ranged from 73 ± 15 to $110 \pm 3\%$.¹⁷² Thus, the performance of the here presented method compares favorably to previously established, alternative approaches.

Table 4-1. Calculated method detection limits (MDLs) and limits of quantitation (LOQs) for fiprole congeners for using either a conventional large-volume laboratory extraction apparatus (LEA) for pre-concentration or the IS2B technology ($n = 7$).

	Chemical	MDL (ng/L)	LOQ (ng/L)	Recovery (%)	RSD (%)	Spike (ng/L)
LEA	Fipronil	0.9	3	72	27	1
	-sulfide	0.7	2	87	23	1
	-sulfone	1.0	3	87	31	1
	-amide	0.8	3	93	25	1
	-desulfinyl	0.05	0.2	74	15	0.1
IS2B	Fipronil	0.7	2	92	24	1
	-sulfide	0.7	2	93	22	1
	-sulfone	0.4	1	86	9	1
	-amide	0.8	3	77	12	1
	-desulfinyl	0.04	0.1	95	13	0.1

Field study

Grab samples of water and sediment were taken from three locations in an undisclosed wetland in the southwestern United States (Figure 4-2); simultaneously with the IS2B deployment, grab samples were taken at the study location. Results are discussed hereafter in the context of the matrix sampled.

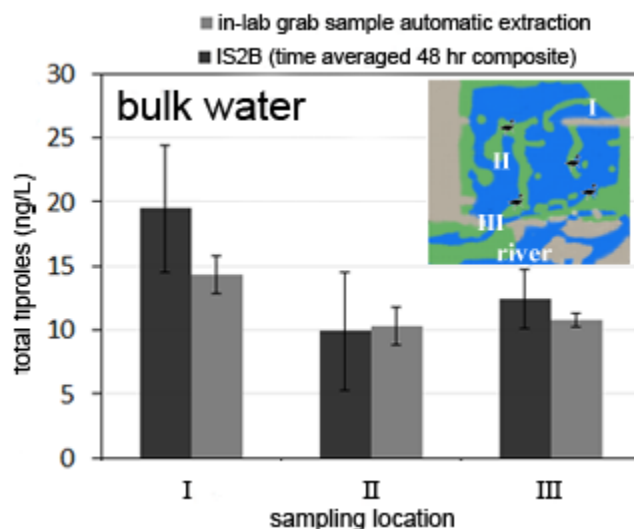


Figure 4-2. Bulk water concentrations of total fiproles obtained for time-discrete grab samples and for time-averaged, 48-hour composites acquired and extracted in situ using the IS2B device. Upper right panel is a schematic of the IS2B field deployment in a constructed wetland in Arizona, USA, showing the flow path of water from sampling locations I (wetland mouth) to II (mid point) to III (outfall into an agricultural irrigation stream). A wastewater treatment plant effluent enters the wetland at location I. The representation of the wetland was drawn in Photoshop by referencing schematics.

Bulk water

Mean total fiprole concentrations obtained from IS2B sampling of bulk water at the wetland ranged from 9.9–18.1 ng/L. Total fiprole concentrations in bulk water derived from in-lab sample concentration ranged from 10.3–13.4 ng/L. In all but one case (fipronil sulfide at location I), individual bulk water fiprole concentrations derived using these two methods were not discernably different (see Table 4-2). Individual fiprole concentrations determined using these two methods also were similar. Concentrations of total fiproles in wetland bulk water, as determined using the IS2B, were similar to results from grab sampling coupled with in-lab concentration of analytes from water samples (Figure 2). Average individual fiprole concentrations, as determined by in situ analyte concentration, were statistically indistinguishable from data obtained using a benchtop,

large-volume, laboratory extraction apparatus (LEA) for the three most abundant congeners: fipronil ($p = 0.27$), fipronil sulfide ($p = 0.26$), and fipronil sulfone ($p = 0.22$). In addition, *in situ* extraction served to detect fipronil-desulfinyl in all three sampling locations at levels near the detection limit of 0.04 ng/g, whereas no peaks were detected using in-lab LEA processing of grab samples taken from locations I and II. In-lab LEA did yield a desulfinyl peak on the chromatogram obtained for sampling at location III, but it was just below the detection limit of 0.05 ng/L. Since the IS2B generates time-averaged composites, it was able to capture transient mass fluxes of one of the analytes that were not observable via analysis of grab samples.

Table 4-2. Concentrations of fiproles in ng/L as determined by *in situ* pre-concentration of samples using the IS2B device, and by grab sampling and extraction of large-volume samples using an automated extraction apparatus in the laboratory.

Chemical		Fipronil	-Sulfide	-Sulfone	-Amide	-Desulfinyl	Total fiproles	
I	BW	IS2B	14.1 ± 3.3	ND (<0.7)	4.0 ± 1.3	ND (<0.8)	0.04 ± 0.14 ^a	18.1 ± 4.6
		LEA	10.0 ± 0.8	ND (<0.7)	3.4 ± 0.5	ND (<0.8)	ND (<0.05)	13.4 ± 1.3
	PW	IS2B**	7.5 ± 1.0	1.4 ± 0.4 ^a	3.7 ± 0.7	ND (<0.8)	ND (<0.04)	12.6 ± 2.1
		LEA	5.3 ± 0.2	1.4 ± 0.5 ^a	1.9 ± 0.7	ND (<0.8)	ND (<0.05)	8.6 ± 1.4
II	BW	IS2B	5.0 ± 2.5	0.8 ± 0.5 ^a	2.3 ± 0.9	1.4 ± 0.7 ^a	0.35 ± 0.16 ^a	9.9 ± 4.6
		LEA	3.0 ± 0.1	2.8 ± 1.1	2.2 ± 0.1	2.3 ± 0.2 ^a	ND (<0.05)	10.3 ± 1.5
	PW	IS2B*	5.6	0.94 ^a	2.9	2.0 ^a	0.3	11.6
III	BW	IS2B	5.4 ± 0.8	0.8 ± 0.1 ^a	3.7 ± 0.9	2.4 ± 0.4 ^a	0.06 ± 0.11 ^a	12.4 ± 2.3
		LEA	4.6 ± 0.2	0.8 ± 0.1 ^a	3.3 ± 0.1	2.0 ± 0.1 ^a	ND (<0.05)	10.7 ± 0.5
	PW	IS2B	4.2 ± 1.4	ND (<0.7)	2.9 ± 1.0	1.9 ± 0.5 ^a	0.09 ± 0.08 ^a	9.1 ± 3.0

^a - values are below the limit of quantitation, and are therefore estimated

Sampling locations I, II, and III are those referenced in Figure 2

BW, bulk water; PW, pore water; LEA, laboratory extraction apparatus (large volume)

Standard deviations shown are calculated from $n=3$, except where indicated

* $n=1$ field replicate (2-day, time-averaged composite)

** $n=2$ field replicates (2-day, time-averaged composite); ± values provided represent maximum/minimum

Pore water

Mean concentrations of total fiproles in pore water, as determined with the IS2B approach at the three sampling locations, ranged from 9.1–12.6 ng/L, whereas mean total fiprole levels determined using in-lab extraction of pore water from sediment from Site A showed an almost identical value of 8.6 ± 1.4 ng/L. The concentrations of individual fiproles in pore water, as determined using the IS2B, were similar to contaminant levels observed in bulk water (see Table 4-2). One explanation for this observation could be the occurrence of short-circuiting of liquids from the bulk water to the pore water intake. However, this scenario is not likely when considering that the volume of pore water sampled by the IS2B (600 mL total) is small compared to the theoretical volume of influence around the pore water inlets (1.8 L), and further considering the very slow pump rate of only 70 $\mu\text{L}/\text{min}$. Since short-circuiting of fluids was never observed during laboratory testing, a second, more plausible explanation is that non-equilibrium conditions were extant at the sampling site. Indeed, surface sediments are known to represent dynamic systems that frequently are not at equilibrium with respect to chemical transfer between sediment and pore water.^{173,174} Probing for such non-equilibrium conditions, we determined the organic carbon content of the sediment (1%) and compared the sediment-associated analyte concentration to what was found in pore water. Calculated based on the pore water concentrations measured in sediment processed in the laboratory, the expected sorbed fipronil concentration was estimated at approximately 350 pg/g dry weight sediment, whereas analytical results from solvent extraction of dry sediment yielded a value of less than the MDL or less than 20 pg/g dw. Taken together, these results strongly suggest that non-equilibrium conditions prevailed between the

sediment and pore water in this highly dynamic sampling location downstream of an urban wastewater treatment plant that is known to experience notable seasonal and diurnal fluctuations in both flow rates and concentrations of water constituents. Our findings are consistent with prior studies by other groups showing that predictive models for estimating sediment concentrations of non-ionic pesticides and other HOCs from aqueous concentrations may overestimate sorbed fractions.¹⁷³⁻¹⁷⁶ Overall, modeling of the fate of pesticides is known to be challenging as environmental factors extant at field sites, such as the one studied here, may not be consistent with the assumptions required to employ equilibrium models.¹⁷⁵

Toxicological implications of monitoring results

Aqueous concentrations of fiproles determined here for a constructed wetland in the southwestern U.S. are lower than those reported previously for urban settings but within one order of magnitude of levels known to be toxic to aquatic biota. Various aquatic organisms are highly susceptible to fipronil, as illustrated by the data compiled in Table 4-3.

Table 4-3. Data on toxicity, occurrence, and persistence of fipronil and three of its degradates.

Compound	<i>Procambarus</i> ^a	<i>Hyaella azteca</i> ^b		<i>Diphetor hageni</i> ^b		¹⁷⁷ OC urban water conc. (µg/L)	Half-life	
	¹⁷⁸ LC ₅₀ (µg/L)	¹⁷¹ LC ₅₀ (µg/L)	¹⁷¹ EC ₅₀ (µg/L)	¹⁷¹ LC ₅₀ (µg/L)	¹⁷¹ EC ₅₀ (µg/L)		¹⁷⁹ Silt loam (days)	¹⁸⁰ Facultative conditions (days)
Fipronil	14.3-19.5	1.3-2.0	0.65-0.83	0.20-0.57	0.11-0.21	0.05-0.39	21±0.15	-
-desulfinyl	68.6	-	-	-	-	0.05-0.13	-	217-497
-sulfide	15.5	1.1-1.7	0.007-0.003	-	-	ND	>200	195-352
-sulfone	11.2	0.35-0.92	0.12-0.31	0.19-0.54	0.055-0.13	0.05-0.19	>200	502-589

^a*Procambarus* species were *clarkii* and *zonangulus*

^bValues for *H. azteca* and *D. hageni* represent the 95% confidence interval
OC, Orange County, California

ND, not detected

Concentrations detected in this study were below the toxic threshold values of reference organisms, and also lower than those detected in urban streams in California.^{167,181,182} In one study where fiproles were quantified in urban runoff in Orange County, median combined fiprole concentrations ranged from 204–440 ng/L.¹⁸¹ Possibly influenced by different land use patterns, these values are an order of magnitude higher than the mean bulk water concentrations determined in this study (10–18 ng/L). Another 6-month monitoring study of the Rhône River in France in 2004 reported no detections of fipronil with a limit of detection of 1 ng/L, a finding that is consistent with the European ban of fipronil application in agriculture.¹⁸³ However, fipronil remains in widespread use in the United States, which explains the detections reported here and by the few additional data available for America.^{167,181,182}

Technology applicability

The IS2B technology is intended as a means for concentrating trace level chemicals in situ, which eliminates the need to transport the large volumes of water needed for in-lab analysis. Whereas the volume of water assayed in this study was 1.2 L in total, sampling of larger or smaller volumes can easily be accomplished, with the selection of the water volume being a function of contaminant concentration, method detection limits, etc. When seeking to process very large volumes of pore water, maintaining equilibrium conditions may impose flow rate limits. Since the pore water inlet is about 15 cm below the sediment-bulk-water interface, a spherical volume of 1.8 L of pore water [$\frac{4}{3} \times \pi \times (7.5 \text{ cm})^3$] represents the upper limit of sediment pore water volume processed. In

contrast to pore water, the volume of surface water that can be sampled is limited only by the capacity of the resin cartridges used, and can be tens, or even hundreds of liters. In this study, cartridges were deployed in series for one of the channel replicates to verify that breakthrough was not occurring.

As performed here, taking of samples with three replicates of each phase (bulk water and pore water) is the preferred deployment mode. This redundancy enables the determination of standard deviations and guarantees the availability of useful data in the event of isolated technical problems, such spillage of extracts during handling in the laboratory. As demonstrated here for fiproles, tubing material should be matched to the chemistry of the analyte to avoid losses from sorption.

Conclusions

The IS2B technology is complementary to established passive sampling and grab sampling strategies by offering several attractive attributes: (i) determination of time-averaged concentrations in triplicate in a single deployment; (ii) concentration measurements for two distinct environmental phases simultaneously (bulk and pore water); (iii) obtained contaminant levels are reflective of the total quantity of mobile, potentially bioaccessible contaminants in surface water and sediments,¹⁵³ concentrations which may differ substantially from the chemical activity of truly dissolved solutes (which are best determined using established passive sampling strategies); (iv) the IS2B concentrates analytes on SPE resins in the field, which eliminates the need to collect large volumes of time-discrete water samples via either grab sampling by hand or by using other automated water collectors; (v) avoidance of the need to transport several

kilograms of wet sediment to the lab for determination of pore water concentrations; (vi) sample handling steps and opportunities for lab contamination are reduced due to in situ analyte concentration; (vii) shipping costs are reduced since the analyte-laden resin cartridge weighs only a fraction of the large mass of water that was extracted in the field; and (viii) the technology is well suited for measurements in highly dynamic environmental compartments where non-equilibrium conditions are expected to prevail.

Methods

Sampler design

Design drawings were produced using SolidWorks ® design software (Dassault Systèmes SOLIDWORKS Corp., Waltham, MA). Active sampling by the *in situ* sampler for biphasic water monitoring (IS2B) device was facilitated by a low-flow, multi-channel peristaltic pump capable of pumping at a continuous rate of $70 \mu\text{L}\cdot\text{min}^{-1}$ or less in each of its six channels. The pump head and motor originated from an ISMATEC Reglo-E Digital 12DC, geared at a ratio of 25:1 (IDEX corp., Oak Harbor, WA). The pump was mounted onto an aluminum frame, fit with custom tubing cartridges for compressing the pump tubing. The pump cartridges were designed to fit on the custom frame, and are identical to those used in another environmental monitoring and assessment tools, the *in situ* microcosm array (ISMA).¹⁸⁴ The pump tubing (0.38 or 0.51 mm inner diameter, ID) consisted of 2-stop PharMed tubing (Saint-Gobain Performance Plastics, Akron, OH), while influent tubing was 6.4 mm ID polytetrafluoroethylene PTFE for the pore water inlet and 1.6 mm PTFE for the bulk water inlet. Effluent tubing is 0.89 mm Viton coupled to 1.6 mm PTFE via a 6-channel manifold. Polyvinylidene fluoride (PVDF) Luer

fittings and all tubing were purchased from Cole Parmer (Vernon Hills, IL). The pump delivers water to an array of preconditioned solid phase extraction cartridges, which were connected to the Luer fittings at the pump tubing outlets by adapters (SPE syringe to male Luer slip fitting) purchased from Sigma Aldrich (St. Louis, MO). All SPE cartridges were purchased from Phenomenex (Torrance, CA). Polystyrene divinylbenzene resin (Strata SDB-L) was chosen for its affinity for hydrophobic, aromatic pesticides like fipronil. The pore water inlet was incorporated into a perforated stainless steel (SS) tube with 1 mm holes, and the tube itself was wrapped in several layers of a SS mesh screen with 30 μm openings. Metal for the inlet apparatus was purchased from Grainger (Lake Forest, IL), and the ASU machine shop fashioned it into a 20-cm inlet spike that could be driven into wet sediment. The unit was contained within an 8.9-cm outer diameter (OD) stainless steel tube, capped at each end by threaded caps with compression fittings for wiring and tubing. Compression fittings were purchased from McMaster-Carr (Santa Fe Springs, CA). A stainless steel Swagelok [®] adaptor for outlet tubing compression was purchased from Swagelok Company (Solon, OH). The peristaltic pump motor was powered by a 12-V Optima Blue Top battery with a power inverter, and controlled by an external ISMATEC MiniClick6 Reglo-E control unit, purchased from IDEX. The 9455 multi-conductor control and instrumentation cables were purchased from Belden (St. Louis, MO). In deployment configuration, the battery, power inverter, cable spool, and control unit were stored in a deck box onshore.

Instruments and analysis

All analytes aside from fipronil-desulfinyl were quantified by performing multiple reaction monitoring (MRM) using liquid chromatography electrospray tandem mass spectrometry (LC-ESI-MS/MS) operating in negative mode. For enhanced method sensitivity, fipronil-desulfinyl was analyzed by MRM using gas chromatography electron ionization tandem mass spectrometry (GC-MS/MS). Details about instrument parameters for methods developed specifically for this study can be found in Appendix C.

Sediment collection and analysis

Prior to the case study, approximately 500 g of wet sediment was collected from a wetland at the specific locations where the sampler was to be deployed. Triplicate aliquots of wet sediment weighing about 1 g each were dried under a nitrogen stream, and subsequently weighed to the nearest milligram. The sediment samples were then extracted with 2 mL of 1:1 hexane/acetone (v/v) in a sonicator for 3 h. The extracts were blown down to dryness and reconstituted in an equal volume of acetonitrile and sonicated for 20 minutes. The resulting samples were filtered with 0.2 μm PTFE filters before analysis via liquid chromatography with tandem mass spectrometry (LC-MS/MS). LC samples were diluted by 50% with LC-MS grade water prior to sample injection.

Benchtop water extraction and analysis

Approximately 10 L of wet sediment was collected from site A, placed into a 19-liter bucket, and stored at 4°C. The perforated IS2B inlet spike was assembled, and three lines from a 6-channel automated SPE unit were wrapped in 30 μm stainless steel mesh and

secured with PTFE tape before being placed inside the perforated tube. The inlet spike was thrust into the sediment far enough to ensure an inlet depth of at least 8 cm. The water was then automatically extracted in triplicate alongside Milli-Q water unspiked controls in duplicate using a large volume automatic solid phase extraction unit, Cartridges were conditioned with 3 mL of acetonitrile, and equilibrated with 3 mL of Milli-Q water. They were then loaded with 200 mL of water, and eluted serially with 2 mL acetonitrile, followed by 2 mL of hexane/acetone (1:1). Serial eluates for each cartridge were combined, and two 0.5 mL aliquots were taken from each sample to be evaporated under nitrogen. One aliquot from each sample was reconstituted to 0.5 mL acetonitrile and diluted to 1.0 mL with water for analysis by LC-MS/MS. The other aliquot of each sample was reconstituted to 0.5 mL hexane for analysis by GC-MS/MS. Overlaying water from the same locations was collected in oven-cleaned 1 L media bottles (~1 L per bottle was collected) and stored at 4°C before being extracted as described above. A field blank consisting of ultrapure reagent grade water transferred to an oven-cleaned bottle onsite was also extracted, and the signal from the field blank chromatogram was subtracted from those of the bulk water and pore water extracts.

Calibration

The IS2B peristaltic pump can be programmed to accommodate various pump tubing diameters; after inputting the tubing diameter and calibrating at a given flow rate, the control unit can be reprogrammed for a different flow rate while maintaining its calibration. Performance was verified by pumping and measuring 1 mL aliquots at a flow rate half that of the calibrated flow rate.

Prior to all tests, the peristaltic pump was rinsed with approximately 150 mL of denatured ethanol and then with an equal volume of 18.2 MΩ water to prime the tubing. The control unit was set to deliver 10 or 20 mL to each of the six channels, and the tubing cartridges were then adjusted to even the flow rates to each channel. Once calibrated, the pump was set to deliver 200 mL of water to each channel at a flow rate of 140 μL/min, and the effluent was captured in pre-weighed 250 mL media bottles.

Field study

The fully assembled IS2B was calibrated for a flow rate of 70 μL•min⁻¹ channel⁻¹ and deployed in a wetlands receiving runoff from a wastewater treatment facility, and from adjacent agricultural fields. The flow rate was chosen in order to minimize the chance for bulk water penetration into the pore spaces as a result of drawdown. A validation study for another pore water sampling device called MINIPOINT indicated that for a sediment with an average porosity of 0.35, flow rates lower than 4000 μL/min did not disturb tracer (Cl⁻) depth profiles, and the lowest flow rate evaluated was 300 μL/min.¹⁸⁵ The total flow rate into the IS2B from the pore spaces in this field deployment was 70 μL/min /channel × 3 channels, or 210 μL/min, which is lower than the lowest flow rate validated for the MINIPOINT. The IS2B simultaneously drew pore water and overlaying bulk water through separate inlets and delivered 200 mL to each of 6 conditioned SDB cartridges at 70 μL/min /channel. The extracted water was released into the bulk water phase, downstream of the bulk water inlet. The SPE cartridges were extracted and the samples processed as described above.

Sediment and water samples were collected concurrent with sampler deployment. One-gram aliquots of sediment were dried, weighed, spiked with 5 ng fipronil des F3 as a surrogate, and extracted with 2 mL hexane/acetone for 30 min in a sonicator. One mL of the supernatant was drawn and evaporated under a nitrogen stream. After solvent exchange into 100% acetonitrile, extracts were filtered with 0.2 μm PTFE, and 500 μL of the filtrate was combined with 500 μL LC-MS grade water for injection into a high-performance liquid chromatography-tandem mass spectrometer (HPLC-MS/MS). Sediment concentration was calculated using equation 4-2:

$$C_{\text{sediment}} = \frac{C_{\text{extract}} \times V_{\text{extract}}}{\text{mass}_{\text{sediment}}} \quad (4-2)$$

The pore water concentration (C_{PW}) then was inferred by normalizing the sediment concentration by the distribution coefficient (K_D) as described in equation 4-3:

$$C_{PW} = \frac{C_{\text{sediment}}}{K_D} = \frac{C_{\text{sediment}}}{K_{OC} f_{OC}} \quad (4-3)$$

K_{OC} was estimated using the published linear relationship shown in equation 4-4:¹⁵⁴

$$\text{Log } K_{OC} = 0.903 (\text{Log } K_{OW}) + 0.094 \quad (4-4)$$

The fraction of organic carbon in sediment (f_{OC}) was determined by total organic carbon (TOC) analysis as described in Appendix C.

Statistical data analysis

Comparison of the mean bulk water concentrations as determined by IS2B sampling and grab sampling was done by performing two-tailed t -tests at the 95% confidence interval,

assuming equal variances. The mean bulk water concentration (log-transformed) of each of the three most abundant congeners (fipronil, and the sulfide and sulfone degradates) was calculated separately for each analyte concentration method (IS2B or LEA), using data from all three sampling locations ($n = 12$), and each sample was assessed for normal distribution. Fipronil amide and fipronil-desulfinyl were omitted from the mean comparison analysis because most peak areas were near or below the method detection limit, thereby producing non-normal distributions. Statistical calculations were performed using the Microsoft Office 2010 Data Analysis ToolPak.

TRANSITION 4

Chapter 4 introduced a biphasic, automated, in situ extraction sampling device (the IS2B). The prototype utilized a peristaltic pump and external control box for regulating flow, as well as external batteries to allow for continuous sampling over the course of several days. While the results of the study in Chapter 4 showed that continuous sampling coupled with in situ extraction produces quality data, and that biphasic sampling produced equally strong data, I also identified opportunities for building on the embodiment of the original IS2B device. In Chapter 5, I document the design, manufacture and initial laboratory testing of a second generation IS2B device, termed the mIS2B (m for “miniature”), whose distinguishing features include compactness, reduced weight, as well as a self-contained design providing on-board power and flow regulation, and lower power requirements. While the IS2B prototype in Chapter 4 could perform continuous sampling for days or weeks, the updated design relies on interval sampling in order to maximize battery life, thus yielding *time-averaged*, rather than time-integrated concentrations. The new design further integrates a syringe pump design, and more robust connector components including Swagelok fittings.

CHAPTER 5

A SECOND GENERATION IN SITU SAMPLER FOR BIPHASIC WATER MONITORING (mIS2B)

ABSTRACT

A compact design of the previously described In Situ Sampler for Biphasic Water Monitoring (IS2B) was developed with the goal of providing a more compact device featuring an onboard power supply. An evaluation of IS2B user-end experience, durability, and failure modes indicated that the bulkhead fittings, interior chassis, pump, shell, power supply, flow control and tubing would benefit from modification. The new design has replaced the peristaltic pump with a syringe pump, the stainless steel shell with a PVC shell, and the off-board power with a 12V onboard battery. The new pump design was evaluated for consistency, and showed a percent error of 3% ($n = 18$ between replicate channels). The onboard battery was able to power the pump continuously for 24 hours, delivering 100 mL to each of 6 channels. In interval mode, the pump was able to sample 200 mL over 24 hours by drawing 2 mL at 4 mL/min every 30 minutes. Additionally, the front-end tubing was replaced with shorter tubing lengths, in either polytetrafluoroethylene (PTFE) or 316 stainless steel. The new design solves some of the user-end issues, most notably the fragility of the chasses and the external power requirements. It also addresses concerns with loss in front-end tubing of hydrophobic analytes.

Introduction

Water sampling technologies must be suited to the target compounds, matrix, and application. The most prevalent water sampling techniques are manual grab sampling, and automated water collection (using ISCO samplers, for example). When characterization of the pollutant mass distribution across the water-sediment interface of contaminated surface water systems is the goal of a monitoring or risk assessment study, it is necessary then to sample and analyze both the sediment pore water and overlaying water column near the phase boundary. One common goal in environmental risk assessment is bioavailability determination, wherein the hazards posed to aquatic organisms are estimated using either biological or chemical means.¹⁸⁶ Benthic aquatic organisms are often of concern in aquatic ecology risk assessment, due to their interactions with contaminated sediments, and the interface where there is a high flux of contaminants into the pore water spaces and the overlaying water column.¹⁸⁷ These organisms are also sources of contaminant uptake for bulk water-dwelling macrobiota, such as fish. Therefore, contaminant bioavailability in sediment pore waters, and near the sediment-water interface is important. A common means of assessing contaminant bioavailability is to use a chemical proxy to mimic biotic exposure and uptake; this is commonly done using passive samplers.¹⁸⁸⁻¹⁹⁰ Sediment bioavailability estimations have also been done by mild solvent extraction,¹⁹¹ but it is commonly accepted that pore water concentrations are most relevant, as some research suggests.¹⁹²

“Active” pore water sampling devices are not commonly used in the assessment of hydrophobic organic contaminants (HOCs) in sediments; a few studies have utilized Rhizon samplers for this purpose, but Rhizon samplers are typically equipped with

syringes or vacuum pumps for taking grab samples.¹⁹³ The In Situ Sampler for Biphasic Water Monitoring (IS2B – also known as the In Situ Sampler for Bioavailability Assessment) was developed as a time-integrative sampler that could draw water from both the sediment pore spaces and overlaying water column in river, lake, estuarine, or oceanic systems.^{86,194} Equipped with a peristaltic pump and on-line solid-phase extraction cartridges, this device could continuously extract hundreds of milliliters of water from both the pore and bulk water phases simultaneously over the course of days, or even weeks. However, the prototype of this device had some shortcomings that made its operation cumbersome: bulkhead fittings were prone to leaking; continuous, low-flow pumping was energy-intensive, and required the use of several external deep cycle absorbent glass mat (AGM) batteries; the control unit for setting flow rates was also external, and had to be deployed along with the sampler and stored in a deck box onshore; internal components were prone to becoming dislodged during construction; some internal construction materials were not strong or robust, and were easily broken; front-end tubing (ahead of the sorbent) could act as a sink for HOC adsorption.

This study deals with a second generation sampler design aimed at addressing these shortcomings to produce a more robust device.

Results and discussion

A functional mIS2B prototype was designed by the Center for Environmental Security at Arizona State University (ASU). The prototype was constructed by the ASU machine shop.

The first prototype built in 2012 consisted of a stainless steel shell, approximately 80 cm long, and 9 cm in diameter. The end caps were also stainless steel, with stainless steel fittings. Each end cap had four threaded holes for bulkhead fittings, through which wires and tubing could pass when deployed under water. The interior consisted of a peristaltic pump utilizing rubber tubing connected to plastic manifolds and an array of up to 6 solid phase extraction (SPE) cartridges in parallel. A stainless steel spike constructed of perforated tubing with 0.635 cm polytetrafluorethylene (PTFE) tubing mounted inside served as a pore water inlet. The bulk water inlet consisted of 0.635 cm PTFE tubing that protruded into the zone near the sediment-water interface. Both inlets, as well as a power/data cable passed through the endcaps via compression bulkhead fittings (McMaster-Carr, Santa Fe Springs, CA). External power was supplied by an array of 12V AGM batteries in parallel, and flow control was provided by an external control box. Each of the components listed were redesigned and incorporated into a new device.

Water-tightness

The original IS2B endcaps had 4 threaded holes each. Two endcaps therefore had 8 holes for bulkhead fittings. Unused holes were plugged with blank stainless steel nuts.

Considering that for the configuration that used the most bulkhead passages, only 3 holes were used, so the 5 extra holes were redundant, and therefore unnecessary sources of potential leaks. Indeed, when leak-tested, the blank nuts did in fact leak under 140 kPa (14 m H₂O) pressure.

As a result, the new design contained only as many bulkhead passages as were maximally necessary. Due to other design considerations, this actually increased the total number of

bulkhead passages from 8 to 10. Furthermore, all fittings were changed to stainless steel Swagelok fittings (7/16-20 straight threading, with a sealing O-ring on each). Bulk water inlet fittings were 0.3175 cm bore-through fittings, with stainless steel ferrules. Pore water inlet fittings were 0.635 cm bore-through fittings, with PTFE ferrules.

Cartridge and manifold mounting system

The aluminum mounting rods of the original IS2B were often bent, even twisted around each other when the device was being constructed or deconstructed. The manifold and cartridge mounting system was therefore eliminated. The manifolds and SPE cartridge caddies were redundant with the inclusion of a different pump system, and were also eliminated entirely.

Pump

The peristaltic pump utilized more energy than did the syringe pump when operated in either continuous or interval mode. The syringe pump design was therefore selected for the new design. Subsequent testing of the new pump design yielded the results shown in Table 5-1. The average % error is less than 10%

Table 5-1. Syringe pump channel precision, expressed as percent error ($n = 18$).

Channel	Target (mL)	Time (h)	Delivered (mL)	Percent Error
1	100	24	98.2	2
2	100	24	99.2	1
3	100	24	94.8	5
4	100	24	97.2	3
5	100	24	100.2	0
6	100	24	99.9	0
1	200	48	196.4	2
2	200	48	198.5	1
3	200	48	170.4	15
4	200	48	190.8	5
5	200	48	198.8	1
6	200	48	199.3	0
1	200	48	198.1	1
2	200	48	197.4	1
3	200	48	196.0	2
4	200	48	177.8	11
5	200	48	199.1	0
6	200	48	199.6	0
Average				3

Power supply

While off-board battery power has the benefits of greater longevity because higher capacity, deep cycle batteries can be used, it has the drawback that it requires the transport of multiple, heavy batteries to and from deployment locations. Furthermore, off-board batteries have to be stored on-shore in a deck box during deployments, essentially tethering the sampler close to the shoreline. In addition to these concerns, off-board batteries increase the visual profile of the sampler, making it more susceptible to vandalism and theft in the field.

For these reasons, on-board power options were investigated, and the longevity of two 12V batteries were explored (7Ah and 12Ah). The 7Ah battery lasted only about 30 hours under slow, continuous operation, and did not complete its entire cycle. The 12Ah battery has been tested under interval sampling conditions (2 mL every 30 minutes), and was able to pump 100 mL of water in 24 hours, consuming less than 30% of its total capacity. It also pumped 200 mL in 48 hours, consuming less than 60% of its total capacity.

Materials

The IS2B shell material was replaced with polyvinylchloride (PVC). The buoyancy of the device was calculated utilizing a PVC shell, and with the inclusion of the battery and other internal parts, the buoyancy was determined to be less than neutral. This material selection made the shell much easier to manipulate. Internal parts are either aluminum or stainless steel. The mounting slide for the pump and battery was constructed of stainless steel to reduce buoyancy. Other internal parts are aluminum, to keep cost and weight down.

Stainless steel was selected for the pore water inlet tubing, and PTFE Swagelok ferrules were mated to it; steel ferrules would be impossible to remove from the tubing once the Swagelok nut was twisted on. PTFE was selected for the bulk water inlet tubing, and steel ferrules were mated to it.

Completed device design

A SolidWorks assembly of the completed sampler design is presented in Figure 5-1, and the fully constructed device is presented in Figure 5-2.

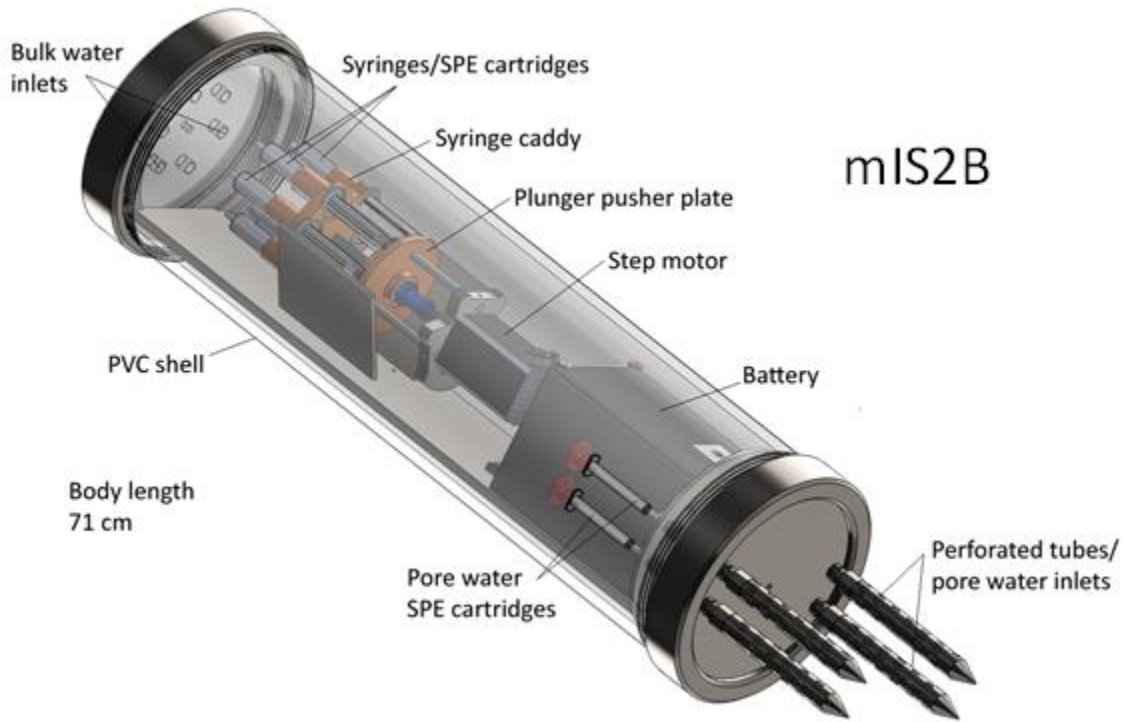


Figure 5-1. Diagram of the mIS2B.



Figure 5-2. Pictures showing the internal components (panel a), including the syringes, pump motor, battery, and pore water extraction cartridges. Panel b shows the completed construction with a clear PVC shell. Panel c shows several individual components, including (from bottom to top) the stainless steel bottom cap, acrylic top cap, step motor, interior chassis, O-rings, battery, and shell. Panel d shows the constructed top tap with Swagelok fittings (for bulk water intake).

Interior tubing

The internal plumbing was intended to be comprised primarily of flexible rubber tubing, for every flow path downstream of extraction resin. The bulk water inlet tubing was intended to be PTFE, because it is in front of the resin, while the pore water inlet tubing was intended to be flexible rubber (Viton, or similar). The tubing schematics are shown in Figure 5-3.

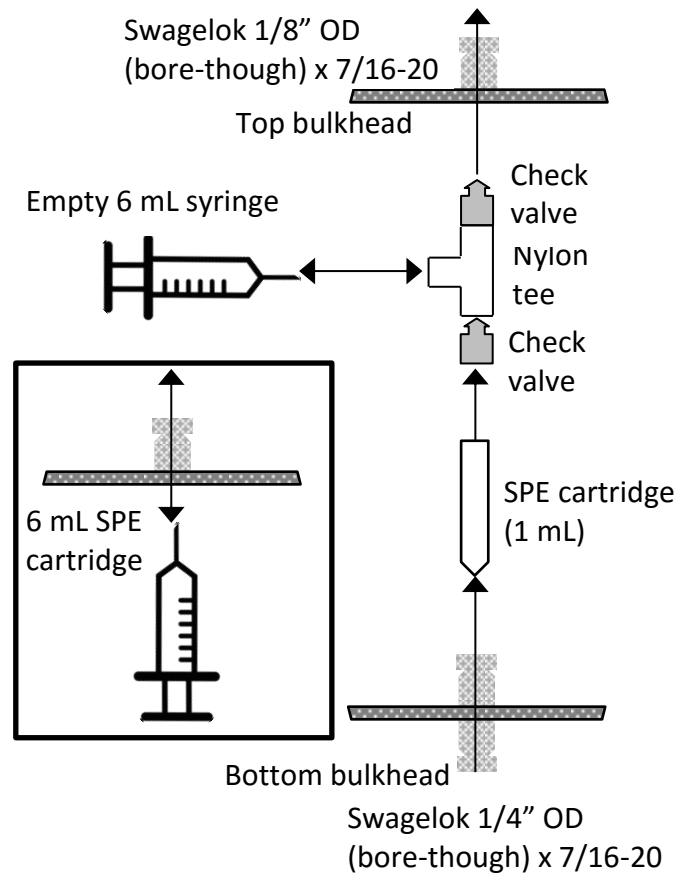


Figure 5-3. IS2B fluid flow diagram. Water from pore spaces passes through the bottom bulkhead fitting, and then through a 1 mL SPE cartridge, after which it is discharged into the overlaying water column (top). Check valves prevent backflow of extracted water, or introduction. For bulk water extraction (insert), water is drawn directly from the water column into 6 mL SPE cartridges, and then discharged back into the column after being extracted on the sorptive resin.

In addition, front-end tubing was replaced with either stainless steel or PTFE, with only 8-10 cm of PTFE tubing upstream of the bulk water extraction cartridges, and 15-20 cm of stainless steel tubing ahead of the pore water extraction cartridges. This change eliminates the much longer front-end tubing of the original design, some of which was flexible rubber peristaltic pump tubing.

Research applications

The mIS2B can capture time-discrete samples at various intervals over the course of several days. It can extract up to 500 mL of bulk water with up to 6 replicates, or of pore water with up to 4 replicates. Data produced using this sampling method will be *time-averaged*, representing average temporal concentrations over the entire sampling period. It can also be operated for up to 48 hours continuously, extracting up to 300 mL of water. Data produced using this method will be *time-integrated*. Sorbents can be selected to extract any number of target analytes, including HOCs, metals, cations, anions, and polar organic compounds.

The sampler can be deployed in any surface water system, and can be fully submerged. Maximum deployment depths are unknown. Since there are no external components, the mIS2B can be submerged as far away from shore as desired.

Methods

All computer aided design was done using SolidWorks ® design software (Dassault Systèmes SOLIDWORKS Corp., Waltham, MA). The new prototype design was generated after examining the original IS2B design and highlighting probable and confirmed failure modes, and user-end problems.

Water-tightness

Bulkhead fittings were tested for water tightness by immersing the IS2B in a pressure chamber at 20 Psi (140 kPa) and systematically examining the potential leak points.

Endcap water-tightness was not examined, as the seal provided by rubber O-rings should adequately fill any gaps in order to maintain water-tightness at realistic pressures.

Cartridge and manifold mounting system

Cartridges and manifolds on the IS2B were mounted on two parallel aluminum rods, and held in place by set screws. Considering the ease with which the mounting rods could be bent, it was determined the cartridge and manifold mounting system should be redesigned. Several design options were explored, including bulkhead-mounting of both manifolds and cartridge caddies, elimination of manifolds, and direct mounting SPE cartridges to a syringe pump.

Pump

The original IS2B pump was a low-flow peristaltic pump ISMATEC Reglo-E Digital 12DC, geared at a ratio of 25:1 (IDEX corp., Oak Harbor, WA). This pump option was explored alongside a syringe pump option, wherein the pump motor was a stepper motor with an integrated controller (model number CO-4118S-09-RO 0.9A, Lin Engineering, Morgan Hill, CA). These options were compared in terms of power usage, cost, and ease of operation, and a cost-benefit analysis was done to choose the best option. The selected pump system was then tested for consistency between 6 parallel channels. Consistency was evaluated using equation 5-1, where \bar{V} is the average volume delivered to 6 channels and V_p is the expected volume programmed into the pump controller.

$$\%Error = \frac{|\bar{v} - v_p|}{v_p} \times 100\% \quad (5-1)$$

Power supply

Options for both off-board and on-board power were examined. Off-board power involved the use of several 12V batteries in parallel, with the option of solar panel trickle charge. On-board power involved the use of a small 12V, 7 amp-hour (Ah) or 12 Ah battery, with the option of a micro solar panel for trickle charge. Both 7Ah and 12 Ah batteries were tested for longevity under the relevant power loads used by a pump motor. The motor was programmed to operate for 24 and 48 hours under both continuous and intermittent sampling conditions, and the battery life was observed. Continuous sampling conditions were as follows: 200 mL sampled at 0.15 mL/min. Intermittent sampling conditions were as follows: 100 mL and 200 mL sampled in 2 mL increments every 30 minutes, at a burst sample draw rate of 4 mL/min for 30 seconds.

Materials

The original IS2B design utilized a stainless steel shell, with primarily aluminum interior parts. Other, less expensive material options were explored for the redesign, as well as the functional tradeoffs for material changes for various parts, including the shell, end caps, tubing, fittings, and interior mounting mechanisms.

TRANSITION 5

Chapter 3 documented a sampling and analytical method suitable for assessing the fate of fiproles in wastewater streams. The following section, Chapter 6, revisits this method and applies it in a mass balance study, wherein the fiprole removal efficiency of a conventional wastewater treatment plant and engineered wetland is assessed. The sampling protocol called for flow-weighted sampling, and in order to generate the most accurate mass load data possible, an array of samplers was programmed for intermittent flow-weighted sampling that portioned composite samples according to predicted hourly flow rate. This flow-weighted sampling method is in contrast to the more commonly used method of flow-triggered sampling by use of a bubble flow-meter.

The mass balance study described in Chapter 6 elucidates the fate of fiproles in a wastewater treatment plant and engineered wetland, providing valuable information regarding environmental mass loads of fiproles in wastewater process flows including treatment plant and wetland effluents.

CHAPTER 6

MASS BALANCE OF FIPRONIL AND ASSESSMENT OF RELATIVE TOXICITY OF PROCESS STREAMS DURING CONVENTIONAL WASTEWATER AND WETLAND TREATMENT

ABSTRACT

The attenuation of the pesticide fipronil and its major degradates (fiproles) in a conventional wastewater treatment plant and downstream wetland was determined. Analysis of flow-weighted composite samples by liquid and gas chromatography/tandem mass spectrometry showed the occurrence of fipronil (12–31 ng/L) in raw sewage, primary effluent, secondary effluent, chlorinated effluent, and wetland effluent. Mean daily mass loads of total fiprole congeners in raw sewage and tertiary effluent after chlorination were statistically indistinguishable ($p = 0.29$; $n = 10$). Fipronil mass was reduced ($25 \pm 3\%$; $p = 0.00025$; $n = 10$) but associated toxicity loss was balanced by formation of toxic fipronil degradates, rendering conventional treatment unfit for reducing overall fiprole toxicity. Both fipronil and total fiprole masses were reduced in the wetland at rates of $44 \pm 4\%$ and $47 \pm 13\%$, respectively. Total fiproles in plant effluent (28 ± 6 ng/L as fipronil) were within an order of magnitude of half-maximal effective concentrations (EC_{50}) of non-target invertebrates. Per-capita masses in plant effluent and biosolids of 1.6 and 0.05 mg/person/year suggest nationwide emissions in the range of 520 and 17 kg/year, respectively. This is the first systematic assessment of fiprole fate during full-scale conventional wastewater and constructed wetland treatment.

Introduction

Fipronil and its congeners (fiproles) are phenylpyrazole insecticides used in a variety of pest control products, including seed coatings, roach and ant bait, flea and tick topical treatments, and termiticides. Fiproles have been implicated as potential contributors to colony collapse disorder of honeybee populations.^{165,195,196} With lethal dosages (LD₅₀) of 4-13 ng/bee,^{16,197,198} fipronil is extremely toxic to honeybees, which play a critical ecosystem function, and also provide an added economic value to the United States crop industry of \$5-14 billion.¹⁹⁹ Fipronil is the parent compound of several similarly potent degradates (including the sulfide, sulfone, amide, and desulfinyl variants), and it has been directly implicated in the sharp decline in crawfish populations in southern Louisiana as a result of pesticide application to rice paddies.^{200,201} Fiproles also are toxic to some non-target vertebrates, including fish and gallinaceous birds.¹⁸ Due in part to its likely role in pollinator poisoning and its effects on aquatic wildlife, China placed heavy restrictions on fipronil's use in 2009,²⁰² and the European Union followed suit in 2013.²⁰³

As a result of its widespread use, fipronil has been detected in urban waterways, and in rural rivers.^{135,143} In a survey of urban waters in Orange County, California, fipronil and fipronil sulfone exceeded benchmarks in over 70% of samples ($n = 94$).¹⁴³ In another study of fiprole contamination in the Mermentau and Calcasieu River Basins in the United States, fipronil, fipronil sulfide, and fipronil sulfone were detected in 78.0, 90.0, and 81.7% of surveyed samples, respectively.²⁰¹ These compounds were also shown to have accumulated in sediments in the same area (100% detection).²⁰¹ Fipronil, like other neurotoxic insecticides (e.g., imidacloprid), has been linked to wildlife population

declines, with a notable impact on biological diversity.¹³⁴ Numerous studies have investigated fiprole impacts on copepods,²⁰⁴ fish,^{205,206} gallinaceous birds,¹⁸ and reptiles.²⁰⁷ Among the suspected sources of fiprole contamination are agricultural runoff,²⁰⁸ urban runoff,¹³ and treated wastewater.^{24,25,138} The fate of several halogenated emerging contaminants in wastewater treatment plants has heretofore been evaluated, and most of these substances display significant recalcitrance.^{24,209-212} The only study employing a mass balance approach for fipronil reported $18 \pm 22\%$ removal; a large margin of error prevented any firm conclusions as to whether fipronil was removed at all, and lacking analytics for major transformation products prevented gaining a better understanding of the formation of toxic fiprole congeners in the wastewater treatment train.²⁴ We hypothesized that loss of fipronil during wastewater treatment may occur, but does not necessarily imply a reduction of the total fiprole toxicity, due to potential formation of equally or more potent congeners.

The primary objective of this study was therefore to assess the fate of fiproles, namely fipronil, fipronil sulfide, fipronil sulfone, fipronil amide, and fipronil-desulfinyl, in a conventional wastewater treatment plant by performing mass balances for conventional treatment unit operations of a full scale U.S. wastewater treatment plant (WWTP), and for a constructed wetland located downstream.

Results and discussion

Fiprole fate and mass balances across a representative conventional treatment train

In the wastewater treatment train selected for extensive monitoring, fipronil was present in raw sewage at an average daily concentration of 17 to 31 ng/L and exited in disinfected treated effluent at levels of 13 to 21 ng/L. The sulfide, amide, and desulfinyl degradates were detected in most WWTP process streams at low levels (0.7–8 ng/L) (see Figure D-2). A mass balance of total fiproles through the treatment train indicated that as a group, fiproles were conserved throughout (Figure 6-1). A five-day mass load of total fiproles entering and exiting the treatment train yielded 77 ± 11 and 69 ± 6 mmol, respectively; fiprole mass loads in primary and secondary effluent were similar to those in the primary influent stream, suggesting conveyance of the contaminants through the treatment train (Figure 6-2).

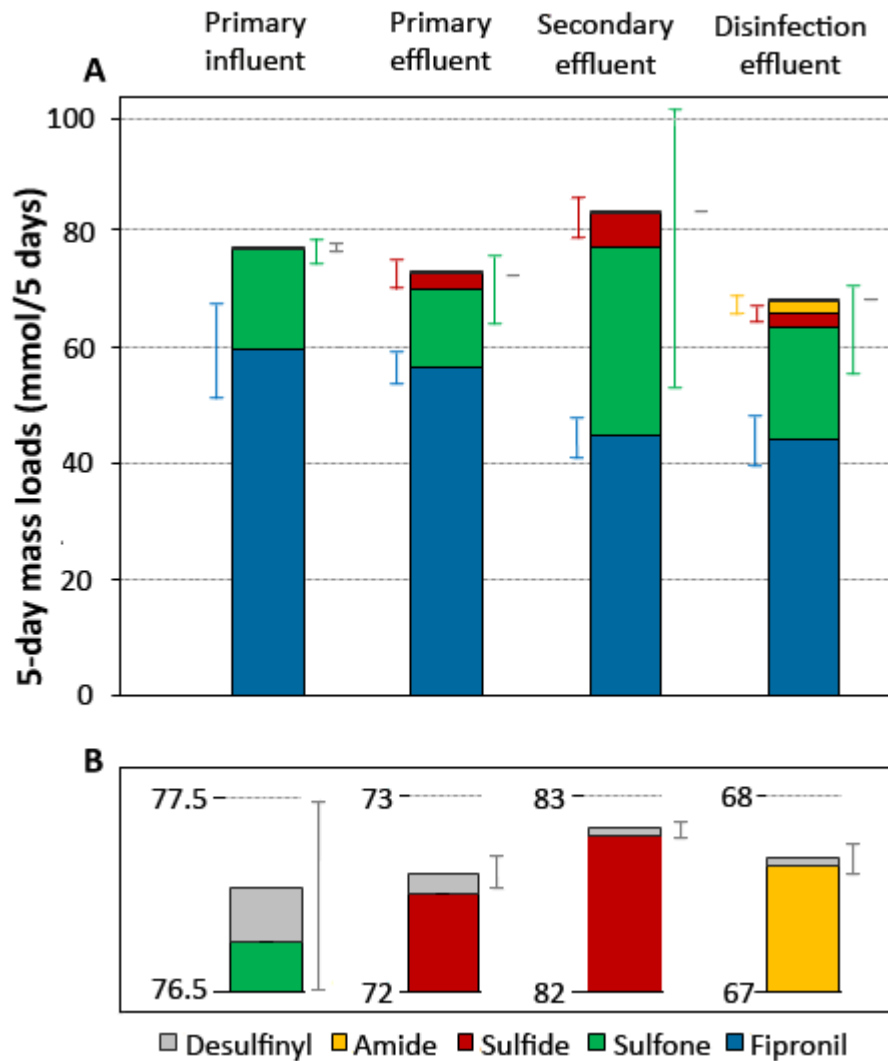


Figure 6-1. (A) Fipronil loads (in mmol) in wastewater streams over the course of five days. Direction of water flow is from left to right, (primary influent to disinfection basin effluent). Error bars represent high and low values from two experimental replicates. (B) Enlarged portions of the histogram in panel A, in order to make fipronil-desulfinyl masses visible. Fipronil-desulfinyl concentrations are estimated, near the detection limit. Sludge streams ($n = 2$) are omitted, as their mass contributions are negligible.

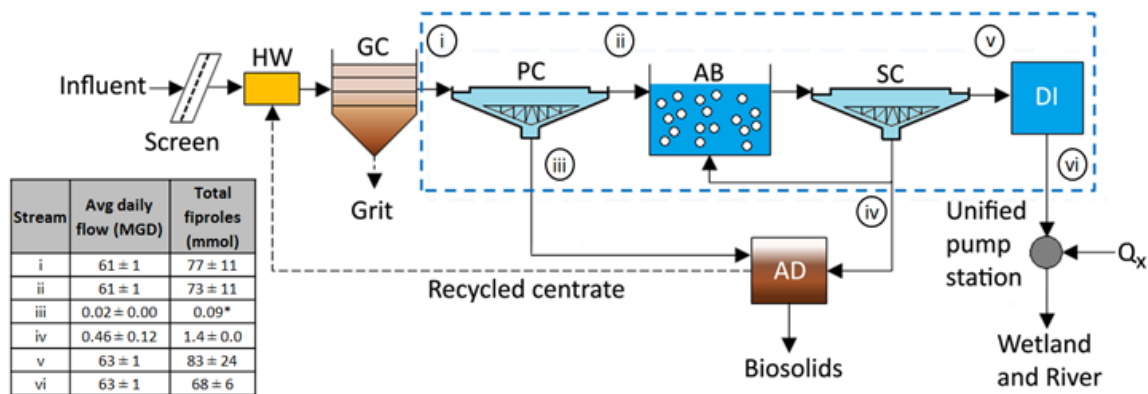


Figure 6-2. Flow diagram of the wastewater treatment train. Labeled streams i, ii, iii, iv, v, and vi indicate primary influent, primary effluent, primary sludge, waste activated sludge, secondary effluent, and disinfection basin effluent, respectively. Total five-day fiprole loads (in mmol) for the sampled streams are given in the table at left. ($n = 2$ experimental replicates per composite) *Primary sludge (stream iii) was taken as a 1-L grab sample each day during the five day sampling period, which yielded only one experimental replicate, and only one five-day sum, so no error is given. Biosolids were sampled 21 days after the water sampling campaign began, in order to account for the solids retention time in the anaerobic digesters. Combined flow from other treatment trains is indicated by Q_x . HW, headworks; GC, Grit chamber; PC, Primary clarifiers; AB, Aeration basins; SC, Secondary clarifiers; DI, Disinfection basin; AD, anaerobic digesters/centrifuges/dewatering systems. The dotted box indicates the control volume around the treatment train.

Overlapping error bars and a two-tailed t -test (95% confidence level) revealed that the mean daily influent and effluent masses of total fiproles were statistically indistinguishable ($p = 0.29$), implying that conventional wastewater treatment is ineffective at converting fiproles beyond the four immediate degradates studied herein (sulfone, sulfide, amide, desulfinyl). Limited settling of fiproles occurred in the primary and secondary clarifiers, despite their considerably high logarithmic n -octanol-water partitioning coefficients ($\log K_{OW} \approx 4.0$ -5.4).^{81,169} While total fiproles experienced no appreciable mass loss during passage through the treatment train, fipronil was transformed at a rate of approximately 25%, with about 1% being removed from water by the solids in waste activated and primary sludge (Figure 6-3).

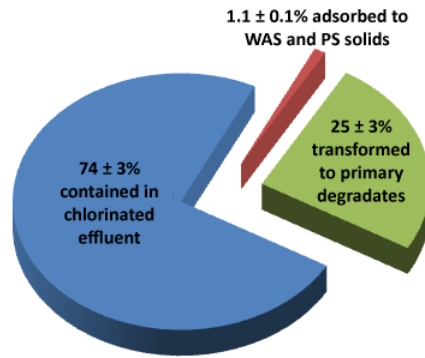


Figure 6-3. Mass balance for parent compound fipronil over five days in a wastewater treatment train from primary through tertiary treatment.

This result is in agreement with and refines prior estimates from a 2009 study, in which fipronil was found to be removed from a similar U.S. conventional wastewater treatment plant at a rate of $18 \pm 22\%$; in that work, the considerable analytical error did not allow the unambiguous identification of differences between influent and effluent concentrations, and a detailed analyses of the effectiveness of individual unit operations was not undertaken.²⁴

Mathematical modeling using EPISuite indicated that fipronil is expected to have a total aqueous removal rate of 30% during wastewater treatment, with only 0.32% removed by biodegradation, and the rest by sludge adsorption.⁸¹ The results of this empirical study show approximately 1% removal by sludge adsorption, and 25% removal by biodegradation. These observed discrepancies between empirical and modeling data are not unexpected. Biodegradation is a complex process that is ill-suited for parameterization with simplistic models. Sorption modeling typically considers only hydrophobic interactions when estimating distribution coefficients (K_D), and further relies on K_{OW} determinations that are known to have order-of-magnitude margins of

error;²¹³ organic carbon partitioning coefficients (K_{OC}) are often estimated from K_{OW} values, and there is also an order-of-magnitude error inherent in this estimation.²¹⁴

Mass balance across all parallel WWTP treatment trains

Approximately 58% of the flow and 48% of the total fiprole mass discharged by the wastewater facility was directed to an engineered wetland located immediately downstream, whereas 43% of fiprole mass was distributed to a power plant, and 9% was sequestered in biosolids. Average daily mass loads of fiproles in the WWTP inputs and outputs were 33.2 ± 5.6 mmol and 37.6 ± 7.3 mmol, respectively (see Figure 6-4, panel A).

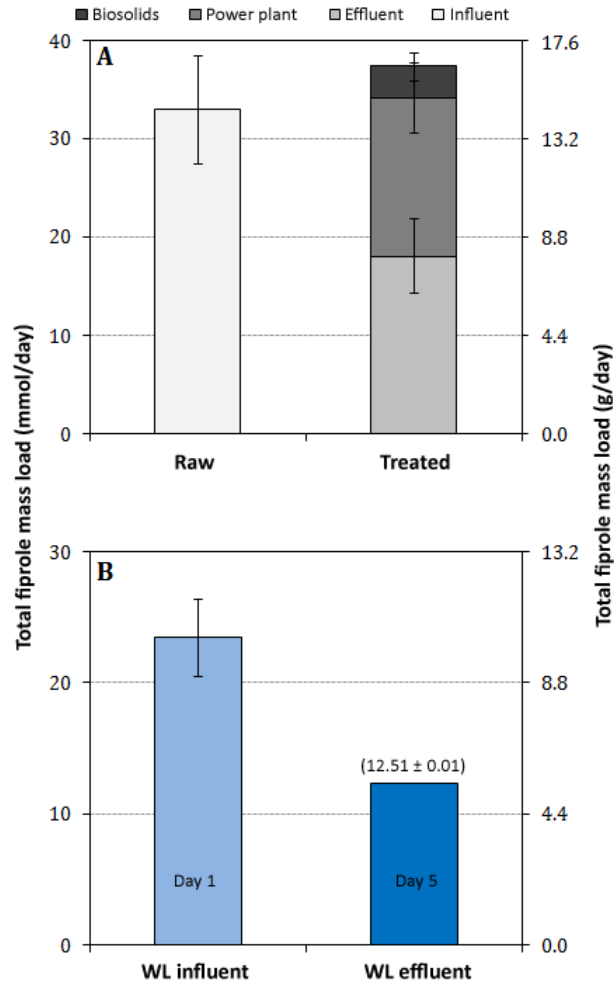


Figure 6-4. (A) Average daily mass loads of fiproles over five days, where error bars represent standard deviations ($n = 10$). (B) Daily mass loads of wetland (WL) influent and effluent streams on days 1 and 5, respectively, where error bars represent max/min values ($n = 2$); the hydraulic retention time of the wetland was 4.7 days. The right-hand y-axis is expressed as grams of fipronil per day.

Similar to the individual treatment train, the daily mean input and output masses of the entire WWTP were not significantly different ($n = 10$, $p = 0.14$), indicating complete lack of, or only insignificant removal of total fiproles. The computed error in reported masses is cumulative, accounting for variability of calibration in flow meters used to measure flow rates, of recovery rates during extraction, of estimated solids retention time of anaerobic digesters, and of instrument response.

Relative abundance of fipronil congeners in input and output streams underwent little change. The stream composition was approximately 75% fipronil, 1% fipronil sulfide, 21-22% fipronil sulfone, 0-4% fipronil amide, and 1-2% fipronil-desulfinyl. However, the mass ratio of sulfone degradate to parental fipronil in waste activated sludge was about 0.74, whereas in primary influent, the same ratio was much lower at about 0.3; this implies that fipronil sulfone was formed in either the aeration basins or in the secondary clarifiers. If the solids retention time in the clarifiers enabled the conversion of fipronil to fipronil sulfone, then this pattern should also be seen in the primary sludge, but it is not (sulfone/parent ratio = 0.14). Considering that fipronil sulfone is an oxidative byproduct of fipronil, the evidence suggests that the sulfone degradate was formed during aerobic digestion. Furthermore, in biosolids, the proportions of the congeners were roughly as follows: 15% fipronil, 65% fipronil sulfide, 9% fipronil sulfone, 1% fipronil amide, and 9% fipronil-desulfinyl. The dominant congener in biosolids was the sulfide degradate.

Wetland mass balance

The wetland downstream of the WWTP had a hydraulic retention time (HRT) of about 4.7 days, so the mass load into the wetland on the first day of sampling should correspond with the mass load out of the wetland 4 to 5 days later. A mass balance on the wetland (Figure 6-4, panel B) using the first day's influent mass load and the fifth day's effluent mass load indicates that fiproles were attenuated in the wetland at a rate of $47 \pm 13\%$.

Over the five-day period, the average effluent concentrations of total fiproles were about 24% lower than the influent concentrations ($n = 10$, $p = 2 \cdot 10^{-5}$). The discrepancy between mass and concentration changes can be accounted for by evapotranspiration (the effluent

flow rate is about 87% of the influent flow rate) and daily mass load deviations over the five day period not captured by the mass balance (the wetland mass balance only uses the first and fifth day mass loads to account for the wetland's hydraulic retention time, while the average concentration over five days accounts for all five days of sampling, wherein concentration fluctuations occurred).

Relative toxicity

Hazard quotients were calculated for process streams in the studied treatment train, including primary influent, disinfection basin effluent, wetland influent, and wetland effluent. For *Procambarus clarkii*, these values were 0.0022 ± 0.00038 , 0.0018 ± 0.00045 , 0.0020 ± 0.00040 , and 0.0015 ± 0.00032 , respectively. The mean HQ (*Procambarus clarkii*) of the primary influent stream was compared with the effluent from disinfection, the wetland influent, and the wetland effluent using a two-tailed *t*-test ($n = 10$) assuming equal variances; *p*-values for these analyses were 0.09, 0.27, and 0.0003, respectively. For *Hyaella azteca*, the primary influent, disinfection basin effluent, wetland influent, and wetland effluent HQs were 0.019 ± 0.0033 , 0.017 ± 0.0041 , 0.018 ± 0.0036 , and 0.014 ± 0.0028 , respectively. Testing for statistical differences in the means of the HQs of disinfected effluent, wetland influent, and wetland effluent process streams with the primary influent stream yielded *p*-values of 0.22, 0.51, and 0.0017, respectively. Thus, passage of water through wetland reduced the toxic load of fiproles but conventional wastewater treatment did not.

Study implications and future research needs

Considering that the toxic load inherent to total fiproles was left essentially unattenuated by conventional wastewater treatment, the next best opportunity to control harmful exposures of aquatic biota and ecosystems is to limit use and loading of raw wastewater with the parent pesticide, fipronil. Although mechanisms of fiprole toxicity to ecosystems were not evaluated here, it has been demonstrated that fiproles can be taken up by angiosperms, transported through their xylem, and deposited on pollen and seedlings.^{21,22,215} Bees and other pollinating insects may be exposed to fiproles upon direct application via treated seeds, and upon application of biosolids on land used to grow flowering plants. Indeed, one survey in France showed total fiprole levels in pollen as high as 8.3 ng/g.²¹⁶

The wastewater treatment plant in this study discharges an estimated 7.9 g/day of total fiproles (as fipronil) into the wetland, with 34–60 % estimated to be attenuated. To what extent fiproles are taken up by plant and animal life is not well understood, and likely varies by fiprole congener and exposed species. Fipronil producers recommend no more than 0.050 lb (23 g) of active ingredient to be applied annually per acre of land for varied uses such as mole cricket control. The water exiting the wetland discharges an estimated total fiprole load of 5.2 g/day. Biosolids produced by the treatment plant contribute a total fiprole load of 1.4 ± 0.7 g/day, mostly in the form of fipronil sulfide. The quantity of fiproles discharged from this single treatment plant in a given year is approximately 2.9 kg. Linearly extrapolated to encompass the entire United States, the corresponding order-of-magnitude estimate suggests that approximately 500 kg of fiproles (as fipronil) are

released into the environment every year by wastewater treatment plants in the United States. Of course, this estimate is subject to a large degree of uncertainty, due to unknown variation in fiprole loads in wastewater effluents around the country, and differences in treatment regimes that may impact removal efficiency.

To put the above estimates into perspective, we compared the estimated annual fiprole mass discharge in U.S. wastewater effluents to the total volumes of fiproles used in California and the United Kingdom. In 2011, sales of fipronil in the State of California amounted to about 18 tonnes.²¹⁷ The population of California in 2011 was approximately 38 million people, and if the total fipronil discharge from wastewater facilities per capita per year is scaled to the population of California, the estimated fipronil load in treated wastewater in California in 2011 could be estimated at about 0.06 tonnes. Thus, the estimated mass of fipronil in California wastewater would account for about 0.34% of fipronil mass purchased. It is difficult to ascertain what fraction of the total fipronil market is represented by wastewater discharge, due to the limited availability of information regarding the quantities of fipronil used for agricultural and other purposes. However, information about the quantities of pesticides used in agriculture is available at the Department for Environment, Food, and Rural Affairs (Defra) in the United Kingdom. According to Defra, peak agricultural use of fipronil in the Great Britain was 124 kg/year total in 2005 and 2006.²¹⁸ Since the 2013 ban, agricultural use dropped to 16 kg/year.²¹⁸ The ratio of the estimated fiprole discharge (as fipronil) in U.S. wastewater effluents to the total agricultural market volume in the UK is 520 kg/124 kg, or 4.2-fold the overall mass of fipronil purchased in the U.K at peak use.

While the amount of fipronil inadvertently discharged into the environment in the form of treated wastewater is considerable, it is unclear how wastewater contributes to fiprole loads on angiosperm pollen, body burdens of aquatic organisms, or toxicological effects in other non-target organisms. Further research is needed to determine whether and to what extent fiprole loading in wastewater effluents can impact plants and non-target organisms. Fipronil is among the most potent insecticides on the market, with toxicity to honeybees over 6,000-times greater than that of the banned pesticide DDT (27,000 vs. 4.2 ng/bee).^{219,220} Acute lethal doses (LD₅₀) for numerous non-target invertebrates also are in the ng range per organism.^{18,25,197,204,219} Some studies have shown that indirect exposure to certain insecticides may have adverse effects on vertebrate organisms, as well. As an example, the presence of the neonicotinoid imidacloprid at concentrations of about 20 ng/L was correlated with a 3% decline in insectivorous bird populations in Holland.²²¹ A study in Madagascar indicated that insectivorous lizards and birds are exposed to fiproles through the food chain, due to the fact that their diets consisted largely of the target organism (termites), and that they experienced sublethal effects.²²² In order to fill in this information gap, it would be necessary to evaluate the bioaccumulative and toxic effects of fiproles at various levels of the food chain. In addition, the plants in environments impacted by sources containing fiproles can be evaluated for uptake and xylem transport by extracting and analyzing pollen and leaves, as described by one study in France, wherein fiprole residues were detected in 13% of randomly selected pollen load samples in honeybee hives.²²

It is currently uncertain whether the levels released into the environment via wastewater effluent may cause accumulation of fiproles in sediments and elicit acute toxic effects.

The present study showed only that fiproles are remarkably resilient to degradation in wastewater treatment plants, where there are abundant potential mechanisms for their removal (e.g., aerobic digestion, anaerobic digestion, chemical oxidation). Considering the half-life of fipronil in water and sediments is typically on the order of several days (more than 200 days in the case of the sulfone, sulfide, and desulfinyl congeners),^{179,180} and considering the paucity of knowledge about the ecological impacts of both direct and indirect discharge of fiproles into the environment, a more extensive longitudinal study of the transport of fiproles in surface waters and their fate in sediments, combined with biomonitoring studies, may help to illuminate potential associations between wildlife population changes and the presence of fiproles in the environment.

Materials and methods

Solvents and standards

Analytical grade solvents (water, acetonitrile) were obtained from Thermo Fisher Scientific (Waltham, MA USA) and EMD Millipore (Billerica, MA USA). Neat analytical standards of fipronil and fipronil-desulfinyl were purchased from Sigma Aldrich (St. Louis, MO), while neat standards of fipronil sulfide, sulfone, and amide were produced by Bayer and BASF (Ludwigshafen, Germany). Isotopically labeled fipronil (¹³C₂¹⁵N₂-fipronil) was purchased from Toronto Research Chemicals, Incorporated (Toronto, Ontario Canada).

Sampling campaign

The wastewater treatment plant located in the southwestern U.S. is comprised of several individual conventional treatment trains operated in parallel. We systematically assessed the fiprole reduction capability of one representative treatment train, as well as the entire treatment plant and a constructed wetland located downstream. Automatic samplers were deployed at the following locations along the treatment train to capture: primary influent, primary effluent, secondary effluent, return activated sludge, disinfection basin effluent, wetland influent, and wetland effluent. Primary sludge was obtained by grab sampling. Sampling was carried out over five consecutive days, from 12 PM on Thursday through 12 PM the following Tuesday. The ISCO 6700 and 6712 samplers (Teledyne Technologies, Thousand Oaks, CA USA) were programmed for flow-weighted composite sampling. In order to obtain flow-weighted composites, the samplers were programmed to sample multiples of 20 mL every hour. The fraction of the total composite volume sampled any given hour was proportionate to the deviation from daily average flow into the plant (as determined by hourly flow data over a period of 21 days). More details on sampler programming can be found in Appendix D and Figure D-1. At 12 PM each day, the composite from the prior day was replaced with an empty 2.5-L amber bottle. Primary sludge was sampled once per day at 9 AM, using a 1-L bottle. Biosolids were taken as grab samples in 40-mL glass vials, starting 21 days after the first day of the water sampling campaign, in order to account for the solids retention time in the anaerobic digesters.

Instruments and analysis

All fiproles except fipronil-desulfinyl were separated by liquid chromatography, and detected and quantified by negative electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS). Fipronil-desulfinyl displayed a significantly lower detection limit by gas chromatography electron impact tandem mass spectrometry (GC-EI-MS/MS), and was therefore analyzed using a GC-MS/MS instead. Liquid chromatography mass spectrometric analyses were done using a Shimadzu Prominence HPLC (Shimadzu Scientific, Kyoto, Japan) controlled by Analyst 1.5 software (Applied Biosystems, Framingham, MA) coupled to an ABSciex API-4000 MS/MS (Applied Biosystems, Framingham, MA). Liquid chromatographic separation was achieved by an XBridge C₈-column (3.5 μm particle size, 4.6 × 150 mm; Waters Corporation, Milford, MA). The mobile phase consisted of 50% acetonitrile (ACN) and 50% water flowing at a rate of 1 mL/min with a total runtime of 10 min, and a gradient profile of 10% ACN/min to 95%. Analytes were introduced into the mass spectrometer using an electrospray ionization probe operating in negative mode, and multiple reaction monitoring (MRM) was used for qualitative analysis. Optimized conditions for the ionization and a fragmentation of the analytes are specified in Appendix D. Quantitation of fipronil was done using isotope dilution and an 8-point calibration curve, with matrix spikes using ¹³C₂¹⁵N₂-fipronil. Quantitation of other fiproles was done using the standard addition method with four analysis sample spike levels. Gas chromatographic mass spectrometric analyses were performed on an Agilent 7890 GC coupled to an Agilent 7000 triple quad MS (Agilent Technologies, Santa Clara, CA) operating in positive mode, and MRM was used for

qualitative analysis. More details on analytical instrument parameters and quality control, including limit of detection determination, can be found in Appendix D.

Solids collection and analysis

Solid samples were extracted using a modified version of EPA method 1699. Ten milliliter aliquots of refrigerated, homogenized water samples were transferred to 15-mL centrifuge tubes, and were subsequently centrifuged at 3500 ×g. The supernatants were then decanted and discarded. The remaining solids were dried, weighed, spiked with 20 ng labeled fipronil, extracted with 10 mL of acetone, and set on a rotary shaker at 60 rpm for 24 hours. The extraction mixture was centrifuged again, and the solvent was collected in a glass vial. After a second extraction with 10 mL of acetone, the serial extracts were combined, evaporated under nitrogen to near dryness, and reconstituted with 6 mL of hexane. Sample cleanup was done using 1g/6 mL Sep-Pak® (Waters Corporation, Milford, MA) cartridges containing Florisil. The cartridges were conditioned with 6 mL dichloromethane, 6 mL acetone, and 6 mL of hexane before the samples were loaded. Once loaded, the cartridges were dried under vacuum and exhaustively eluted with dichloromethane and acetone (1:1 v/v). The solvent mixture was switched to either 50% acetonitrile in water for LC-MS/MS analysis, or 100% hexane for GC-MS/MS analysis. Total suspended solids (TSS) for each stream was determined by dividing the solids mass of the samples described above by the 10 mL wet volume.

Water extraction and analysis

Fiproles were extracted from 500 mL aliquots of wastewater and wetland water (in duplicate for all streams except primary sludge) using automated, high-volume solid phase extraction. Extraction was carried out using cartridges containing polystyrene divinylbenzene resin modified with pyrrolidone (500 mg/3mL Strata X and Strata XL, Phenomenex, Torrance, CA USA) installed on an Autotrace 280 (Thermo Scientific Dionex, Sunnydale, CA USA). Water samples were spiked with 20 ng $^{13}\text{C}_2^{15}\text{N}_2$ -fipronil prior to extraction via SPE. The resin was eluted with 5% formic acid in methanol, and then aliquots of these extracts were reconstituted to either 50% methanol in water (for LC analysis) or 100% hexane (for GC analysis). Water samples with high TSS like waste activated sludge (WAS) and primary sludge (PS) were centrifuged at 7500 x g, and 500 mL of the supernatants was decanted and extracted as described. Analyte mass on the solid fraction of those streams was determined as described in the previous section, and the weighted mass contribution of the solids was added to that of the water to determine the total mass of fiproles in WAS and PS.

Calculations

Automatic samplers were programmed to take a number of 20 mL incremental samples within the first few minutes of a given hour. The total desired composite sample volume for one day was 2500 mL. The number of 20 mL increment samples taken in a given hour was calculated using equation 6-1.

$$N_{20\text{ mL}}(t) = \frac{2500\text{ mL}}{20\text{ mL} \times 24} \times \frac{Q(t)}{\bar{Q}} \quad (6-1)$$

Where $N_{20\text{ mL}}(t)$ is the number of 20 mL increments in the first few minutes of a given hour t , $Q(t)$ is the measured flow rate at hour t , and \bar{Q} is the average daily flow rate over the course of 21 days.

Mass loads for fipronil and total fiproles in process streams were determined by multiplying determined concentrations with the flow rates for corresponding days. A combination of daily average flows (12 AM to 12 AM) and monitored hourly flows is reported (see supporting information).

Applying a steady state assumption (accumulation = 0), the mass balance over the treatment train was calculated as shown in equation 6-2.

$$\begin{aligned}
 & \underbrace{\sum_{t=1}^{t=5} Q_{1'inf}(t)C_{1'inf}(t)\Delta t}_{\text{Primary influent}} - \underbrace{\sum_{t=1}^{t=5} Q_{DIEff}(t)C_{DIEff}(t)\Delta t}_{\text{Disinfection effluent}} - \underbrace{\sum_{t=1}^{t=5} Q_{PS}(t)[f_{PS_s}\rho_{PS_s}C_{PS_s} + C_{PS_w}(t)]\Delta t}_{\text{Primary sludge (effluent)}} - \\
 & \underbrace{\sum_{t=1}^{t=5} Q_{WAS}(t)[f_{WAS_s}\rho_{WAS_s}C_{WAS_s}(t) + C_{WAS_w}(t)]\Delta t}_{\text{Waste activated sludge (effluent)}} = \underbrace{m_{converted}}_{\text{Reacted}}
 \end{aligned}
 \tag{6-2}$$

The bracketed terms (primary influent, etc.) represent the total mass load through each respective stream over a five day period, where Q is flow rate (L/d), C is concentration (ng/L), t is time (d), f is the mass fraction of solids in a stream ($\text{g}_{\text{solid}}/\text{g}_{\text{wastewater}}$), and $m_{converted}$ is the mass not accounted for in all influent and effluent streams, assumed to be transformed (ng). The notations $1'inf$, $DIEff$, PS , and WAS respectively refer to primary influent, disinfection basin effluent, primary sludge, and waste activated sludge.

Subscripts *s* and *w* refer to solid and water, respectively. Individual fiprole masses were first converted to mmol before being added together to compute total fiproles. The flow rate of WAS was not directly measured, but was instead obtained by subtracting the return activated sludge (RAS) flow rate from the secondary effluent flow rate.

Equation 6-3 illustrates the method for performing a mass balance over the wastewater treatment plant, accounting for total influent and effluent streams, and biosolids. The total plant influent mass was estimated using the product of the treatment train primary influent concentration ($C_{1'inf}$) and total plant influent flow rate (Q_{tot}). The effluent streams from the plant were directed to the downstream wetland (*WL*) and a power plant (*PP*). The biosolids, or dewatered sludge (*DWS*), concentrations were given in units of $\mu\text{g/g}$, and DWS production rates were expressed in units of mass per day (g/d). The total mass of fiproles converted ($m_{total_converted}$) represents the mass of fiproles that presumably reacted or degraded during treatment through the entire plant.

$$\sum_{t=1}^{t=5} Q_{tot}(t)C_{1'inf}(t)\Delta t - \sum_{t=1}^{t=5} Q_{WL_inf}(t)C_{WL_inf}(t)\Delta t - \sum_{t=1}^{t=5} Q_{PP}(t)C_{PP}(t)\Delta t - \sum_{t=1}^{t=5} Q_{DWS}(t)C_{DWS}(t)\Delta t = m_{total_converted}$$

(6-3)

Equation 6-4 was used to calculate the conversion of fiproles in the wetland.

$$\sum_{t=1}^{t=5} Q_{WL_inf}(t)C_{WL_inf}(t)\Delta t - \sum_{t=1}^{t=5} Q_{WL_eff}(t)C_{WL_eff}(t)\Delta t = m_{WL_converted} \quad (6-4)$$

Equation 6-5 shows the calculation for obtaining nationwide estimates of fiprole emissions from wastewater effluents or biosolids (m_{USA}). The average daily fiprole emissions in wastewater effluent or biosolids over five days (\bar{M}) in kmol/d was divided by the total flow into the plant over 5 days (\bar{Q}) in liters, then multiplied by the average number of liters of wastewater per capita as determined by Mayer et al (292 L/d/person)²²³, the total population of the United States (318.9 million persons), 365 d/yr, and the molar mass of fipronil in tonnes/kmol.

$$m_{USA} = \frac{\bar{M}}{\bar{Q}} \times 292 \frac{L}{pers \cdot d} \times 318.9 \times 10^6 \text{ pers} \times 365 \frac{d}{yr} \times 0.43715 \frac{tonnes}{kmol} \quad (6-5)$$

It should be noted that an effluent stream feeding a nearby power plant was not directly sampled, but since it was split off from the plant effluent, the concentration in that stream was assumed to be the same as the concentration in the plant effluent. Total fiprole masses and concentrations were converted to fipronil equivalents by multiplying them by the relative molar mass of fipronil.

Equation 6-6 was used to calculate the species-specific hazard quotient (HQ_x) of the influent and effluent wastewater streams, using methods established in literature. (Stark & Banks) There are three fipronil congeners (fipronil, fipronil sulfone, and fipronil

sulfide) accounted for in the calculation, indicated in the equation by i . The desulfinyl and amide byproducts were omitted due to their less significant occurrence and toxicity. Influent or effluent stream concentrations are indicated by C_{stream} . The HQs of the influent and effluent streams were then compared to determine whether treatment affects the toxicity of wastewater.

$$HQ_x = \sum_{i=1}^3 \left(\frac{C_{stream_i}}{LC_{50i}} \right)_x \quad (6-6)$$

Toxicity indices were calculated for two arthropod species, *Hyaella azteca* and *Procambarus clarkii*, using the half-maximal lethal concentrations (LC_{50}) for the various fiprole congeners. These species were chosen due to the availability of aqueous toxicity data. The *Procambarus* LC_{50} values used in this calculation were 14.3, 11.2, and 15.5 $\mu\text{g/L}$ for fipronil, fipronil sulfone, and fipronil sulfide, respectively.²⁰⁰ The *Hyaella* LC_{50} values used in this calculation were 1.6, 1.4, and 0.59 $\mu\text{g/L}$ for fipronil, fipronil sulfone, and fipronil sulfide, respectively.²⁵

TRANSITION 6

The previous chapters provide a considerable body of work including a review of sampling technologies and their various potential applications, a sensitive method for analyzing fiproles in wastewater matrices, the development and validation of a novel water sampler for HOC assessment across the sediment-water interface, a development of a derivative device featuring a more compact and robust design, and a mass balance of fiproles across a wastewater treatment plant and wetland. In the next and final section of this thesis, Chapter 7, I reflect on the strengths and limitations of the methods and technologies provided and consider what type of follow-up work is recommended in order to fill still existing data gaps.

CHAPTER 7

RESEARCH IMPLICATIONS AND RECOMMENDATIONS

Sampling to assess fipronil in surface water and wastewater

The ultimate goal of environmental monitoring is to help ensure the integrity of the environment and the health and wellbeing of human and ecosystem populations.²²⁴ This goal hinges on useful environmental assessment strategies, which involve three basic tools: (1) determination of contaminant mass loading (including emissions/discharges from industrial, municipal, and agricultural sources), (2) determinations of contaminant distribution and concentration, and (3) an assessment of temporal and spatial trends. Each of these tools relies on accurate, representative data, and on the relevance of mathematical models used to estimate environmental impacts. In Chapters 2 and 4, I discussed the importance of sampling for achieving these objectives. Chapter 2 illustrated the fact that active sampling is under-utilized for assessment of hydrophobic organic contaminants, and that in situ extraction using automated active sampling can (1) mitigate sample handling issues, and (2) provide time-integrated or time-averaged data without the need to manually collect grab samples. Chapter 4 introduced a novel sampler designed for the purpose of providing data relevant to assessing the distribution of contaminants across the sediment-water interface, and the likely exposure levels to benthic and water column-dwelling organisms.

Contaminant mass loads

While numerous studies have utilized non-flow-weighted sampling for mass load and mass balance calculations,^{109,110} the best way to determine mass loads in systems with

variable flow is to collect flow-weighted samples; even if flow is relatively consistent, actual mass loading in most water streams probably is not, which means that time-discrete sampling is inadequate for mass load calculations. Chapter 6 utilized flow-weighted sampling to perform a mass balance for fipronil, and ISCO samplers had the programmability to allow for flow-weighted sampling. As a result, environmental mass loads of fipronil were calculated at a level of accuracy not previously attained.

Average contaminant concentrations

Time-integrated or time-averaged sampling gives an indication of the time-weighted average concentration (TWAC) in a water system, information that is indicative of how toxic the water is to susceptible organisms suffering chronic exposures. The IS2B was developed as a means to perform this kind of sampling, with the added benefit of offering simultaneous sampling of bulk water and pore water with multiple field replicates. Of the automated samplers discussed in Chapter 2, the water collection devices (e.g., ISCO) were capable of producing field replicates of time-averaged composites, but none of the reviewed in situ extraction samplers had this feature. The IS2B is the first active, pumping sampler with all of these features combined.

Assessment of contaminant distribution

Among the many considerations in contaminant distribution assessment is the partitioning of pollutants between sediments, the pore water, and the flux into the water column. The IS2B performs simultaneous in situ extraction of both the pore water and bulk water, which helps to provide data relevant to (1) contaminant distribution and (2) benthic species exposure. While there are numerous studies attempting to link

contaminant concentrations to bioavailability, the connection may be more complex than most studies seem to indicate. Indeed, some researchers argue that passive uptake via pore water exposure may or may not be the primary exposure route for benthic organisms: it depends on the species and the contaminant.^{225,226} And yet, a number of studies use chemical surrogates (e.g., passive samplers) to mimic organism uptake in pore water.²²⁷ This disagreement about the relative importance of pore water versus bulk water versus sediment may be resolved with more research into the contaminant uptake mechanisms of various benthic organisms. Samplers like the IS2B and mIS2B can be used to conduct research on contaminant bioavailability for organisms in both the sediment pore water and overlaying water column to illuminate correlations between biotic uptake and the distribution of contaminants between the two respective compartments (see Figure 7-1).

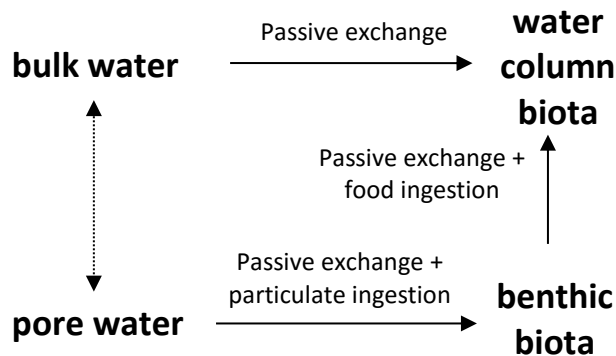


Figure 7-1. Routes of biotic contaminant uptake.

Coupling contaminant distribution data with biomonitoring studies using model organisms like *Lumbriculus variegatus* and *Pimephales promelas*, future research can focus on the uptake pathways for persistent organic pollutants like fipronil, as well as

their long-term ecological effects. If a convincing relationship between biotic uptake and the “available” fraction of contaminant in the various compartments is found, then the IS2B technology can be a useful bioavailability assessment tool.

Fipronil in wastewater

Only a handful of studies have quantified fipronil in wastewater treatment plants; Chapter 6 was the first study to assess the fate of fipronil *and* its primary degradates in a wastewater treatment plant. A systematic screening of wastewater streams from locations all over the country would provide a clearer picture of the actual total fiprole discharge from wastewater effluents. Furthermore, considering that fipronil is attenuated in a wetland, but not in a treatment plant indicates that there are mechanisms for degradation or accumulation that exist in a wetland that are absent in a wastewater treatment plant. It is possible that the much greater retention time of a wetland allows for mechanisms to become effective (e.g., hydrolysis, photodegradation, biodegradation) that are of limited or insignificant impact in the wastewater treatment plant, as not enough time or exposure is being provided to transform these compounds to a measurable degree.

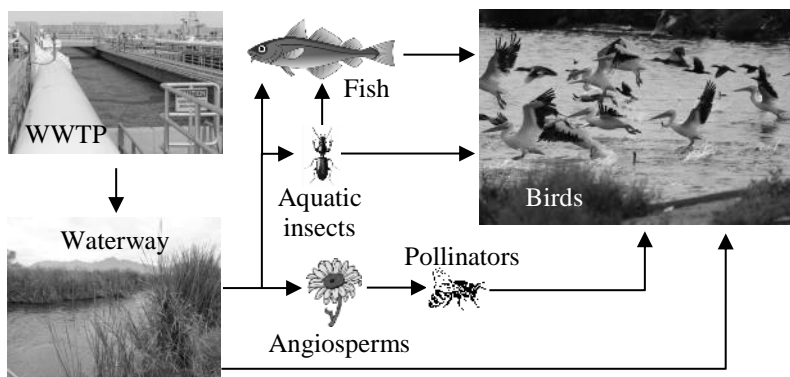


Figure7-2. Potential routes of biotic exposure to pesticides from wastewater effluents. All artwork is either public domain, or photography by the author.

Fiproles are highly toxic to invertebrates, and they are known to be persistent. It is therefore important to know the means of removal for these compounds in the wetland described in Chapter 6. They may be removed by either chemical or physical processes. If they are accumulating in flora and fauna (see Figure 7-2), then there are implications for ecological impact that need to be further explored. As one of several possible contributors to honeybee colony collapse disorder, potential uptake of pesticides in angiosperms exposed to contaminated wastewater is a major concern. Findings of another study (unpublished) at the Center for Environmental Security at Arizona State University indicate that at least three neonicotinoids also can be found in wastewater effluents. There is a possibility of cumulative, synergistic, and antagonistic effects on non-target organisms exposed to these pesticides. Monitoring campaigns aimed at tracking the total fiprole load in wastewater effluents as they enter an ecosystem can elucidate this issue.

This thesis presents qualitative and quantitative data on fiproles that are relevant to regulatory agencies, as it provides information regarding the occurrence and fate of potentially high-impact emerging contaminants in wastewater treatment plants and wastewater-impacted waters. Additionally, the sampling tools and analysis strategies described in this thesis can be more broadly applied to assess the ecological risks posed by trace level emerging contaminants, such as neonicotinoids, organophosphates, and other pesticides.

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APPENDIX A

SUPPLEMENTAL MATERIAL FOR CHAPTER 2

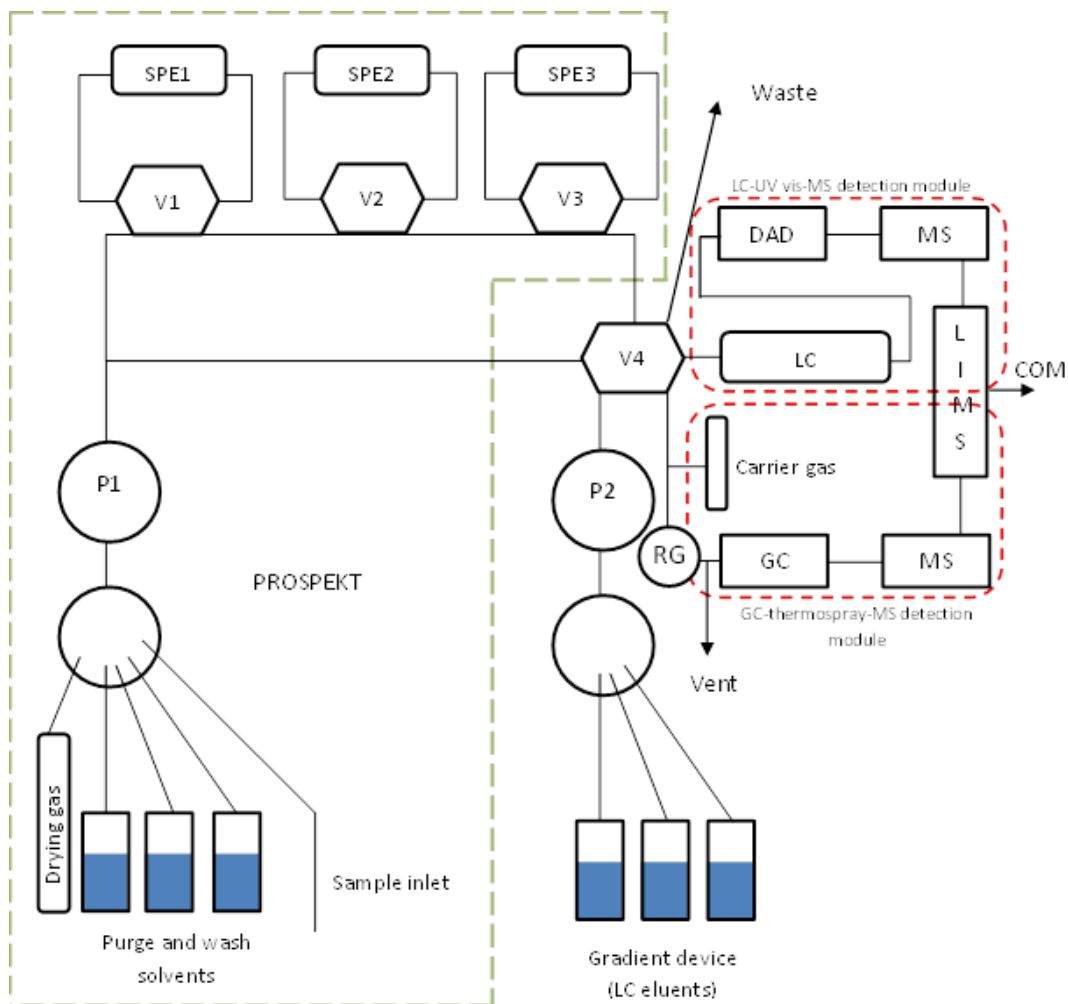


Figure A-1. SAMOS design. SPE1-3 = preconcentration cartridges; V1-4 = automatic switching valves; P1 = sample inlet and SPE solvent pump; P2 = LC high pressure pump; LC = liq. chromatograph; GC = gas chromatograph; MS = mass spec.; LIMS = laboratory information management system; DAD = diode array detector; COM = communications link; RG = retention gap. Design from van Hout and Brinkman (1994).

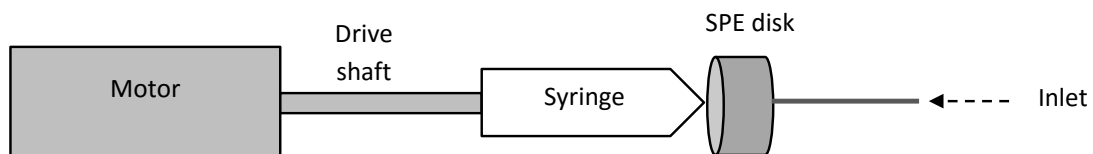


Figure A-2. A modified in situ SPE syringe sampler. A motor (battery-powered) drives a rod that retracts and pulls sample water into the syringe through an SPE cartridge or disk.

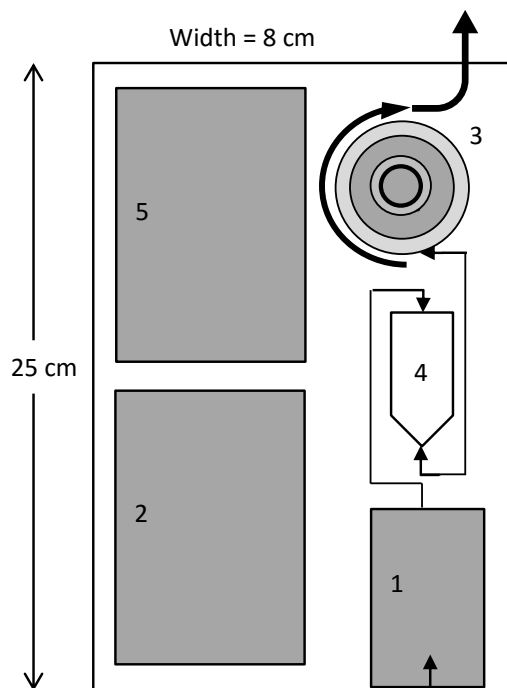


Figure A-3. CFIS unit. 1 = filter; 2 = batteries; 3 = peristaltic pump; 4 = PDMS Twister™ bars in glass cell; 5 = microchip.

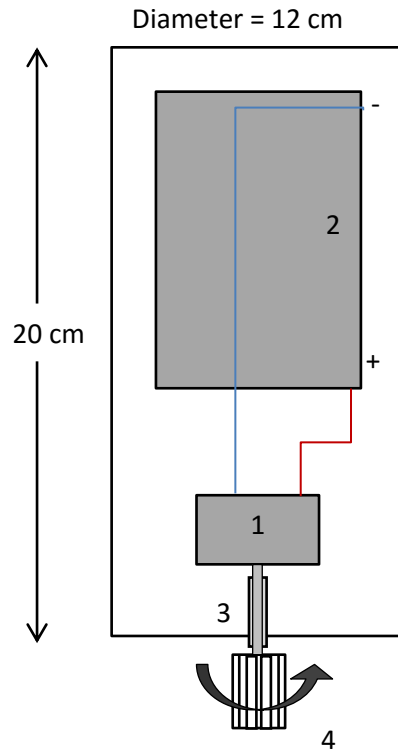


Figure A-4. CSS unit. 1 = motor; 2 = battery; 3 = stern tube; 4 = PDMS Twister™ bars.

APPENDIX B

SUPPLEMENTAL MATERIAL FOR CHAPTER 3

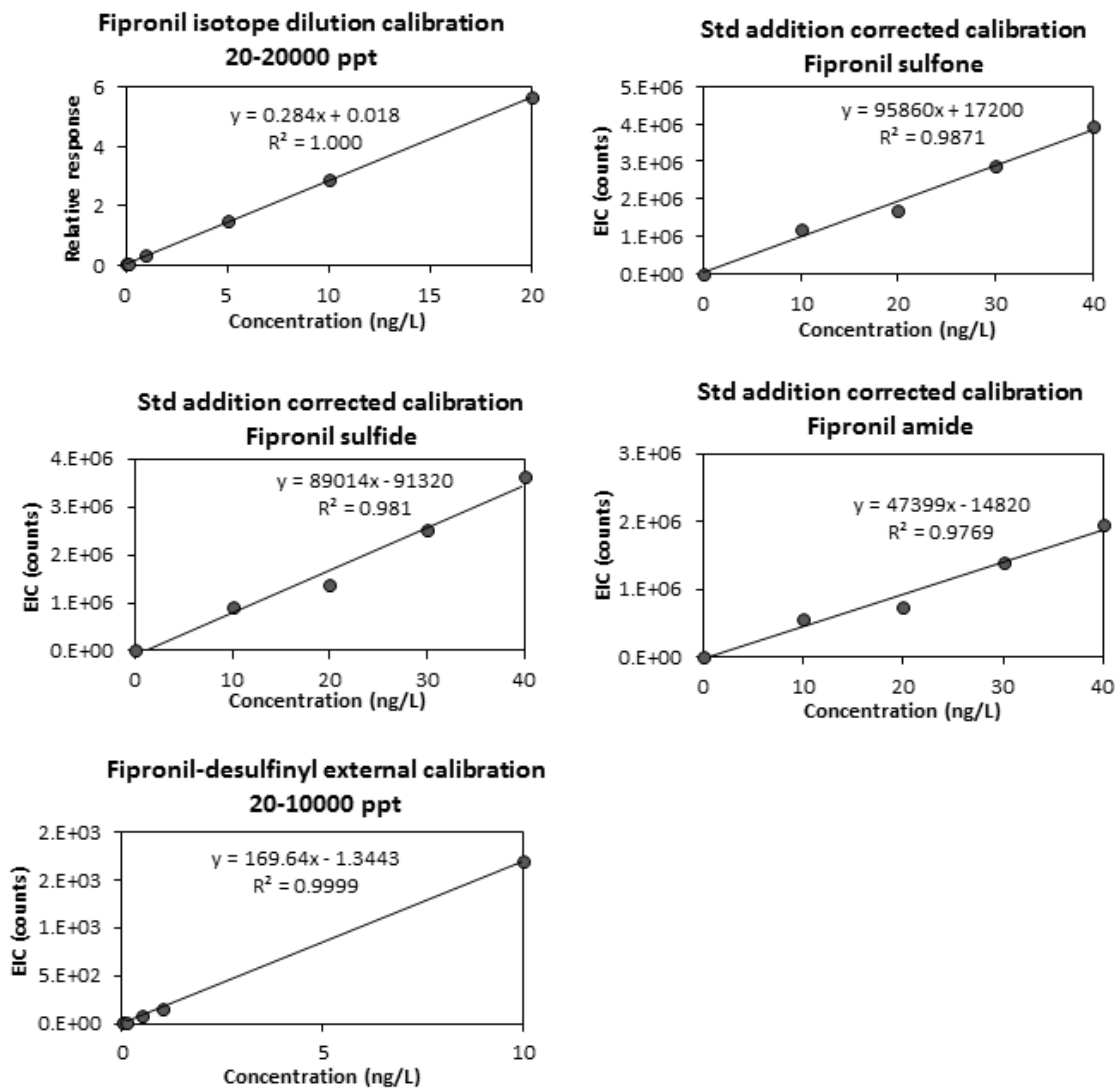


Figure B-1. Sample calibration curves for quantitation of fiproles in wastewater and wetland water matrices. Standard addition curves are for sludge extracts. Similar curves were generated for wastewater extracts to correct for matrix effects (not shown).

Instruments and analysis

Table B-1. Optimized mass spectrometry parameters.

Analyte	Fipronil		-sulfide		-sulfone		-amide		-desulfinyl	
Q1 mass (amu)	435	435	419	419	451	451	387	387	388	388
Q3 mass (amu)	330	250	383	262	415	282	351	282	333	281
Dwell (ms)	80	80	80	80	80	80	80	80	150	150
DP (V)	-70	-70	-75	-75	-70	-70	-70	-70	NA	NA
CE (arbitrary units)	-24	-38	-18	-40	-40	-40	-40	-40	25	30

Environmental samples

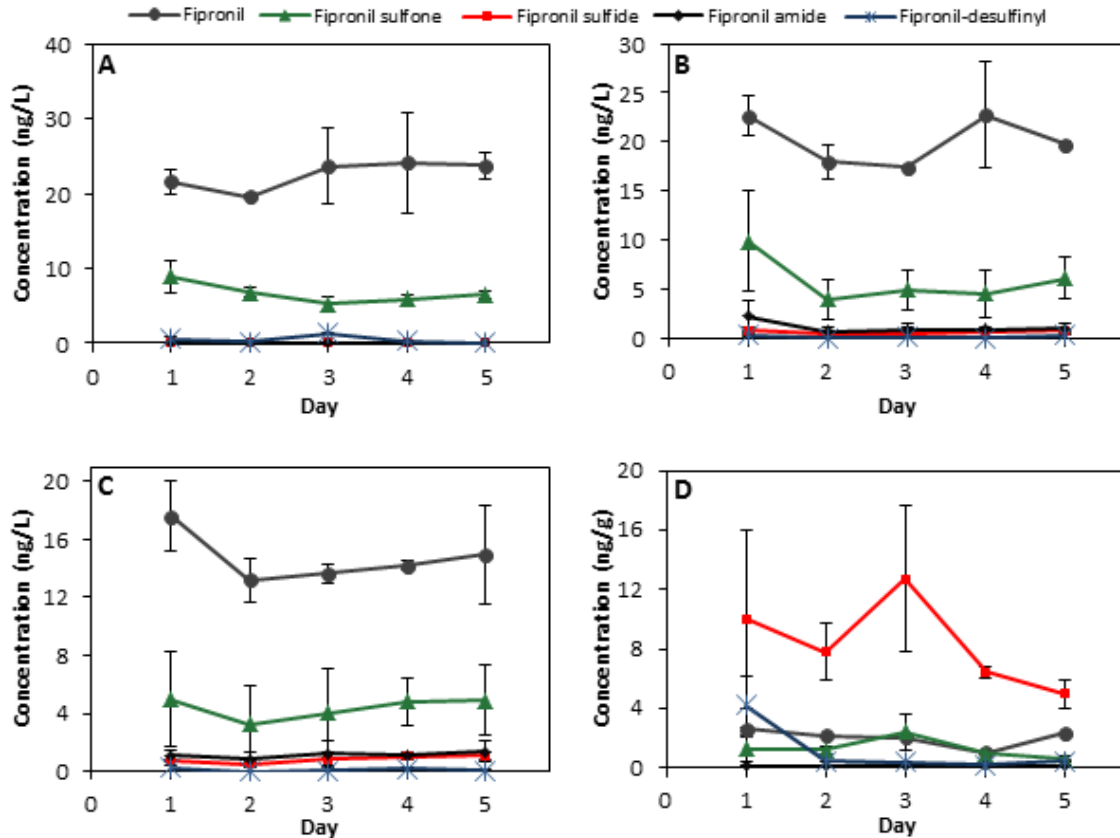


Figure B-2. Concentration profiles in (A) wastewater influent, (B) wastewater effluent, (C) wetland effluent, and (D) biosolids over the course of five days of sampling. Error bars represent min/max values ($n = 2$).

APPENDIX C

SUPPLEMENTAL MATERIAL FOR CHAPTER 4

Quality Assurance

Solvents and standards. Neat analytical standards of fipronil and fipronil-desulfinyl were purchased from Sigma Aldrich (St. Louis, MO). Neat analytical standards of fipronil sulfide, sulfone, and amide were manufactured by Bayer and Basf (Ludwigshafen, Germany). Organic solvents and Fluka brand liquid chromatography mass spectrometry (LCMS) grade water were purchased from Sigma Aldrich, while Optima brand LCMS grade water was purchased from Fisher Scientific (Fair Lawn, NJ). Fipronil des F3 (a fipronil analog) was purchased from Dr. Ehrenstorfer labs (Augsburg, Germany). Individual standard solutions of the target compounds were prepared by dissolving 10 mg neat standard into 10.0 mL of acetonitrile, or toluene, in the case of fipronil desulfinyl. Solutions were then vortexed until dissolution was complete, yielding 1.0 g/L standards from which serial dilution produced commingled standard solutions ranging from 5 mg/L to 1 µg/L in acetonitrile. Separate standards of fipronil desulfinyl were prepared for GC-MS/MS calibration in hexane.

Pump performance. The IS2B peristaltic pump was calibrated prior to each analysis. Two replicate benchtop tests were performed to ascertain the precision of the pump. Results are shown in Table C-1. Multiple trials with this device indicated that one type of tubing (PharMed) provided greater consistency in pump performance than did others (e.g., Viton), probably due to the tendency of the latter to deform permanently when pinched by the pump rollers. During the recovery tests, the ISMATEC control unit was set to deliver 200 mL at a pump rate of 140 µL/min/channel/ to each of the six channels in two consecutive runs (n = 12).

Sample collection. Sediment field blanks were collected from locations about 50 yards from the edge of the wetland. The sediment was not impacted by the wastewater effluent, and was therefore used as a quality control. Water field blanks were DI water samples transported from Arizona State University to the wetland, and transferred there into ashed media bottles.

Analytics. Calibration standard response accuracy had to be within 20% of expected values. Level 1 QA/QC for quantitation of fiproles was performed using lab control spikes. The absolute recovery of spiked mass was compared to “clean” calibration standards in 1:1 acetonitrile/water (for LC-MS/MS analysis) or 100% hexane (for GC-MS/MS analysis), and these results are displayed in Table C-2. Unspiked equipment blanks were used as controls, and the method of quantitation required subtraction of the equipment blank signal from that of the spiked samples.

Recovery tests. Water laden with dissolved organic carbon (DOC) was generated by adding 100 mg potassium citrate to 3 L of 18.2 M Ω (Milli-Q) water. The water was spiked to 300 ppm (v/v) with Kathon CG/ICP biocide and stored at room temperature in ashed amber media bottles. 2000 mL was transferred to a 2-L ashed media bottle and was spiked with 20 ng (nominal concentration 10 ng/L) of the fipronil parent compound, along with the sulfide, sulfone, and amide degradates. A separate 2-L sample of water was spiked with 2 ng (nominal concentration 1 ng/L) with fipronil-desulfinyl. Both samples were extracted in separate tests as described below.

For bench top extraction, the sampler was assembled with two 3-channel PTFE manifolds for water inlet, and six 1-mL SDB-L SPE cartridges (25 mg of resin), conditioned and rinsed with acetonitrile and LCMS grade water, respectively. Both IS2B inlet tubes were placed into the spiked lab-created water with the IS2B control unit set to deliver 200 mL at 140 μ L/min/channel. The effluent tubes from the SPE cartridge were each placed into separate weighed 1000 mL media bottles. At the end of the pumping period, the SPE cartridges were rinsed with 1 mL LCMS water, and eluted with 1 mL of acetonitrile, followed by 1 mL of 1:1 hexane/acetone. The serial eluates from each channel were combined, divided into two 1 mL aliquots, evaporated under nitrogen, and one set of aliquots was reconstituted to 1 mL of acetonitrile (ACN), while the other was reconstituted to 1 mL hexane. The resulting ACN solutions were diluted by 50% with water, and the ACN/H₂O samples were analyzed by LC-MS/MS for fipronil and the sulfide, sulfone, and amide degradate, while the samples in hexane were analyzed by GC-MS/MS for the desulfinyl degradate. In order to determine the background concentrations of the five analytes in the matrix, 200 mL of lab-created unspiked DOC-laden water was extracted by the IS2B in triplicate, along with 200 mL of 18.2 M Ω water (in triplicate). Absolute recoveries were calculated by the background subtraction method.

After the recovery test, the pump calibration was assessed by comparing the set volume on the control unit with the volumes collected in the effluent capture bottles. The volumes were determined by dividing the mass difference between the empty and full bottles by the density of water.

A similar procedure was used to determine the recovery efficiency using an AutoTrace 280 by Dionex (Sunnyvale, CA). The AutoTrace was loaded with 500 mg/3mL SDB

cartridges (8 replicates total), which were conditioned as described above. 200 mL of spiked DOC-laden water with 1 ng/L of targets was loaded onto each cartridge at 1 mL/min, and eluted serially with 2 mL of acetonitrile and 2 mL of hexane/acetone (1:1) at 1 mL/min. The eluates were commingled and blown down to dryness under nitrogen before being reconstituted to 2 mL of acetonitrile. These samples were split for GC-MS/MS analysis and LC-MS/MS analysis. LC samples were diluted by 50% with LCMS grade water prior to analysis. GC samples were solvent switched to hexane prior to analysis.

Method Detection Limit. A sample of lab-generated water (as described above) was used to determine the baseline signal for each analyte. Nine replicate samples were generated, and two were subsequently omitted, resulting in six degrees of freedom. The method detection limit (MDL) was calculated as described by the Environmental Protection Agency.¹⁴⁵ This method was used to determine the MDL using both the AutoTrace and IS2B preconcentration devices. Since the IS2B and AutoTrace each have six channels, the process was run twice: once with three spiked replicates and three unspiked controls, and once with six spiked replicates. A student's t-value (99% confidence interval) of 3.14 was used, and was multiplied by the standard deviation of 7 replicates. The calculated MDLs were checked against the following criteria:

MDL < spike level

Spike level < 10 x MDL

70 % < Absolute recovery < 130%

Signal-to-noise ratio < 10

Porewater filtration. One concern about sampling porewater *in situ* was that mobile particulates would clog the frits of SPE cartridges and inhibit flow. This concern was addressed by (1) visually inspecting the quality of the filtered porewater (Figure C-1), and (2) by measuring volumes of filtered porewater delivered to cartridges.

Sample preparation

IS2B. The IS2B was set to deliver 200 mL at 70 $\mu\text{L}/\text{min}$ and 140 $\mu\text{L}/\text{min}$ to 25 mg polystyrene divinylbenzene (SDB) cartridges. In both a lab and field test, 100 mg C18 cartridges were placed downstream of the SDB in order to ascertain whether any analyte mass passed through the initial SDB cartridges. The cartridges were eluted serially with 1 mL acetonitrile and 1 mL 1:1 hexane/acetone. The breakthrough cartridge eluates indicated no fiproles broke through the initial SDB cartridges.

In-lab water sample extractions. The AutoTrace 280 was equipped with 500 mg/3 mL SDB cartridges. The AutoTrace program is as follows:

1. Condition cartridge with 4.0 mL of acetonitrile into aqueous waste.
2. Condition cartridge with 2.0 mL Milli-Q water.
3. Load 200.0 mL of sample onto cartridge.
4. Rinse cartridge with 2.0 mL of Milli-Q water into aqueous waste.
5. Dry cartridge with nitrogen gas for 10.0 minutes.
6. Soak and collect 0.5 mL fraction using acetonitrile.
7. Collect 2.0 mL fraction into sample tube using acetonitrile.
8. Collect 2.0 mL fraction into sample tube using 1:1 hexane/acetone.

All eluates were solvent-switched to either 1:1 acetonitrile/water or 100% hexane for LC-MS/MS or GC-MS/MS analysis, respectively.

Instruments and analysis

TOC of sediment samples was analyzed using a Shimadzu TOC Solid Sample Module SSM-5000A (Shimadzu Scientific Instruments, Inc., Columbia, MD), while TOC of water samples was assessed using a Shimadzu TOC-5000 analyzer. Fipronil and the sulfide, sulfone, and amide degradates were quantified using liquid chromatography negative electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) with background signal subtraction. Fipronil-desulfinyl was quantified using gas chromatography tandem mass spectrometry (GC-MS/MS) with background signal subtraction. LC mass spectrometric analyses were performed using an API-4000 MS/MS (Applied Biosystems, Framingham, MA) coupled to a Shimadzu Prominence HPLC controlled by Analyst 1.5 software (Applied Biosystems, Framingham, MA). Separation was done using an Ultra IBD column (5 μm particle size, 2.1 \times 150 mm; Restek Corporation, Bellefonte, PA). The mobile phase consisted of 40% acetonitrile and 60% water flowing at a rate of 400 $\mu\text{L}/\text{min}$ with a total runtime of 12 min, with a gradient profile of 10% ACN/min starting at $t = 1.00$ min. Analytes were introduced into the mass spectrometer using an electrospray ionization probe operating in negative mode, and multiple reaction monitoring (MRM) was used for qualitative analysis. Optimized conditions for the ionization and fragmentation of the analytes are specified below. Quantitation was performed using a 5 point calibration curve in 1:1 acetonitrile/water. GC mass spectrometric analysis was performed using an Agilent 7890 gas chromatograph coupled to an Agilent 7000 triple quadrupole mass spectrometer (Agilent

Technologies, Santa Clara, CA) operating in positive mode, and MRM was used for qualitative analysis. Absolute recovery of all compounds was performed by using 4- or 5-point calibration curves and subtracting the concentration in the unspiked matrices from those of the spiked matrices. Equipment blanks using 18.2 M Ω (Milli-Q) water were run prior to all deployments, and grab sample controls included field blanks of Milli-Q water.

Table C-1. IS2B peristaltic pump calibration. In two replicate runs, each of six channels was calibrated to deliver 200 mL at 140 μ L/min. Individual channel volumes were measured by mass, assuming a fluid density of 1.0 g/mL.

Channel	Vol delivered (mL)	Abs error (mL)	%Error
Trial 1			
1	203.7	3.7	2%
2	190.8	-9.2	5%
3	202.8	2.8	1%
4	219.7	19.7	10%
5	195.6	-4.4	2%
6	180.8	-19.2	10%
Trial 2			
1	219.1	19.1	10%
2	187.1	-12.9	6%
3	216.5	16.5	8%
4	219.6	19.6	10%
5	192.9	-7.1	4%
6	211.0	11.0	6%
Avg	203.3	3.3	6%
StdDev	13.9		3%

Table C-2. IS2B absolute recoveries of fiproles from lab water (n = 8).

Chemical	Spike level (ng/L)	Recovery (%)	SD (%)
Fipronil	10	103	15
-sulfide	10	82	14
- sulfone	10	89	13
-amide	10	90	14
-desulfinyl	1	110	18

Matrix:

33 mg/L potassium citrate

300 ppm Kathon

Milli-Q water

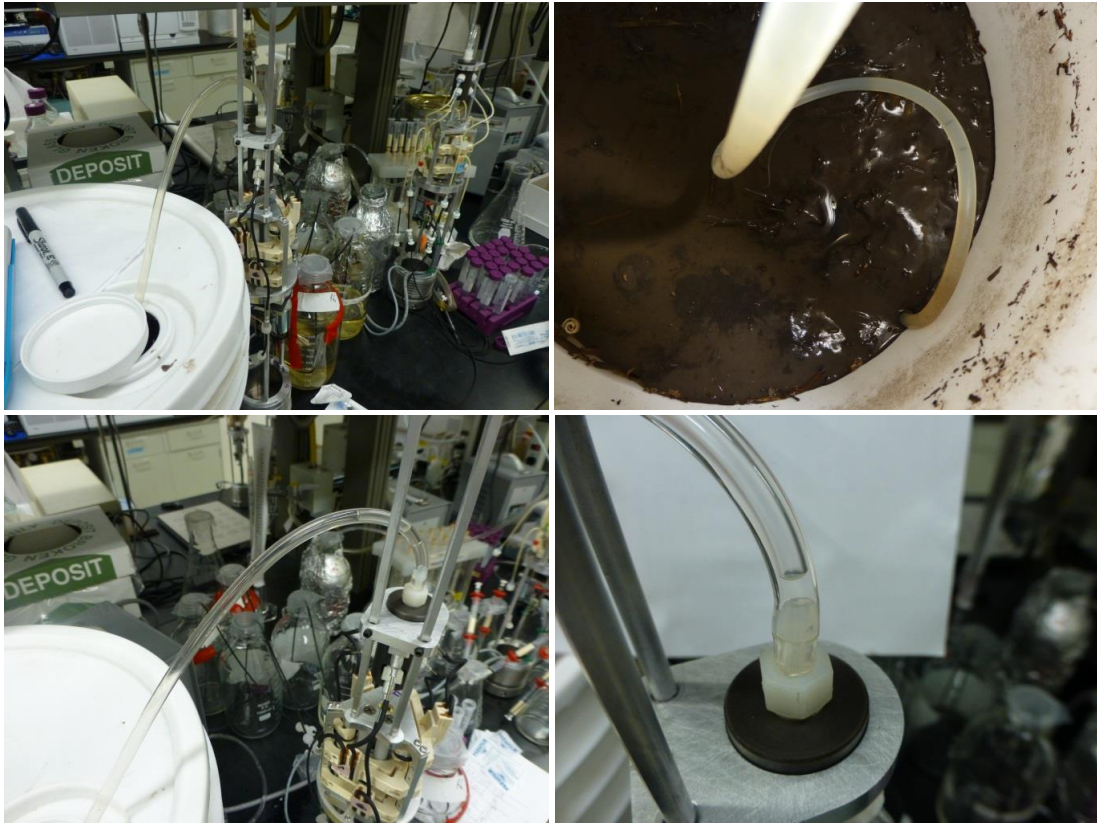


Figure C-1. The IS2B inlet immersed in high-OC sediment (> 30% OC), drawing water at 100 $\mu\text{L}/\text{min}$. The clear plastic tubing shown carries the filtered pore water. Plastic tubing shown is for demonstration purposes only. Actual inlet tubing is PTFE.

APPENDIX D

SUPPLEMENTAL MATERIAL FOR CHAPTER 6

Sampling campaign. Since the sampling campaign ran from 12 PM to 12 PM daily, the flows from the two days overlapping each sampling day were averaged. For example, the first day of sampling was Thursday 12 pm through Friday 12 pm, and the average flow data for Thursday and Friday represented the average flow for those respective days from 12 am to 12 am. Therefore, the daily flow rates on Thursday and Friday were averaged to ascertain the flow rate represented by the 12 pm through 12 pm sampling period. Figure D-1 shows the hourly division of sample volumes, collected in 20 mL increments, to generate a total daily composite with a volume of 2.5 L.

Analytical quality control. Method detection limits were determined by analyzing seven spiked surrogate matrix replicates and employing the USEPA's recommended analysis for determination of limits of detection. Solid and water aliquots were spiked with five native fiproles and 20 ng of labeled fipronil prior to extraction via SPE. Spike levels for each analyte were chosen to reflect a signal to noise ratio between 3:1 and 10:1, and the concentrations were estimated using a 6-point calibration curve. The standard deviation using 6 degrees of freedom was multiplied by the appropriate student's t_{99} value, providing an estimate of the lowest concentration detectable and identifiable with 99% confidence.

Since all samples of wastewater and archived sludge exhibited peaks reflective of the presence of fipronil, "clean" surrogate matrices were generated using peat moss and peat moss slurry. USEPA method 1694 recommends using this surrogate matrix as a proxy for the acidic fraction of biosolids for quality assurance in the absence of a true clean

reference matrix. Limits of detection in surrogate wastewater ranged from 46 to 773 pg/L, while for surrogate biosolids, they ranged from 19 to 242 pg/g (dry weight). Relative recovery of fipronil was $116 \pm 14\%$ in water, and $120 \pm 13\%$ in solids. Absolute recoveries of individual fiproles from water ranged from $60 \pm 14\%$ to $101 \pm 19\%$ (overall average recoveries for all fiproles was $78 \pm 20\%$), while absolute recoveries of individual fiproles from solids ranged from $48 \pm 18\%$ to $90 \pm 21\%$ (overall average recoveries for all fiproles was $73 \pm 28\%$).

All water and solids samples were spiked with 20 ng of labeled fipronil prior to extraction, and final fipronil concentrations were quantified using isotope dilution. Other fiprole concentrations were assessed using standard addition with either three or four calibration points generated from sample extracts spiked just prior to instrument analysis. Method development indicated that nearly all losses were due to matrix effects, and standard addition and isotope dilution proved to mitigate the quantitative effects of these losses. All samples were quantified by background subtraction of method blank controls.

Statistical analyses. In order to determine whether there was a change in wastewater stream mass loads from influent to effluent, the average daily mass loads in influent and effluent streams during the five day sampling campaign were compared using a two-tailed *t*-test, assuming equal variances. Ten data points for each stream were assessed for normal distribution, and the means were compared at a 95% confidence interval, using the Microsoft Excel 2010 Data Analysis Toolpak.

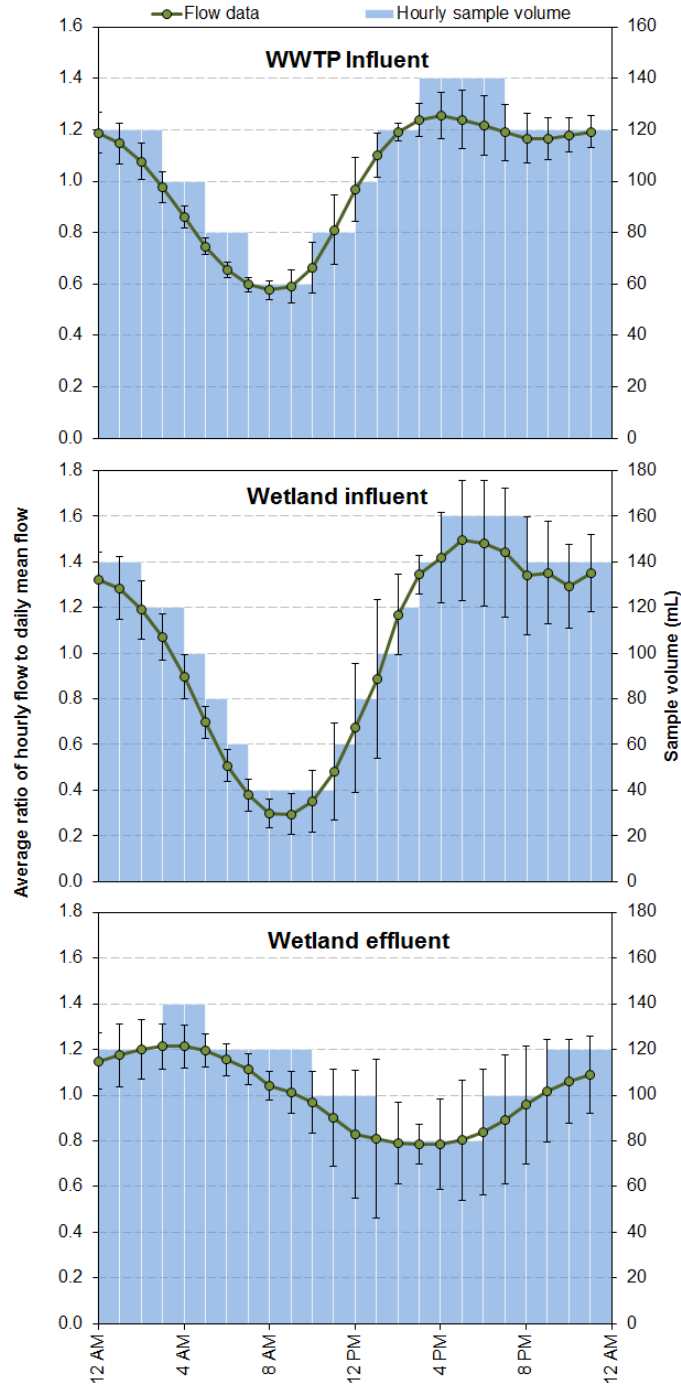


Figure D-1. Diurnal flow patterns obtained by 21 days of hourly flow data. Flow patterns were used to program automatic samplers for flow-weighted sampling; hourly increment volumes for a given hour are shown on the right-hand axis (hourly volumes were multiples of 20 mL increments). Error bars represent standard deviation ($n = 21$).

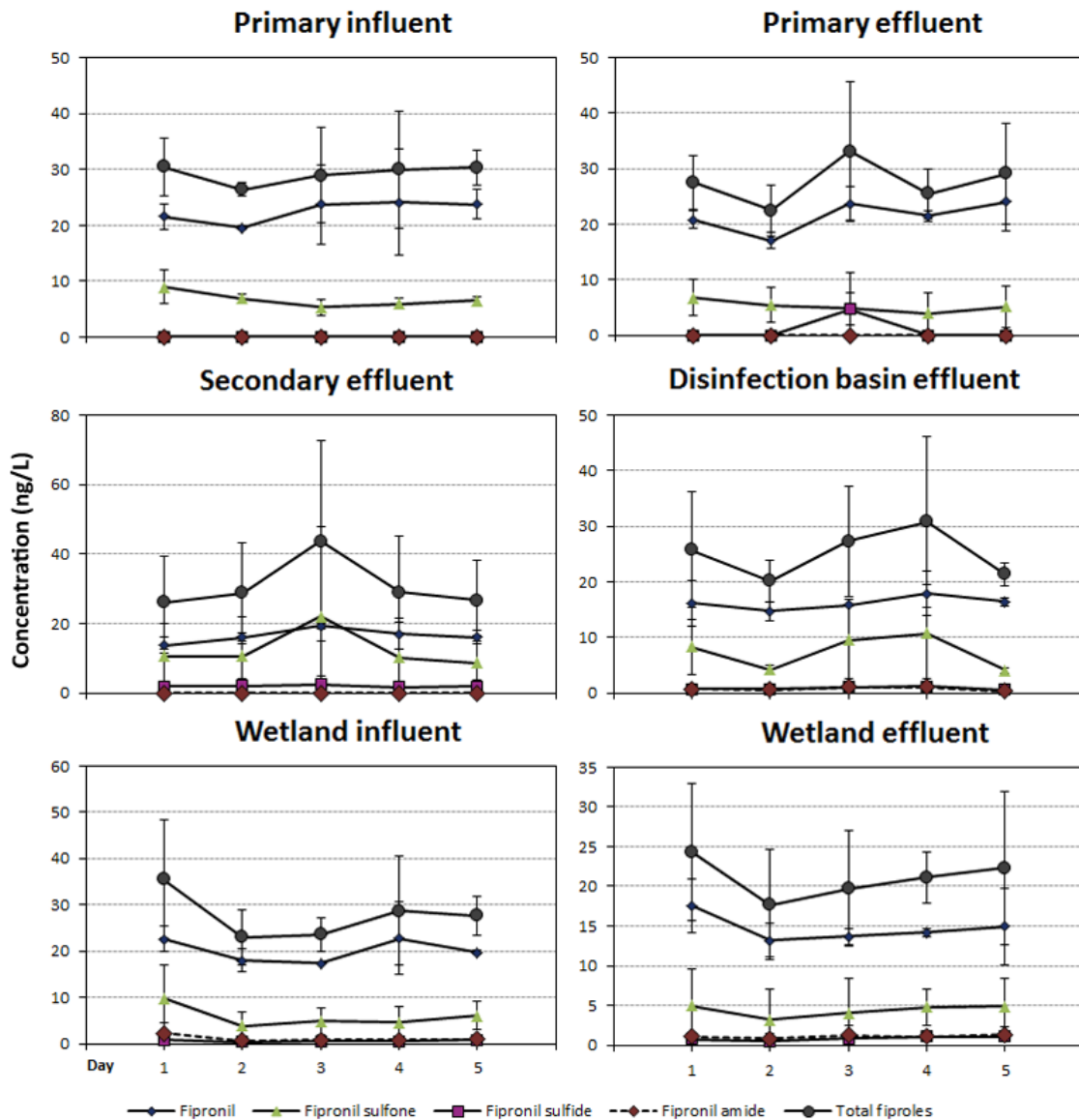


Figure D-2. Daily fiprole concentrations by stream. Error bars represent max/min measurements ($n = 2$ experimental replicates).