METHODS FOR PREDICTING ENVIRONMENTALLY-RELEVANT CONCENTRATIONS OF PULSED AQUATIC EXPOSURES

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METHODS FOR PREDICTING ENVIRONMENTALLY-RELEVANT CONCENTRATIONS OF PULSED AQUATIC EXPOSURES

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Abstract: Most current-use pesticides have short half-lives in the water column and thus the most relevant exposure scenarios for many aquatic organisms are pulsed exposures. However, it is frequently challenging to measure exposure due to rapid dissipation of contaminants from water and reduced bioavailability. Therefore, my objective was to evaluate methods for measuring environmentally-relevant exposures associated with pulsed events. The first study utilized a modeling approach to compare the effectiveness of discrete verses integrative sampling methods for predicting toxicologically-relevant pulsed concentrations. Differences between discrete point samples and integrative samples were highest at low sampling frequencies where discrete point samples required higher sampling frequencies to ensure median values > 50% and no sampling events reporting < 10% of the true 96-h time-wighted average concentration as compared to integrative methods. The second study implemented a biomonitoring approach using *Helisoma trivolvis* snail tissue residues to predict fungicide water concentrations. Although snails have high fungicide tolerance and tissue resides can be detected long after water concentrations drop below detection limits, passive elimination means that residues can only be used to assess whether exposure has occurred. Integrative sampling methods that continuously sample freely dissolved contaminants over time intervals (such as integrative passive samplers) have been demonstrated to be a promising measurement technique. Three different integrative passive sampler configurations were evaluated under different flow and pulsed exposure conditions for measurement of current-use pesticides (n=19), polyaromatic hydrocarbons (n=10), and personal care products (n=5). Results show that reducing membrane limitations allowed for rapid, integrative accumulation of analytes spanning a broad range of hydrophobicities (log *Kow* 1.5-7.6) even under pulsed conditions. The consequence of reducing membrane limitation was that sampling rates became flow dependent. As such, the last study evaluated various performance reference compounds (PRCs) as methods for *in-situ* flow corrections for analyte accumulation. Results suggested that multiple PRCs may be required to account for a wide range of flow dependencies. Additionally, although PRC corrections reduced the variability when in-situ conditions differed from laboratory calibrations (e.g. static verses moderate flow), applying PRC corrections under similar flow conditions increases variability in estimated values.

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CHAPTER I

INTRODUCTION

Surface waters may become contaminated through inputs from a variety of sources including application of agrochemicals, surface runoff, chemical spills, or discharge of industrial effluents (Allan et al., 2008; Ashauer and Brown, 2013). Toxicological responses are driven by the magnitude and frequency of exposure to the bioavailable fraction of contaminants (i.e. freely dissolved in water). Predicting the intensity and length of these episodic events is challenging because complex environmental factors influence exposure kinetics (Hickie et al., 1995; Landrum et al., 2013). As such, aquatic exposures are frequently pulsed, episodic, fluctuating, or intermittent rather than continuous concentrations (Ashauer and Brown, 2013; Reinert et al., 2002). This is also true for hydrophobic contaminants that are considered persistent in the environment yet have short aquatic half-lives and readily partition to sediments (Morrison et al., 2013; Yang et al., 2006) and other organic matter resulting in a pulsed exposure to aquatic organisms residing above the benthos (Landrum et al., 1984; Maul et al., 2008). Despite short exposure periods, pulsed exposures can elicit significant toxicological responses (Morrison et al., 2013; Reinert et al., 2002). Therefore, developing effective sampling technologies capable of measuring short-term exposures (eight days or less) is important for providing toxicologically-relevant exposure concentrations for contaminants that rapidly pulse through aquatic systems.

Traditionally, monitoring of aquatic systems entails discrete sampling of surface waters, which provides information on current contaminant levels; however, these discrete or “snapshot”

measurements may not be accurate indicators of exposure for compounds that pulse through the system (Stuer-Lauridsen, 2005). Additionally, each grab sample typically requires extensive extraction (potentially including cleanup steps), transport, and analysis of large volumes to achieve detection limits of trace contaminants (Stuer-Lauridsen, 2005; Vrana et al., 2005). Differences in apparent freely dissolved, bioavailable fractions, of contaminants can also be observed based on whether or not the samples are pre-treated (i.e. filtered versus whole water samples) and can be dependent on the type of dissolved organic carbon (Landrum et al., 2013). Thus, high frequency water sampling is usually resource prohibitive and the results may be hard to interpret. By accounting for intra- and inter- day contaminant fluctuations, time-weighted average (TWA) measurements may provide better estimates of exposure concentrations as compared to discrete sampling of surface waters. Discrete sampling can provide estimates of TWA concentrations; however, for contaminants that rapidly dissipate or pulse through the water column, extensive spot or automated sampling is required (Alvarez et al., 2004; Alvarez et al., 2005; Morrison et al., 2013; Vrana et al., 2014).

Biomonitoring is an alternative method to grab sampling where body residue concentrations are measured in biological samplers (i.e. organisms) collected from field sites to predict environmental exposure. Organism body burdens are frequently used in standardized sediment quality tests (OECD, 2010; USEPA, 2000) and biomonitoring (Coogan and La Point, 2008; Druart et al., 2011; Goldberg, 1986; Sabaliunas et al., 1998; Smalling et al., 2015) especially for contaminants known to persist and bioaccumulate in the environment. Although synthetic passive samplers have recently had the advantage over measuring chemical residues in organisms due to cleaner sample matrices, the development of better analytical extraction and cleanup protocols and increased instrumentation sensitivity has greatly enhanced our ability to measure tissue concentrations (Anastassiades et al., 2003). Previous work has demonstrated that tissue concentrations can be analyzed in mussels (Liscio et al., 2009; Sabaliunas et al., 1998) and snails (Coogan and La Point, 2008; Druart et al., 2011) to indicate environmental pollution. Mussels receive greater attention in biomonitoring programs (as compared to snails) because they actively filter water, resulting higher

contaminant accumulation rates. However, their use is limited to coastal waters and streams due to absence of indigenous populations in ephemeral water bodies (e.g. depressional wetlands). Snails may provide a reasonable alternative in these systems.

Deployment of passive sampling devices is also becoming a viable alternative to discrete sampling due to its ability to measure bioavailable water fractions over time (Alvarez et al., 2004; Harman et al., 2012; Stuer-Lauridsen, 2005). Current integrative passive samplers such as polar organic chemical integrative samplers (POCIS) continuously accumulate freely dissolved contaminants throughout their deployment and have been demonstrated to be a reliable technique for providing time-weighted average (TWA) concentrations of some organic contaminants (Alvarez et al., 2004; Vrana et al., 2005). Assuming an infinite sink, two processes primarily regulate uptake of an integrative sampler: 1) Diffusion through the aqueous boundary layer (ABL) and 2) Permeation through the sampler membrane. The thickness of the ABL changes with flow across the sampler thus sampling rates can be highly influenced by variations in flow (Harman et al., 2012; Vrana et al., 2005). Ultimately, whichever process has the slowest kinetics determines the sampling rate (Seethapathy et al., 2008).

The classical POCIS configuration utilizes microporous polyethersulfone (PES) membranes (0.1 μm pore size) which Alvarez et al. (Alvarez et al., 2004) selected over several other membranes based on high sampling rates of polar substances, minimal biofouling, and durability. This membrane has helped establish POCIS as a valuable tool for providing TWA concentrations of polar contaminants; however, limitations of diffusion limiting membrane cause inherent problems for accumulation of hydrophobic contaminants. First, the properties of the PES membranes generally restricts accumulation to hydrophilic chemicals with octanol-water partitioning coefficients (log *Kow*) less than four due to low accumulation coefficients for more hydrophobic substances (i.e. log *Kow* > 4) (Ahrens et al., 2015; Alvarez et al., 2004; Belles et al., 2014a; Harman et al., 2008). Although some hydrophobic chemicals accumulate in POCIS, the diffusion limiting membrane generally causes a lag-effect in the initial accumulation kinetics (Belles et al., 2014a). This is particularly problematic

for hydrophobic chemicals because they have the greatest potential to pulse through the water column and partition to sediment and biota. As such, the classical POCIS configuration may under predict actual exposure concentrations for more hydrophobic contaminants due to limited or delayed accumulation. Second, POCIS are typically deployed for 30 (or more) days and have long integration times with linear accumulation of many polar contaminants greater than 28 d (Alvarez et al., 2004; Belden et al., 2015). Although this is beneficial for polar contaminants that tend to stay in the water column, the opposite is true for contaminants that are acutely toxic and quickly pulse through aquatic systems. For these compounds, 4-8 d TWA concentrations would likely be more comparable to toxicological endpoints. Collectively, the lag-effect phenomenon and long deployment times present major challenges for predicting water concentrations of hydrophobic substances using the classical POCIS configuration.

One of the challenges for utilizing POCIS-style samplers is that changes in flow across sampler membranes can dramatically effect analyte sampling rates (Birch et al., 2013; Booij et al., 1998; Charlestra et al., 2012; Harman et al., 2012; Li et al., 2010a; Li et al., 2010b; Vermeirssen et al., 2008; Vermeirssen et al., 2009; Vrana et al., 2005). Performance reference compounds (PRCs) have been suggested as a viable method to improve calibration for POCIS-style samplers by providing in-situ flow calibrations has been (Belles et al., 2014a; Belles et al., 2014b; Harman et al., 2012; Mazzella et al., 2010). Equilibrium-based passive samplers have benefitted from PRCs as stable isotope compounds can be spiked into samplers prior to deployment and dissipation occurs proportional with uptake (Huckins et al., 2002). However, the integrative nature of POCIS-style samplers presents a challenge for PRCs due to minimal fugacity out of stable isotope compounds (Harman et al., 2012; Mazzella et al., 2010). Regardless, several PRCs have been suggested to have sufficient fugacity out of classical POCIS devices including desisopropyl atrazine-d_5 (Belles et al., 2014a; Belles et al., 2014b; Mazzella et al., 2007; Mazzella et al., 2010) and caffeine-C_{13} (Belden et al., 2015; Belles et al., 2014a). These PRCs are especially important for adjusting *Rs* when analyte

accumulation controlled by the aqueous boundary layer rather than diffusion through the membrane (Belles et al., 2014a; Belles et al., 2014b).

The following research chapters (Chapters II, III, IV, and V) describe my research exploring methods for measuring pulsed aquatic exposures in the environment and some of the challenges faced with implementing different sampling methodologies. The overall research question arose from my Master's Thesis where our results suggest that despite short persistence in the water column, environmentally-relevant pulsed exposures can still elicit significant toxicity (Morrison et al., 2013). Regardless, interpretation of environmental fate and toxicological responses demonstrated that changes in water concentration over time described toxicity and TWA concentrations of measured water concentrations produced the similar LC50 (median lethal concentration) values as water-only exposures. As such, my PhD research has focused on methods to accurately quantify TWA water concentrations of pulsed exposures to facilitate measurement of toxicologically-relevant exposure concentrations.

The first research chapter (Chapter II) lays the ground work by comparing the effectiveness of discrete point sampling and integrative sampling methods predicting the peak 96-h TWA water concentrations while varying sampling frequencies and a range of contaminant water half-lives (t_{50} = 0.5, 2, and 8 d). These comparisons were accomplished through simulations (coded in R 3.2.2) which assumed sampling frequencies and timing for both discrete and integrative samples based on a study designs that would likely be used to monitor and predict peak exposure concentrations.

Investigations detailed in Chapter III took a biomonitoring approach for measuring pulsed exposures using *Helisoma trivolvis* pond snails. This snail species is a pulmonate snail species ubiquitously found throughout North America, inhabiting a variety of moderately eutrophic, permanent, and ephemeral freshwaters (Russell-Hunter et al., 1984). Due to their ubiquitous presence and abundance in areas of interest throughout the typical growing season, snails are good candidate organisms for biomonitoring of shallow, ephemeral water bodies (e.g. embedded cropland wetlands). This chapter focuses on evaluating the effectiveness of snails for biomonitoring of current-use

fungicides (i.e. pyraclostrobin and metconazole) by describing chemical sensitivities (i.e. acute toxicological responses) and toxicokinetic modeling (i.e. accumulation and elimination).

Finally, Chapters IV and V evaluate the use of integrative passive samplers for measuring pulsed aquatic exposures. Previous research has suggested that adjustment of sampler design to reduce diffusion membrane limitations is possible (Belles et al., 2014a). Specifically, replacing the microporous-PES membrane with nylon mesh screens with 30 µm openings allows greater flow through the sampler and reduced diffusion membrane limitations (Belles et al., 2014a). Belles et al. (Belles et al., 2014a) called this sampler configuration the "Nylon POCIS"; however we will refer to this design as the Nylon Organic Chemical Integrative Sampler (NOCIS) to emphasize the obvious advantage of this modification for increased sampling rates for hydrophobic contaminants. As opposed to the classical POCIS configuration, nylon configuration could be used for integrative measurement of hydrophobic contaminants pulsing through the water column; ultimately expanding the utility of the sampler by providing toxicologically relevant exposure data and better risk assessments for more hydrophobic substances. Results in Chapter IV describe the calibration of two NOCIS configurations in addition to the newly developed Sentinel Sampler (ABSMaterials, Inc., Wooster, OH). The objective was to confirm linear accumulation and integration of analytes during short-term exposures (i.e. < 8 d). Chapter V expands from this research to evaluate in-situ methods for estimating flow across sampler membranes. This research is critical for environmental applications to account for variations in flow that could occur when samplers are deployed in the field.

Author Contributions

The following chapters were written in manuscript format for publication purposes. Several chapters have been submitted and may appear below with contents "As accepted" or "As submitted" based on the manuscript status when this dissertation was submitted to the graduate college. I am the first author on these manuscripts as I designed and conducted the experiments, analyzed the results, and wrote the major extent of the text.

Dr. Jason Belden is co-authored on all manuscripts. As my major advisor, Dr. Belden was paramount for developing research questions and above all, obtaining external funding the helped provide supplies for this research. Dr. Barney Luttbeg is co-authored on Chapter II as our modeling expert. Without his expertise, we could not have brought this idea to fruition.

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CHAPTER II

COMPARISONS OF DISCRETE AND INTEGRATIVE SAMPLING ACCURACY IN ESTIMATING PULSED AQUATIC EXPOSURES

The following chapter appears as submitted to *Environmental Pollution*:

Morrison, S.A., B. Luttbeg, and J.B. Belden (2016). Comparisons of discrete and integrative sampling accuracy in estimating pulsed aquatic exposures. *Environ Pollut* (Submitted).

Abstract

Most current-use pesticides have short half-lives in the water column and thus the most relevant exposure scenarios for many aquatic organisms are pulsed exposures. Quantifying exposure using discrete water samples may not be accurate as few studies are able to sample frequently enough to accurately determine time-weighted average (TWA) concentrations. Integrative sampling methods that continuously sample freely dissolved contaminants over time intervals (such as integrative passive samplers) have been demonstrated to be a promising measurement technique. We conducted several modeling scenarios to test the assumption that integrative methods may require many less samples for accurate estimation of peak 96-h TWA. We compared the accuracies of discrete point samples and integrative samples while varying sampling frequencies and a range of contaminant water half-lives (t_{50} = 0.5, 2, and 8 d). Simulated results suggest that regardless of sampling methodology, increased sample frequency resulted in better estimates of the peak 96-h TWA. Differences between discrete point samples and integrative samples were

greatest at low sampling frequencies where discrete point samples required higher sampling frequencies to ensure median values > 50% and no sampling events reporting < 10% of the true 96-h TWA as compared to integrative methods. Additionally, integrative samples reduced the frequency of extreme values < 10% of the true value and never exceeded the actual peak 96-h TWA. Overall, integrative methods are the more accurate method for monitoring contaminants with short water half-lives due to reduced frequency of extreme values, especially with uncertainties around the timing of pulsed events.

Key Words: Environmental Monitoring, Integrative Sampling, Pulsed Exposure, Spot Sampling

Capsule

Integrative methods provided better predictions of the true 96-h TWA concentration when water dissipation was rapid, especially with uncertainties around the timing of pulsed events.

1. Introduction

Surface waters may become contaminated through inputs from a variety of sources including application of agrochemicals, surface runoff, chemical spills, or discharge of industrial and municipal effluents (Allan et al., 2008; Ashauer and Brown, 2013). The reality of monitoring surface waters is that contamination frequently occurs through pulsed, episodic, fluctuating, or intermittent exposures rather than continuous concentrations (Ashauer and Brown, 2013; Reinert et al., 2002). Predicting the intensity and length of these episodic events is challenging because complex environmental factors influence exposure kinetics (Hickie et al., 1995; Landrum et al., 2013). Aquatic risk assessments typically entail discrete sampling of surface waters, which provides information on current contaminant levels; however, these discrete or "snapshot" measurements may not be accurate indicators of exposure under fluctuating water concentrations (Soderstrom et al., 2009; Stuer-Lauridsen, 2005). Exposure assessments generally relate predicted environmental exposure concentrations to biological effects obtained from laboratory experiments that are conducted with relatively stable water concentrations; however,

environmentally-relevant exposures rarely occur under stable contaminant regimes (Boxall et al., 2002).

By accounting for intra- and inter- day contaminant fluctuations, time-weighted average (TWA) measurements may provide better estimates of exposure concentrations as compared to discrete sampling of surface waters. Discrete sampling can provide estimates of TWA concentrations; however, for contaminants that rapidly dissipate or pulse through the water column, extensive spot or automated sampling is required (Alvarez et al., 2004; Alvarez et al., 2005; Morrison et al., 2013; Soderstrom et al., 2009; Vrana et al., 2014). Integrative passive sampling devices are becoming valuable alternatives to discrete sampling by providing TWA measurements of bioavailable water fractions (Alvarez et al., 2004; Harman et al., 2012; Stuer-Lauridsen, 2005). Integrative passive sampling devices like the Polar Organic Chemical Integrative Sampler (POCIS) are promising tools for providing estimates of TWA concentrations because they continuously accumulate freely dissolved contaminants throughout their deployment (Allan et al., 2008; Alvarez et al., 2004). We will not delve into a lengthy review of passive sampling technologies because several published reviews already exist covering numerous sampler configurations, applications, and principles (Harman et al., 2012; Miege et al., 2015; Mills et al., 2014; Namiesnik et al., 2005; Seethapathy et al., 2008; Stuer-Lauridsen, 2005; Vrana et al., 2005).

It is generally assumed that TWA measurements provide better monitoring data; however, discrete point samples may provide suitable measurements under relatively stable contaminant concentrations. Moreover, long-term TWA measurements (i.e. 14-28 days), which are frequently collected with POCIS, may have the unintended consequence of diluting out shorter pulses (i.e. 12-96 hours) that could be of toxicological significance. Although integrative passive sampling devices have been extensively used in field monitoring scenarios for TWA water concentrations, limited investigations have focused on quantitatively evaluating the performance of discrete point samples as compared to integrative samples for measuring pulsed

aquatic exposures. The overall objective of this research was to investigate the impact of sampling methods (e.g. discrete verses integrative) and sample frequency on the accuracy and precision of measured concentrations obtained during simulated pulsed exposures. A modeling exercise was conducted to provide quantitative comparisons between discrete point samples and integrative samples. Varying water dissipation rates and sampling frequencies were programed to evaluate the prediction accuracy of three sampling methods compared to the actual peak 96-h TWA.

2. Methods

2.1 Exposure scenario description

We made several assumptions pertaining to physiochemical properties, toxicity, sample preparation, and analysis. The environmental impact of the contaminants was considered to occur through acute toxicity with the primary exposure route via water. The peak 96-h TWA concentration was chosen as the best metric of exposure as it closely relates to toxicological values obtained from standard toxicity tests that frequently report the 96-h LC50 (median lethal concentration) (USEPA, 2000). Thus, the sampling method that accurately and consistently estimated this value would be considered the best method for exposure assessments. We assumed that technology was available to collect both discrete (i.e. grab) and integrative (i.e. TWA) samples. Sample processing and analysis was considered as the limiting step in collecting the environmental data. Thus, the number of analytical measurements was compared across sampling methods and frequencies. All technologies were considered to have similar analytical accuracies following the collection event. Other considerations (potentially two trips to deploy an integrative sampler versus a single trip for a grab sample) were not considered.

We assumed that a contamination pulse would start at a random point within a 10-d time period and thus the peak 96-h TWA would be finished by 14 d, which is the time frame during which monitoring would occur (Fig. 1). The pulsed water concentrations were created using a first order elimination model so that

$$C_t = C_0 \exp(-k_e t) \quad (1)$$

where C_t is the water concentration at time (t), C_0 is the peak concentration of the pulse, and k_e is dissipation rate from water. Three water dissipation rates were chosen to be representative of a range of contaminants. The three dissipation rates increased by factors of four and corresponded to water half-lives (t_{50}) of 0.5, 2, and 8 d. Therefore, the exact value varied in accordance with the water dissipation rate within the given scenario.

2.2 Sampling design

For the simulations (coded in R 3.2.2) we divided the 14 d window into one-minute intervals. The starting point of the contamination was randomly drawn from a uniform distribution ranging from 1 to 14,400 minutes (the end of day 10). Initial concentration of the contaminant was 100 and exponentially decayed each minute. The actual peak 96-h TWA was calculated by taking the average of the concentration over the 5760-minute interval starting at the initial release of the contaminant. The actual peak 96-h TWA concentrations for 0.5, 2, and 8 d water half-lives (t_{50}) were 18.0, 54.1, and 84.5 µg/l, respectively (Table 1).

During each pulsed scenario, three types of sampling methods were considered (i.e. one discrete point sample and two integrative) as well as four different sampling frequencies (1, 2, 3, or 7 data points collected; Fig. 1). Sampling frequencies and timing for both discrete and integrative samples were based on a study designs that would likely be used to monitor and predict peak exposure concentrations. For the discrete sampling and single sample case, the sampling was done at the end of day 10 to assure that sampling occurred after contamination occurred. For the two-sample scenario, discrete sampling occurred at the end of days 5 and 10. For the three-sample scenario, discrete sampling occurred at the end of days 4, 8, and 12. And for the seven-sample scenario, discrete sampling occurred at the end of days 2, 4, 6, 8, 10, and 12. For each sample, the concentration measured was taken from the actual concentration of the contaminant at that time point with no error.

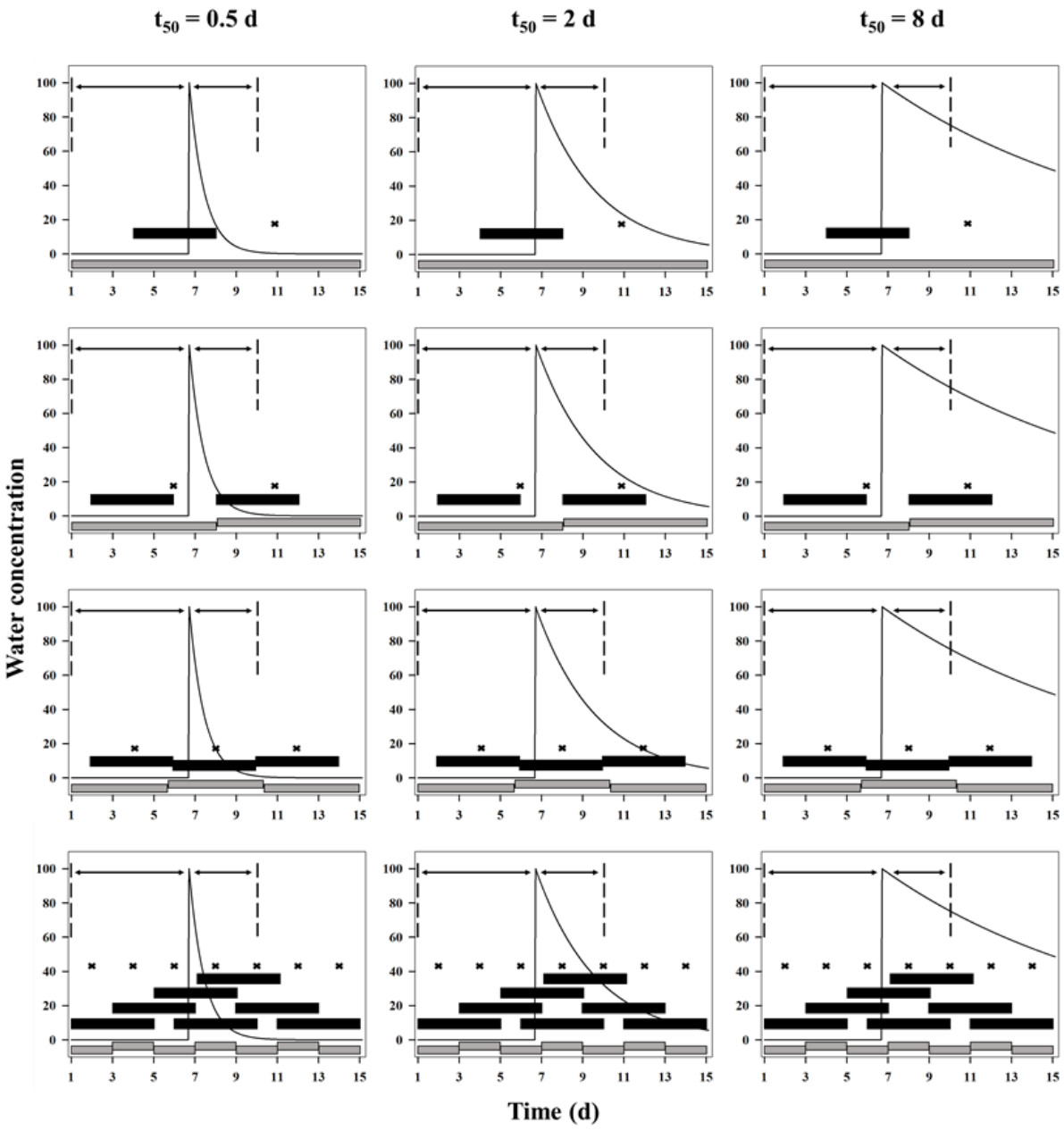

Fig. 1. Graphical representation showing placement of discrete point samples (X), fixed duration integrative samples (black), and full integrative samples (grey). Water half-lives increase from left two right and sampling frequencies increase from top to bottom. Initiation of the pulse was allowed to occur within the first 10-d of exposure (vertical dashed line). Numbers depicting time represent the beginning of the indicated day.

The first integrative method, defined as full integrative sampling (FIS), sampled the entire 14 d window with no overlap. Therefore, for scenarios with more than one sample, consecutive sampling periods evenly divided the 14 d interval with no overlap (Fig. 1). The measured concentration was the average real concentration across the range of the measurement with no error.

The second integrative method, defined as fixed duration integrative sampling (DIS), consisted of 96-h periods. These windows were arranged within the 14 d exposure window to provide the best coverage based on the number of measurements within the sampling regime (Fig. 1). This resulted in overlapping samples when seven samples were taken. For the one-sample scenario, the DIS measurement was taken from the beginning of day 4 through the end of day 7. The two-sample scenario measured from beginning of day 3 through the end of day 6 and day 8 through day 11. The three-sample scenario measured from beginning of day 2 through the end of day 5, day 6 through 9, and day 11 through 14. The seven-sample scenario had samples evenly distributed over the entire 14-d exposure (Fig. 1). In each case, the measured concentration was the average real concentration across the range of the measurement with no error.

2.3 Estimating the actual 96-h TWA

When sampling regimes consisted of one, two, or three measurements, the highest singular value obtained for each sampling method was used as the 96-h TWA estimate. Increased sampling regimes to seven measurements resulted in the frequency of discrete point samples and FIS measurements exceeding 96-h intervals; therefore, the highest two values were averaged. The seven sample regimes also resulted in overlapping intervals for DIS measurements (i.e. 96-h integration). The highest single DIS measurement was compared against the actual peak 96-h TWA.

2.4 Data analysis

To visualize differences resulting from changes in water half-lives and sampling frequencies, estimate values from modeled scenarios (n=1,000 replicates) were provided as box

and whisker plots using SigmaPlot 10.0 (Systat Software, CA, USA) with median (solid horizontal line), mean (dotted horizontal line), inner quartile range (25^{th} and 75^{th} percentiles; box), the 10^{th} and 90^{th} percentiles (whiskers), and outliers (dots). Environmental data sets with few high values and many low values are typically positively skewed which can influence the mean and standard deviation; however, median values are largely unaffected by outliers (Helsel, 2005). Due to the high numbers of non-detect values at lower sampling frequencies (Table 1), median values are the most appropriate summary statistic to evaluate sampling methodologies (Helsel, 2005).

Table 1. Median estimates from model runs (n=1,000) for all sampling methodologies and frequencies with quantitative comparisons to the actual peak 96-h time-weighted average (TWA). DIS = Fixed Duration Integrative Samples; FIS = Full Integrative Samples.

Sample Frequency	Actual Peak 96-h TWA	Discrete Point Samples		DIS		FIS	
		Median	Percent of 96-h TWA (%)	Median	Percent of 96-h TWA (%)	Median	Percent of 96-h TWA (%)
Water half-life: t_{50} = 0.5 d							
n=1	17.96	0	0	3.5	19.5	5.1	28.4
n=2	17.96	2.9	16.1	16	89.1	10	55.7
n=3	17.96	3.7	20.6	16	89.1	15	83.5
n=7	17.96	13	72.4	17.8	99.1	17.8	99.1
Water half-life: t_{50} = 2 d							
n=1	54.10	0	0	18	33.3	15	27.7
n=2	54.10	42	77.6	38	70.2	31	57.3
n=3	54.10	44	81.3	41	75.8	40	73.9
n=7	54.10	53	98.0	49	90.6	49	90.6
Water half-life: t_{50} = 8 d							
n=1	84.51	0	0	32	37.9	24	28.4
n=2	84.51	81	95.8	57	67.4	48	56.8
n=3	84.51	81	95.8	63	74.5	60	71.0
n=7	84.51	84	99.4	75	88.7	74	87.6

3. Results

Predicted TWA median, mean, and concentration distributions varied substantially between sampling methods and frequencies (Fig. 2). Predictably, estimated TWA concentrations became more accurate and had less variability with higher sampling frequencies and longer aquatic half-lives.

3.1 Discrete point samples

Short water half-lives (t_{50} = 0.5 d) resulted in higher chances of underestimating the peak 96-h TWA as compared to longer half-lives, especially at sampling frequencies less than seven (Fig. 2). Additionally, discrete point samples were the only sampling methodology to yield values greater than the peak 96-h TWA (Fig. 2, Table 2).

Discrete point samples had the greatest variance; however, this variation was substantially decreased as both sampling frequency and aquatic half-lives (t_{50}) increased (Fig. 2). Across all water half-lives, decreasing the sampling frequency to one sample resulted in median predictions of zero (Table 1). Moreover, scenarios with only one collected sample produced the most results (> 59%) that were less than 10% of the peak 96-h TWA, which suggests a strong chance of receiving a false negative when predicting the presence of a toxicologically significant exposure event (Table 2). Conversely, sample frequencies of seven provided much better predictions (median values > 72%) of the true value (Table 1) and always yielded results > 10% of the peak 96-h TWA (Table 2). If threshold values of acceptance were set for median values > 50% and no sampling events reporting < 10%, then required sampling frequencies for each aquatic half-life (i.e. 0.5, 2, and 8 d) would be 7, 3, and 3, respectively.

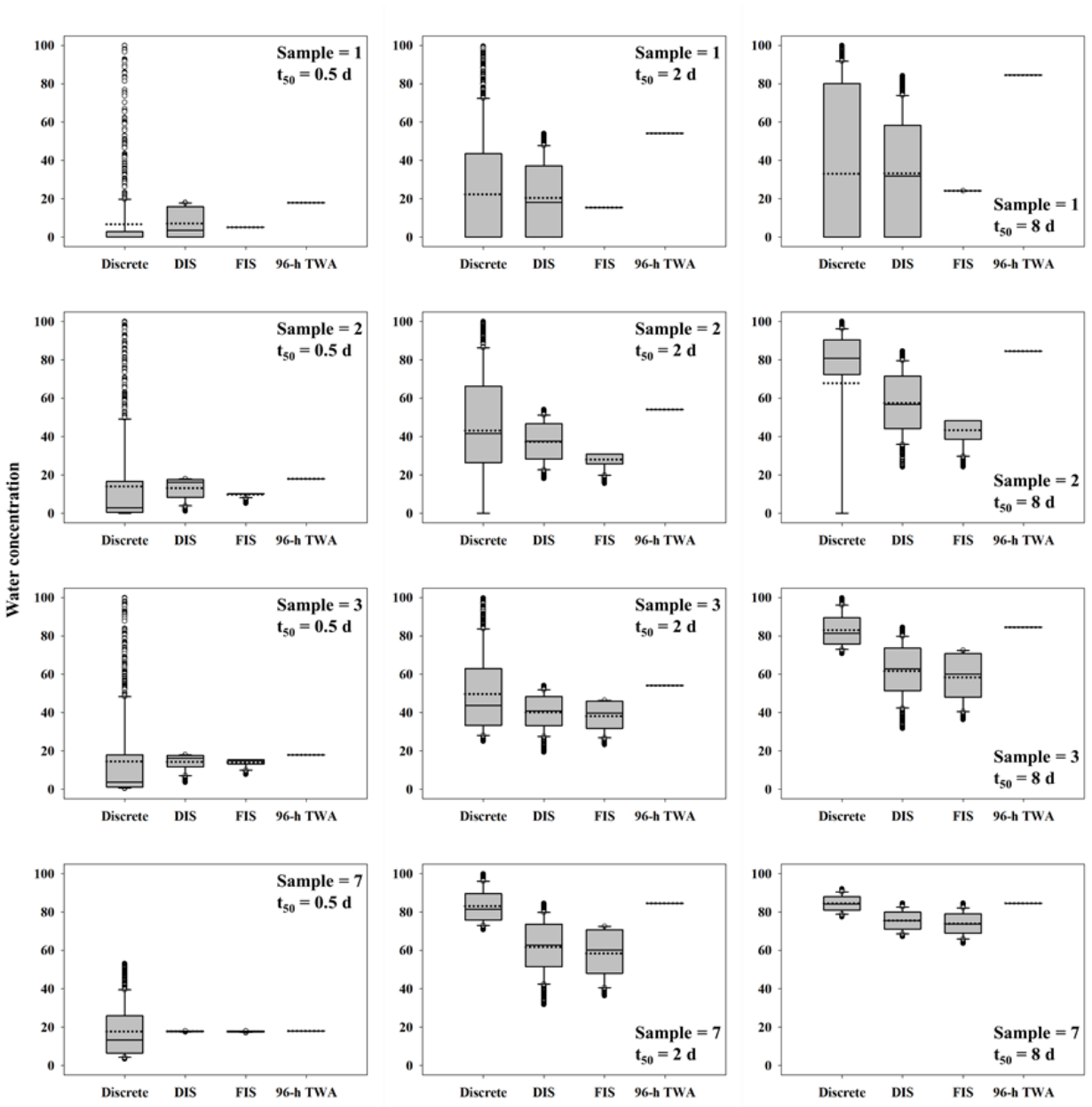

Fig. 2. Summary statistics resulting from modeling scenarios (n = 1,000) comparing discrete point samples (Discrete), fixed duration integrative samples (DIS), and full integrative samples (FIS) with varying sampling frequencies. Model estimates provided as box and whisker plots with median (solid horizontal line), mean (dotted horizontal line), inner quartile range (25^{th} and 75^{th} percentiles; box), the 10^{th} and 90^{th} percentiles (whiskers), and outliers (dots). Graphs are arranged to mirror the graphical representation of modeled scenarios as depicted in Figure 1 with water half-lives of t_{50} = 0.5 d (left column), t_{50} = 2 d (middle column), and t_{50} = 8 d (right column). Sample frequency increases from top to bottom.

Table 2. Evaluation of measurement distributions all replicate model runs (n=1,000). The sample frequency (expressed as percentage of values) are given for the total values below 10% of the peak 96-h time-weighted average (TWA), total greater than the peak, and the total of non-detect values for each sampling methodology and frequency. DIS = Fixed Duration Integrative Samples; FIS = Full Integrative Samples.

	Percentage of non-detect (i.e. zero) values (%)			Percentage of values <10% peak 96-h TWA (%)			Percentage of values greater than peak 96-h TWA (%)		
Sample Frequency	Discrete Point Sample	DIS	FIS	Discrete Point Sample	DIS	FIS	Discrete Point Sample	DIS	FIS
	Water half-life: t_{50} = 0.5 d								
n=1	60.2	30.2	0	71.3	45.0	0.0	10.8	0	0
n=2	20.3	0	0	42.4	3.8	0	23.8	0	0
n=3	0	0	0	33.8	0	0	25.0	0	0
n=7	0	0	0	0	0	0	38.0	0	0
	Water half-life: t_{50} = 2 d								
n=1	59.3	30.8	0	59.3	32.9	0	18.1	0	0
n=2	21.3	0	0	21.3	0	0	35.2	0	0
n=3	0	0	0	0	0	0	33.5	0	0
n=7	0	0	0	0	0	0	46.9	0	0
	Water half-life: t_{50} = 8 d								
n=1	60.9	29.1	0	60.9	32.7	0	19.1	0	0
n=2	20.1	0	0	20.1	0	0	39.6	0	0
n=3	0	0	0	0	0	0	39.3	0	0
n=7	0	0	0	0	0	0	47.4	0	0

3.2 Fully integrative samples (FIS)

Across all water half-lives investigated, variation in model estimates for FIS methods decreased as sampling frequency increased (Fig. 2). Moreover, decreasing the sampling frequency to one sample resulted in the lowest median predictions (28%) of the peak 96-h TWA (Table 1). Because FIS methods covered the entire 14-d exposure window, FIS methods always yielded values greater than 10% of the peak 96-h TWA for all sampling frequencies, even under short aquatic half-lives and reduced sample frequencies (Table 2). Sampling frequencies of seven always resulted in median values of greater than 87% of the peak 96-h TWA (Table 1). If threshold values of acceptance were set for median values > 50% and no sampling events

reporting < 10%, then required FIS sampling frequencies for each environmental half-life (0.5, 2, and 8 d) would be 2, 2, and 2, respectively.

3.3 Fixed duration integrative samples (DIS)

Similar to FIS methods, variation in model estimates for DIS methods decreased as sampling frequency increased, across all water half-lives (Fig. 2). Across all aquatic half-lives, decreasing sampling frequency to one sample resulted in the lowest prediction accuracy (i.e. < 40%) of the peak 96-h TWA (Table 1). Regardless of aquatic half-life, sample frequency of one resulted in the greatest number of non-detect values (30%) and values registering < 10% of the peak 96-h TWA (> 32%; Table 2). Sampling frequencies containing seven samples provided better estimates (> 88%) of the true value (Table 1) and always yielded results > 10% of the peak 96-h TWA (Table 2). Moreover, as sampling frequency decreased, estimates obtained from DIS methods produced slightly better predictions of the peak 96-h TWA than FIS methods across all water dissipation rates (Table 1). Regardless, similar sample frequencies would be required to achieve acceptable median values of > 50% and no sampling events reporting < 10%, where DIS sampling frequencies for each environmental half-life (0.5, 2, and 8 d) would be 2, 2, and 2, respectively.

4. Discussion

Maximum contaminant exposure concentrations occur immediately following release into aquatic systems (Ashauer and Brown, 2013; Reinert et al., 2002). Obtaining toxicologically-relevant measurements is difficult because aquatic exposures are dynamic with rapidly fluctuating concentrations and uncertainties regarding timing of contamination events (Ashauer and Brown, 2013; Boxall et al., 2002; Reinert et al., 2002). Current monitoring practices employ discrete point sampling, integrative sampling, or a combination of the two methods (Alvarez et al., 2005).

Although discrete point sampling is a common monitoring practice, we found it produced the highest variability in estimates. Specifically, discrete point samples had the highest frequency of non-detect measurements (i.e. zero), the highest frequency of estimates < 10% of the peak 96-h

TWA, and the highest frequency of values greater than peak 96-h TWA (Fig. 2, Table 2). This wide variation in exposure estimates should be expected given that modeled scenarios consisted of a single pulsed exposure and realistic sampling regimes. Consequently, discrete point samples have the highest probability of completely missing a pulse while simultaneously being the most likely to provide an estimate exceeding the peak 96-h TWA. As compared to integrative methods that only required two samples, discrete point estimates required greater sampling frequencies (7, 3, 3 samples) to ensure median values > 50% and no sampling events reporting < 10% (Table 1 and Table 2). Although these trends apply across all aquatic half-lives investigated (i.e. 0.5, 2, and 8 d), differences between discrete point samples and integrative methods were most evident under short aquatic half-lives.

Alvarez et al. (2005) investigated streams receiving agricultural, municipal, and industrial wastewaters and compared contaminant detection frequency for integrative (i.e. POCIS) and discrete methods (i.e. standard water-column sampling) and reported 32 analytes accumulated in POCIS; however, only 24 analytes were detected in discrete point samples. Although POCIS samples accumulated ten unique contaminants, discrete point sample extracts also yielded six contaminants not detected in POCIS (Alvarez et al., 2005). Regardless, the integrative nature of POCIS devices provided the most consistent identification of contaminants from water, most likely due to contaminant pre-concentration from the water to yield residues greater than method detection limits (Alvarez et al., 2005). Modeled scenarios described herein also suggest integrative samples provided better prediction of the peak 96-h TWA than discrete water samples, especially for short aquatic half-lives (Table 1). Although median values for integrative samples were consistently below the peak 96-h TWA, the frequency of extreme values < 10% of the true value or exceeding the true value were reduced as compared to discrete point samples (Table 1 and Table 2). Collectively, modeled data present herein and field data suggest integrative sampling methods likely provide more consistent measurements as compared to discrete point

samples due to inter- and intra- day variations in water concentrations that occur during pulsed exposures.

Results from modeled scenarios also suggest that that discrete point samples can be semi-integrative provided enough samples are obtained and there is adequate contaminant persistence in the water column (Fig. 2, Table 1). This observation is true for all sampling methods investigated. Assuming a single pulsed exposure, contaminants with long water half-lives can be appropriately measured using discrete point samples; however, more than one sample should be obtained within the anticipated exposure window (spaced near the beginning and end) to contaminant detection. This is particularly important because results from modeling scenarios suggest sampling frequency had the greatest influence on the total number of non-detect values as compared to contaminant dissipation rates (Table 2). Although the investigated modeling scenarios implemented single pulsed exposures, scenarios involving continuous contaminant release could cause stable water concentrations even for substances with fast water dissipation. Regardless, integrative sampling technologies (e.g. POCIS) provide a concentrated sample with lower background matrices and typically yield lower detection limits without the need to collect, extract, and filter large water volumes (Alvarez et al., 2004). Logically, increased sample frequency provides a better estimate of the true value (i.e. peak 96-h TWA); however, in practice there is a tradeoff between increased sampling frequency and expense. Increased sampling frequency is even more challenging when investigating sites distributed across a landscape.

The decision to use a particular sampling method should be driven by contaminant-specific physicochemical properties to ensure adequate sampling occurs. Contaminants with shorter water half-lives in the water column should be sampled using integrative methods (Table 1). For short water half-lives, integrative samples provided better estimates of the peak 96-h TWA, fewer values < 10% of the peak, and fewer non-detect values as compared to discrete point samples (Table 1). Moreover, integrative samples measuring a 96-h interval (DIS) generally provided better estimates than integration periods exceeding 96-h (FIS; Table 1). Although DIS

methods provided better estimates at low sampling frequencies (i.e. 1-2), estimates from FIS measurements were the only predictions consistently > 10% of the peak 96-h TWA (Table 1). Conversely, when substances have long aquatic half-lives, discrete point sampling methods produced higher median estimates as compare to both integrative methods. Similar results have been reported for antibiotic concentrations in wastewaters comparing residues in organic chemical diffusive gradient thin film (o-DGT) samplers with automated and discrete grab samples (Chen et al., 2013). Chen et al. (2013), reported grab sample concentrations approximately 3x higher than concentrations found in integrative samples obtained wastewaters with frequent to continuous discharge. Therefore, polar hydrophilic contaminants with long water half-lives that do not readily partition to sediment or other organic matter may not require integrative sampling to obtain toxicologically-relevant concentrations.

Although aquatic pulses are frequently considered for compounds with a short half-life due to hydrolysis or photolysis, other factors can also contribute to rapidly declining water concentrations. For example, contaminant concentrations in aquatic systems are continuously in flux due to intermittent inputs, contaminant dissipation, and flow variations (Ashauer et al., 2006; Edwards and Moore, 2014; Reinert et al., 2002) where pulsed exposures can occur simply by contaminants being diluted in the water column and being transported downstream. Moreover, hydrophobic contaminants (e.g. current use pesticides, polyaromatic hydrocarbons, and some personal care products) that have large octanol-water partitioning coefficients (*Kow*) rapidly partition to organic matter (Landrum et al., 1984; Maul et al., 2008; Morrison et al., 2013; Yang et al., 2006). Hydrophobic contaminants with Log *Kow* > 4 have limited accumulation in classical POCIS configurations due to interactions with the diffusion limiting membrane (Alvarez et al., 2004); however, POCIS-Nylon modifications may facilitate integrative sampling of these substances due to decreased membrane limitation of analyte accumulation (Belles et al., 2014).

Collectively, results from modeled scenarios suggest that regardless of sampling methodology, better estimates of the actual peak 96-h TWA were obtained at higher sampling

frequencies. Across all aquatic half-lives investigated (i.e. 0.5, 2, and 8 d), differences between discrete point samples and integrative samples were greatest at low sampling frequencies where discrete point samples required higher sampling frequencies to ensure median values > 50% and no sampling events reporting < 10% as compared to integrative methods. Therefore, discrete point samples may be appropriate for monitoring contaminants with long water half-lives; however, when pulse initiation is unknown, sufficient samples should be taken to ensure sampling occurs. Integrative samples reduced the frequency of extreme values < 10% of the true value and exceeding the actual peak 96-h TWA. Moreover, FIS measurements provided the only predictions consistently greater than 10% of the peak 96-h TWA. As such, integrative methods may be most appropriate for monitoring contaminants with short water half-lives, especially when monitoring sites are distributed across a landscape and the exact timing of pulsed events is uncertain.

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CHAPTER III

DEVELOPMENT OF *HELISOMA TRIVOLVIS* POND SNAILS AS BIOLOGICAL SAMPLERS FOR BIOMONITORING OF CURRENT-USE PESTICIDES

The following chapter appears as accepted to *Environmental Toxicology and Chemistry*:

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Abstract

Non-target aquatic organisms residing in wetlands are commonly exposed to current-use pesticides through spray drift and runoff. However, it is frequently challenging to measure exposure due to rapid dissipation of pesticides from water and reduced bioavailability. Our hypothesis is that freshwater snails can serve as bioindicators of pesticide exposure based on their capacity to passively accumulate tissue residues. *Helisoma trivolvis* snails were evaluated as biomonitors of pesticide exposure using a fungicide formulation that contains pyraclostrobin and metconazole and is frequently applied to crops surrounding depressional wetlands. Exposure-response studies indicate that *H. trivolvis* are tolerant of pyraclostrobin and metconazole at concentrations >10x those lethal to many aquatic species with a median lethal concentration based on pyraclostrobin of 441 µg/L (95% CI of 359-555 µg/L). Bioconcentration factors ranged from 137-211 mL/g and 39-59 mL/g for pyraclostrobin and metconazole, respectively.

Elimination studies suggested one-compartmental elimination and snail tissue half-lives (t_{50}) of approximately 15 h and 5 h for pyraclostrobin and metconazole, respectively. Modeling derived toxicokinetic parameters in the context of an environmentally-relevant pulsed exposure suggests residues can be measured in snails long after water concentrations fall below detection limits. With high fungicide tolerance, rapid accumulation, and slow elimination, *H. trivolvis* may be viable for biomonitoring of pyraclostrobin and should be investigated for other pesticides.

Key words: Biomonitoring, toxicokinetics, pesticide, *Helisoma trivolvis*

Introduction

Traditionally, monitoring of aquatic systems entails discrete sampling of surface waters, which provides information on current contaminant levels; however, these discrete or "snapshot" measurements may not be accurate indicators of exposure for compounds that pulse through the system [1]. The major complicating factor is dissipation rate from water to suspended sediments [2], sediment beds [3], and other natural organic matter sources such as dissolved organic carbon [4] and detritus [5]. As such, obtaining environmentally-relevant concentrations of pulsed exposures necessitates understanding chemical fate and effective sampling protocols, thus ensuring accurate measurement of the bioavailable fractions [6]. For instance, Morrison et al. [3] demonstrated significant mortality of amphipods (*Hyalella azteca*) following a pulsed exposure to high fungicide concentrations, despite rapid sediment partitioning that resulted in approximately 70% reduction of mass applied to the system within four hours of water column application. Accurate estimation of water concentrations was only possible by making repeated measurements (3, 12, 96, 168 h) of the overlying water throughout the exposure. This level of resolution is difficult to obtain in the field, especially when investigating numerous sites distributed across a landscape. Additionally, many current-use pesticides have short aquatic half-lives and thus the most relevant exposure scenarios for many aquatic organisms are pulsed exposures [7]. Predicting intensity and length of episodic events is challenging because complex environmental factors influence exposure kinetics [6-8].

Deployment of passive sampling devices (PSDs) is becoming a viable alternative to discrete sampling due to its ability to measure bioavailable water fractions over time [1, 9, 10]. Biomonitoring is another alternative method where body residue concentrations are measured in biological samplers (i.e. organisms) collected from field sites to predict environmental exposure. Synthetic PSDs have had the advantage over measuring chemical residues in organisms due to cleaner sample matrices; however, the development of better analytical extraction and cleanup protocols and increased instrumentation sensitivity has greatly enhanced our ability to measure tissue concentrations [11]. Organism body burdens are frequently used in standardized sediment quality tests [12, 13] and biomonitoring [14-18] especially for contaminants known to persist and bioaccumulate in the environment. Previous work has demonstrated that tissue concentrations can be analyzed in mussels [17, 19] and snails [14, 15] to indicate environmental pollution. Mussels receive greater attention in biomonitoring programs (as compared to snails) because they actively filter water, resulting higher contaminant accumulation rates. However, their use is limited to coastal waters and streams due to absence of indigenous populations in ephemeral water bodies (e.g. depressional wetlands). Conversely, sustained colonization of ephemeral waters can be maintained by pulmonate snails through waterfowl dispersal [20] and their innate ability to burrow into moist soil and estivate using atmospheric air for respiration [21].

Helisoma trivolvis (also known as *Planorbella trivolvis*) is a pulmonate snail species ubiquitously found throughout North America, inhabiting a variety of moderately eutrophic, permanent, and ephemeral freshwaters [22]. Coogan and La Point [14] successfully demonstrated that *H. trivolvis* bioaccumulate antimicrobial agents from wastewater treatment plant effluents, which suggests that these snails may also be useful for the biomonitoring of other pollutants. To be considered a viable option for biomonitoring of pesticides in aquatic systems, these snails would need to satisfy several criteria. First, the snails must be tolerant of pesticides at environmentally-relevant concentrations, which would allow collection and subsequent analysis of body residues even if a pulsed exposure significantly impacted other (more sensitive) non-

target species. Second, to account for declining water concentrations, pesticide accumulation would need to occur rapidly. These two criteria must be met to satisfy the requirements of an acceptable organism for biomonitoring, provided that the ultimate goal is to simply assess if prior exposure has occurred. However, if the objective were to estimate water concentrations for risk assessment, pesticide concentrations would also need to be retained for long durations (i.e. slow elimination phase) so that body burdens are easily measurable after water concentrations have approached or fallen below detection limits.

Due to their ubiquitous presence and abundance in areas of interest throughout the typical growing season, snails are good candidate organisms for biomonitoring of shallow, ephemeral water bodies (e.g. embedded cropland wetlands). Because most wetlands within the midcontinent of North America are adjacent to row-crop agriculture [23], aquatic organisms inhabiting these wetlands are at risk for pesticide exposure due to spray drift or unintentional direct spraying. The spray drift model (AgDrift, Stewart Agricultural Research Services) predicts that under certain circumstances (e.g. wind speed, height of pesticide release, etc.), up to 20% of aerially applied agrochemicals can occur 15 m downwind from application [24]. This model prediction was supported in a recent field investigation where 20-30% of the nominal application rate was detected above corn canopies located 15 m outside of the spray zone [25]. Indeed, over a half billion pounds of fungicides, herbicides, and insecticides were applied annually from 1992 to 2011 in attempt to increase crop production and reduce diseases [26]. For example, strobilurin fungicides have been the only effective means of controlling outbreaks of soybean rust (*Phakopsora pachyrhizi*) despite cultural practices of varying planting date, row width, and crop rotation [27, 28]. Despite documented increases in fungicide application and reports of environmentally-relevant toxicity to non-target organisms [3, 29-33], detailed studies pertaining to environmental exposure concentrations are lacking.

The overall goal of this investigation was to evaluate the use of *H. trivolvis* body burdens for assessing aquatic pesticide exposure. This conceptual premise was tested using Headline

AMP fungicide formulation, which contains two active fungicide ingredients, pyraclostrobin and metconazole that make up 13.64% and 5.14% of the formulation, respectively [34]. *H. trivolvis* were evaluated for their biomonitoring capacity through three experiments, each of which was designed to test the previously outlined criteria. First, a toxicity test was performed to investigate fungicide tolerance following a single pulsed exposure. Second, uptake studies were conducted to determine tissue accumulation rates and steady-state concentrations. Finally, elimination studies were conducted to investigate compartmental half-lives, elimination rates, and excretion potential of parent fungicide compounds. In order to frame the results within an environmental context, a modeling exercise was performed, using toxicokinetic parameters characterized in this study, to describe how tissue concentrations change based on an environmentally-relevant pulsed exposure scenario using data obtained from sediment-water microcosms.

Materials and Methods

Test Organisms

Juvenile *Helisoma trivolvis* pond snails (5-8 mm in length) were haphazardly selected from cultures maintained at Oklahoma State University. Cultures were kept in static renewal systems with dechlorinated tap water, coarse coral substrate, and a 16:8 light:dark cycle. Cultured organisms were fed Algae Wafers (Kyorin Inc., NJ, USA) and small broccoli florets *ad libitum*. Mean (± standard deviation) of lipids for cultured snails was 7.0±2 µg/mg (0.8±0.3 % lipid by weight) as determined by a modified method put forth by Van Handel [35] and gravimetrically confirmed using a modified method from Hara and Radin [36]. Refer to Supporting Information for additional method details.

Overall Experimental Design

Experimental units in each study (i.e. toxicity, toxicokinetic, and mass balance investigations) consisted of stainless steel steaming trays (17.8 cm × 10.8 cm × 10.2 cm), 800 mL of dechlorinated tap water, and ten (n=10) snails. Snails were haphazardly collected from cultures, sorted into size classes, and randomly divided amongst experimental units prior to

treatment. Formulation dosing suspensions were mixed (i.e. rigorously vortexed) with deionized water to achieve expected environmental concentrations based on maximum label application rates. Desired exposure concentrations were achieved by adding either 100 µL of suspended fungicide formulation into treated units or 100 µL of deionized water into control units. Water was gently stirred with a glass rod to evenly distribute fungicides within experimental units. The light cycle was kept at 16:8 light:dark and water quality measurements were taken every 24 h for the duration of the experiments. To remove the complication of analyte exposure and accumulation through ingestion, organisms were only provided food during elimination phases of investigations. Temperature averaged 24(±2) °C, pH ranged from 6.9-7.6, hardness 190-220 mg/L as calcium carbonate. Dissolved oxygen concentrations in toxicity tests, accumulation studies, and elimination studies ranged from 6.5-8.7 mg/L. During the parallel mass-balance elimination study where water exchanges were not possible (see *Elimination studies* below), dissolved oxygen was lower, ranging from 4.6-8.6 mg/L, but still above levels expected to cause stress to snails. Mortalities were assessed daily throughout each experiment. Mortality was defined using the following criteria: a) Failure to respond (retract into shell) after gentle prodding with a glass rod, b) Floating or laying on side with no response to gentle prodding, and c) Hemorrhage or bleeding [37]. Snails found dead during any assessment were removed from experimental units and were not included in any toxicokinetic analysis. Snails collected from individual experimental units at designated takedown points were combined and stored frozen at -30 °C until analysis. As such, combined snails from any one experimental unit (i.e. snails remaining at a designated takedown point) constituted one sample.

Toxicity tests

Toxicity tests (96-h in length) were conducted using five exposure concentrations of Headline AMP fungicide, one control treatment, three replicates (n=3) per treatment, and ten snails (n=10) per replicate. A single pulsed exposure was used during toxicity exposures, as opposed to static renewal or flow through systems, to match exposure scenarios established

during previous investigations for determining acute toxicity [3, 29, 31]. Nominal concentrations for pyraclostrobin were 0, 86, 160, 300, 700, and 1000 µg/L which correspond to nominal metconazole concentrations of 0, 32, 60, 111, 260, and 372 µg/L. These exposure concentrations are well below the water solubility for both pyraclostrobin 1,900 µg/L and metconazole 1,600 µg/L [38, 39]. Water concentrations were measured at 4, 24, 48, 72, and 96 h after initial exposure using 15 mL aliquots from each experimental unit. At the conclusion of the study, remaining snails were combined from individual experimental units and sacrificed by freezing in polyethylene test tubes at -30 °C until further analysis. Mortality observed throughout acute toxicity tests resulted in variable number of individuals remaining between treatment levels at the end of the study. Thus, snail wet-weight (not the number of individuals) utilized for body residue analysis is the most important metric. As such in relation to the acute toxicity test, snail weights provided in the Supporting Information correspond to de-shelled wet-weight of snails (living and dead) combined from a single experimental unit within the treatment level at the 96-h mortality assessment.

Accumulation Studies

Two accumulation studies were conducted to determine accumulation rates and steady-state concentrations. Each accumulation study was conducted using Headline AMP fungicide with a constant nominal exposure concentration of 86 µg/L pyraclostrobin and 32 µg/L metconazole. This concentration was chosen based on water-only toxicity test results, which demonstrated minimal mortality at this concentration. Coincidentally these concentrations align with expected maximum environmental concentrations, based on a direct overspray of a shallow wetland (approximately 16 cm deep) and assuming full water incorporation using the maximum application rate for North American corn. During the first study (24-h in length), experimental units were taken down at 2, 4, 7, 14, and 24 h. During designated takedown points, water samples

(15 mL) were extracted from each experimental unit and snails (n=9-10 per unit) were recovered from four treated (n=4) and two control (n=2) replicate unit and stored at -30 °C until analysis.

To fully characterize accumulation and measure steady-state tissue residues, a second study was conducted by extending the exposure duration to last 96 h with takedown points at 3, 6, 12, 24, 48, 72, and 96 h. To ensure stability of water concentrations throughout the extended study, static renewals were performed every 24 h by decanting and gently refilling experimental units with aerated dechlorinated water and immediately re-spiking with Headline AMP. Three treated (n=3) and one control (n=1) experimental unit(s) were sacrificed during designated takedown points. From these experimental units, water samples (20 mL) were extracted and recovered snails (n=9-10 per unit) were stored at -30 °C until analysis.

Elimination Studies

Elimination studies were conducted to determine compartmental half-lives and the duration of time that snail residues remained detectable for Headline AMP fungicides. Control units (n=8) and treated units (n=24) exposed to Headline AMP fungicides with nominal exposure concentrations of 86 µg/L pyraclostrobin and 32 µg/L metconazole were exposed for 96 h to achieve steady-state tissue concentrations based on results from previous accumulation experiments. Each experimental unit contained ten snails (n=10) and daily static water renewals were performed as described above. Following the accumulation phase, snails (n=10) from each experimental unit were collected, rinsed, and transferred to clean experimental units until designated takedown points at 0, 6, 12, 24, 48, 96, 168, and 240 h. During the elimination phase, snails were provided small pieces of algae wafers. Water exchanges were conducted daily to maintain minimal fungicide water concentrations, and ensure that no reuptake occurred as elimination progressed. Three treated (n=3) and one control (n=1) experimental unit(s) were sacrificed during designated takedown points. From these experimental units, water samples (400 mL) were extracted and recovered snails (n=10 per unit) were stored at -30 °C until analysis.

A parallel mass balance elimination study was conducted to evaluate fungicide excretion and potential detoxification pathways. The mass balance of both pyraclostrobin and metconazole was determined by monitoring changes in tissues residues and water concentrations throughout the elimination phase. Similar to the previous elimination study, the 96-h accumulation phase was conducted with daily water exchanges and was immediately followed by an elimination phase. However, unlike the previous elimination study, daily water exchanges were not performed during the elimination phase, which lasted 168 h (seven days). Following the accumulation phase, snails (n=10) were collected, rinsed, and transferred to clean experimental units until designated takedown points at 0, 6, 12, 24, 48, 96, and 168 h. Three treated (n=3) and one control (n=1) experimental unit(s) were sacrificed during designated takedown points. From these experimental units, water samples (400 mL) were extracted and recovered snails (n=10 per unit) were stored at -30 °C until analysis. Due to the toxicokinetic differences of this study caused by reuptake from water, resulting body residues were only used during this mass balance investigation and were not included in any of the toxicokinetic models described below.

Analyte Extraction and Analysis

During designated takedown points, acquired water samples were passed through SampliQ solid phase extraction (SPE) cartridges (Agilent Technologies, CA, USA) immediately following collection. The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method was used for analytical determination of pyraclostrobin and metconazole body resides for all snails recovered during designated takedown points [11, 40]. Analysis of chemical residues was performed using gas chromatography / mass spectrometry (GC/MS; Agilent 5975c), electron ionization, select ion monitoring, matrix matched standards, and deuterated polycyclic aromatic hydrocarbons (chrysene-d_{12} and perylene-d_{12}) as internal standards. Supporting Information provides additional detail for quality control, extraction, and analysis methods.

Mean (± standard deviation; SD) percentage of SPE water recoveries for pyraclostrobin and metconazole were 117.8 ± 12.4% and 102.7 ± 5.0%, respectively. Method recovery for QuEChERS extracts was performed at 15,000 (n=8), 6,000 (n=8), and 600 ng/g (n=8) to ensure quantitative accuracy over a range of fortification levels. Mean (±SD) percentage of recoveries for pyraclostrobin were 80.5 ± 5.0%, 110.3 ± 5.0% and 104.9 ± 8.1% for 15,000, 6,000, and 600 ng/g fortification levels, respectively. Metconazole had similar recoveries with 94.3 ± 16.8%, 102.2 ± 3.4%, and 87.6 ± 5.6% for 15,000, 6,000, and 600 ng/g fortification levels, respectively. Method detection limits for pyraclostrobin and metconazole were 11.2 ng/g and 14.0 ng/g, respectively. *Helisoma trivolvis* body residues for all control (n=28) and laboratory blanks (n=8) were below method detection limits. Mean (±SD) percentage of expected concentrations for all dosing solutions was 110 ± 19% and 102 ± 17% for pyraclostrobin and metconazole, respectively.

Data Analysis

Snail LC50 (median lethal concentration) were calculated using IBM SPSS Statistics Data Editor Version 21 (IBM Corp., NY, USA), which uses Probit algorithms developed for exposure-response analysis by Finney [41]. Metconazole was not considered when calculating the LC50 because previous investigations demonstrated that active strobilurin fungicide ingredients (e.g. pyraclostrobin) were responsible for observed toxicity for both amphipods [3] and amphibians [31] when concurrently applied with environmentally-relevant concentrations of an azole fungicide (e.g. metconazole). As such, calculated LC50s were based solely on time-weighted average (Equation 1) pyraclostrobin concentrations. Time-weighted average water concentrations (C_{TWA}) were achieved by fitting a first order exponential decay function through average water concentrations measured over time and taking the average of the curve, so that

$$C_{TWA} = \frac{\int_a^b f(x)dx}{(b-a)} \quad (1)$$

where a = 0 h, b = 96 h and f(x) = first order exponential decay function [3]. Differences in *H. trivolvis* mortalities and bioconcentration factors (BCFs) were determined with IBM SPSS Statistics Data Editor. Bioconcentration factors obtained from each exposure level of toxicity tests and toxicokinetic investigations were compared using an univariate general linear model to conduct analysis of variance and a Tukey's Honestly Significant Differences test (α=0.05) was used for post hoc analysis.

Accumulation rates of fungicides by *H. trivolvis* snails were calculated by fitting body residue data across time to an one-compartment first-order kinetics model [42] (Sigmaplot 12.0, Systat Software, CA, USA) so that

$$C_a = C_{SS}\left(1 - \exp(-k_e t)\right) \quad (2)$$

where, C_a is the amount of fungicide accumulated in snail tissue (ng/g), C_{SS} is the fungicide concentration (ng/g) when the tissue is at steady-state with the water, and *t* is the exposure duration (h). After sufficient exposure duration, C_{SS} and bioconcentration factors (BCFs) are described by

$$BCF = \frac{C_{SS}}{C_W} = \frac{k_u}{k_e} \quad (3)$$

where C_W is the water concentration (time-weighted average or otherwise; ng/mL), k_u and k_e are accumulation (mL/g/h) and elimination (h^{-1}) rate coefficients. Results obtained from Sigmaplot accumulation regressions were used to calculate k_u, k_e, and "modeled" BCFs for the 96 h uptake experiment using parameter estimates. Equation 3 was also used to calculate "measured" BCF results from analyzed body residue and average water concentration data from the toxicity study, the 96 h uptake study, and the 0 h assessment point for the elimination study. Data from replicate experimental units were averaged and reported with 95% confidence intervals (CI). During elimination, the first-order elimination model (Equation 4) describes the total fungicide concentration in snails at any given time

$$C_a = C_{SS} \exp(-k_e t) \quad (4)$$

where *t* is the elimination time (h) post exposure. Equation 5 was used to calculate elimination half-lives (t_{50}) for compartments based on k_e parameter estimates from model fitting

$$t_{50} = \frac{\ln(0.5)}{k_e} \quad (5)$$

Integrating *H. trivolvis* toxicokinetic data, as described above, with previous water concentration trends observed for pyraclostrobin in sediment-water microcosm studies (Table S1) [3], provided an opportunity to model tissue concentrations under a simplified, but environmentally-relevant, exposure scenario. For environmental realism, the initial C_W was set at the maximum environmentally-relevant concentration of pyraclostrobin (150 µg/L) based on maximum application rates of Headline EC fungicide to North American corn [29]. Water measurements obtained from the sediment-water microcosm exposures reported by Morrison et al. [3] were fit with a first order exponential decay model to determine an environmentally-relevant dissipation rate (k_d) from water (h^{-1}). Water concentration values were generated using Equation 6 while accounting for dissipation rate from water so that

$$C_W(t) = C_W(t-1) \times Q_W \quad \text{and} \quad Q_W = (1 - k_d) \times \Delta t \quad (6)$$

where $C_W(t)$ is the water concentration at time *t* (h), $C_W(t\text{-}1)$ is the previous water concentration, and Q_W is the quantity of analyte remaining in the water for accumulation (i.e. the bioavailable fraction). Modeled snail tissue values were determined using Equation 7 by applying toxicokinetic parameters controlling accumulation from water (k_u) while accounting for elimination (k_e) over time so that

$$C_a(t) = C_W(t) \times k_u + C_a(t-1) \times Q_a \quad \text{and} \quad Q_a = (1 - k_e) \times \Delta t \quad (7)$$

where C_a is dependent on accumulation (k_u) from C_W (Equation 6), $C_a(t\text{-}1)$ is the past exposure history (i.e. previous tissue concentration), and the quantity of analyte remaining (Q_a) from the total fraction previously accumulated (i.e. the net body residue after elimination). Modeled tissue and water values were generated pointwise with one-hour increments using Microsoft Excel;

therefore, $\Delta t = 1$ h for both equations. The model assumes that the system is sufficiently large enough so that uptake and elimination from snail tissues does not influence water values.

Results and Discussion

Toxicity Study

Analyte water concentrations were relatively stable during the 96 h static exposure. Measured concentrations for pyraclostrobin and metconazole decreased from initial (4 h) concentrations by 26 ± 10% and 23 ± 11%, respectively. Time-weighted average water concentrations were within 20% of nominal concentrations for pyraclostrobin; however, metconazole concentrations decreased 30-40% from nominal (Table S2). Measured body residues taken at the 96 h assessment are provided in the Supporting Information (Table S3). Control mortality was less than 5% during toxicity tests and no adverse effects were observed within treated units until 72 h into the exposure. This is a stark contrast to previous research using *Hyalella azteca* amphipods and *Bufo cognatus* tadpoles, in which greater than 80% of observed toxicity occurred within 8 h of exposure [3, 29, 31]. The LC50 (95% CI) based on time-weighted average pyraclostrobin concentrations was 441 µg/L (359-555 µg/L; Figure 1). Estimated LC10 (concentration required to induce 10% mortality) was 150 µg/L (97-197 µg/L; Figure 1). Lethal concentration thresholds were calculated based on pyraclostrobin (not metconazole) concentrations because previous investigations demonstrated that the active strobilurin fungicide ingredients were responsible for observed toxicity when concurrently applied with an azole fungicide [3, 31]. Data suggesting minimal metconazole toxicity to non-target organisms as compared to pyraclostrobin data may be attributed to different modes of action (MOA). The MOA for pyraclostrobin (and other strobilurin fungicides) is a non-specific inhibition of cellular respiration at the mitochondria by binding the Quinone Outside Inhibitors (Qol) and blocking electron transfer between cytochrome b and c complexes [34, 43]. Metconazole inhibits demethylation of sterol biosynthesis, thus disrupting cell membrane synthesis [34, 44]. Although this remains unconfirmed for snails, indirect effects on pyraclostrobin toxicity could occur from

metconazole and/or the additional formulation adjuvants. Regardless, previous investigations have demonstrated that the additional adjuvants of the commercial formulations did not significantly contribute to toxicity compared to individual strobilurin ingredients [3, 31].

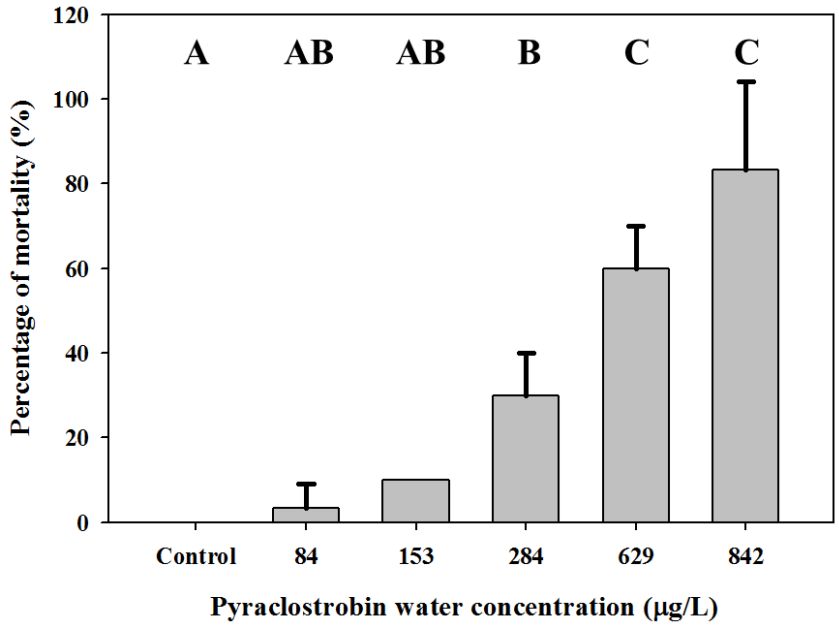

Figure 1. Mean (± standard deviation) percentage of mortality for *Helisoma trivolvis* pond snails following a single pulsed water-only exposure of Headline AMP. Time-weighted average water concentrations are provided for the active strobilurin ingredient, pyraclostrobin. Provided mortalities correspond to the 96-h assessment. Each treatment concentration consisted of three replicates (n=3) and 10 individual snails per experimental unit. Categorical letters represent statistical differences between treatment concentrations ($p < 0.05$).

The estimated LC50 in this study is magnitudes greater than thresholds reported for other aquatic organisms. Bringolf et al. [30] reported 96 h EC50 (the median effective concentration) in freshwater mussels (*Lampsilis siliquoidea*) to be 30 µg/L. LC50s have also been reported for water fleas (*Daphnia magna*) ranging from 14-120 µg/L [33], and amphipods *(H. azteca*) at 21 µg/L [3]. Aquatic vertebrates have also shown sensitivity to pyraclostrobin: bluegills (*Lepomis macrochirus*) have reported LC50s of 11 µg/L [32], while LC50s for Great Plains Toad (*B. cognatus*) tadpoles range from 3.7-10 µg/L [29, 31]. Maximum environmentally-relevant

concentrations of pyraclostrobin are expected to be less than 150 µg/L based on direct overspray of Headline EC fungicide into a shallow wetland (i.e. 16 cm water depth), assuming full water incorporation [29]. Using the same reasoning for the formulation used in this study, Headline AMP fungicide, 86 µg/L is the maximum environmentally-relevant concentration. These concentrations are at or below the LC10 reported in this study. As such, *H. trivolvis* snail populations will persist at any environmentally-relevant concentration of pyraclostrobin while populations of other sensitive species may be adversely affected.

Although limited toxicological studies have been performed using *Helisoma trivolvis* snails, relatively high tolerance has been reported for some current-use pesticides. For instance, exposure to malathion resulted in 24-h LC50s of 268 mg/L (95% CI: 237-297) and 479 mg/L (95% CI: 417-536) for *H. trivolvis* juveniles (3-5 mm) and adults (8-10 mm), respectively [37]. These values are magnitudes greater than the 48-h EC50 (0.011 mg/L) reported for *D. magna* exposed to malathion [45]. No significant change in *H. trivolvis* behavior or survival following a 28 d fenvalerate exposure up to 0.79 µg/L; however, toxicity was observed for more sensitive species within the same system with LC50s for juvenile amphipods (*Gammarus pseudolimnaeus*), mayflies (*Ephemeralla sp.*), and Rhagoinid flies (*Atherix*) of 0.05, 0.022, and 0.12 µg/L, respectively [46]. Similarly, Spehar et al. [47] reported no significant reduction in *H. trivolvis* survival following exposure to permethrin at concentrations up to 0.33 µg/L. Permethrin exposure results in 21-d LC50 and EC50s of 0.03 µg/L and 0.042 µg/L for caddisflies (*Brachycentrus americanus*) and stoneflies (*Pteronarcys dorsata*), respectively [46]. Collectively, data suggests that *H. trivolvis* would likely survive environmentally-relevant fungicide exposures and many other current-use pesticides, whereas other more sensitive species may be affected and potentially absent from the aquatic system.

Toxicokinetic Studies

Based on initial toxicity testing results, 86 µg/L was chosen for accumulation and elimination experiments because less than 10% mortality was observed (Figure 1). Additionally,

tissue residues were well within quantitation limits (Table S3). Daily water renewals provided stable water concentrations for all accumulation phases during toxicokinetic investigations. Measured water concentrations were within 20% of nominal concentrations during accumulation phases for accumulation studies and the accumulation phases of elimination studies (Table S2). By conducting daily water exchanges during the 240 h elimination study, measured water concentrations remained below three percent of accumulation-phase water concentrations for both fungicides. Control mortality was less than 10% throughout uptake experiments, and no mortality was observed during elimination phases of experiments.

Toxicokinetic uptake results from both accumulation experiments are reported together for pyraclostrobin (Figure 2A) and metconazole (Figure 2B). Measured body residues used for modeling are reported in Supporting Information (Table S4). The one-compartment first-order kinetics model (Equation 2) described accumulation well for both pyraclostrobin (R^2 = 0.943, F(1, 10) = 648, p < 0.0001; Figure 2A; Table 1) and metconazole (R^2 = 0.913, F(1, 39) = 410, p < 0.0001; Figure 2B; Table 1). Using model parameter estimates (k_e), snails reach C_{SS} within 60 h of exposure for pyraclostrobin and within 9 h for metconazole (Figure 2).

Toxicokinetic elimination results are reported for pyraclostrobin (Figure 2C) and metconazole (Figure 2D) with measured body residues used for modeling reported in Supporting Information (Table S5). The first-order elimination model (Equation 4) described elimination well for both pyraclostrobin (R^2 = 0.991, F(1, 23) = 2426, p < 0.0001; Figure 2C; Table 1) and metconazole (R^2 =0.990, F(1, 10) = 960, p < 0.0001; Figure 2D; Table 1). Estimated depuration coefficients were similar between accumulation and elimination studies for both fungicides (Table 1), resulting in compartmental half-lives (t_{50}) of approximately 15 h and 5 h for pyraclostrobin and metconazole, respectively (Figure 2). Detection of pyraclostrobin was achieved up to 240 h (10 d) after exposure (Figure 2C; Table S5) which exceeded predictions of reaching detection limits by 168 h based on the predicted half-life in snail tissue. Metconazole was predicted to fall below detection limits by 35 h post exposure; however, detection was only

achieved up to 24 h due to the spacing of takedown points (Figure 2D, Table S5). The discrepancy between actual and predicted detection of pyraclostrobin could suggest elimination occurs via multiple compartments with different elimination rates; however, a single-compartment model was chosen because fitting residue data with a double-compartment bioconcentration model produced non-significant parameter estimates ($p > 0.05$) for the second compartment.

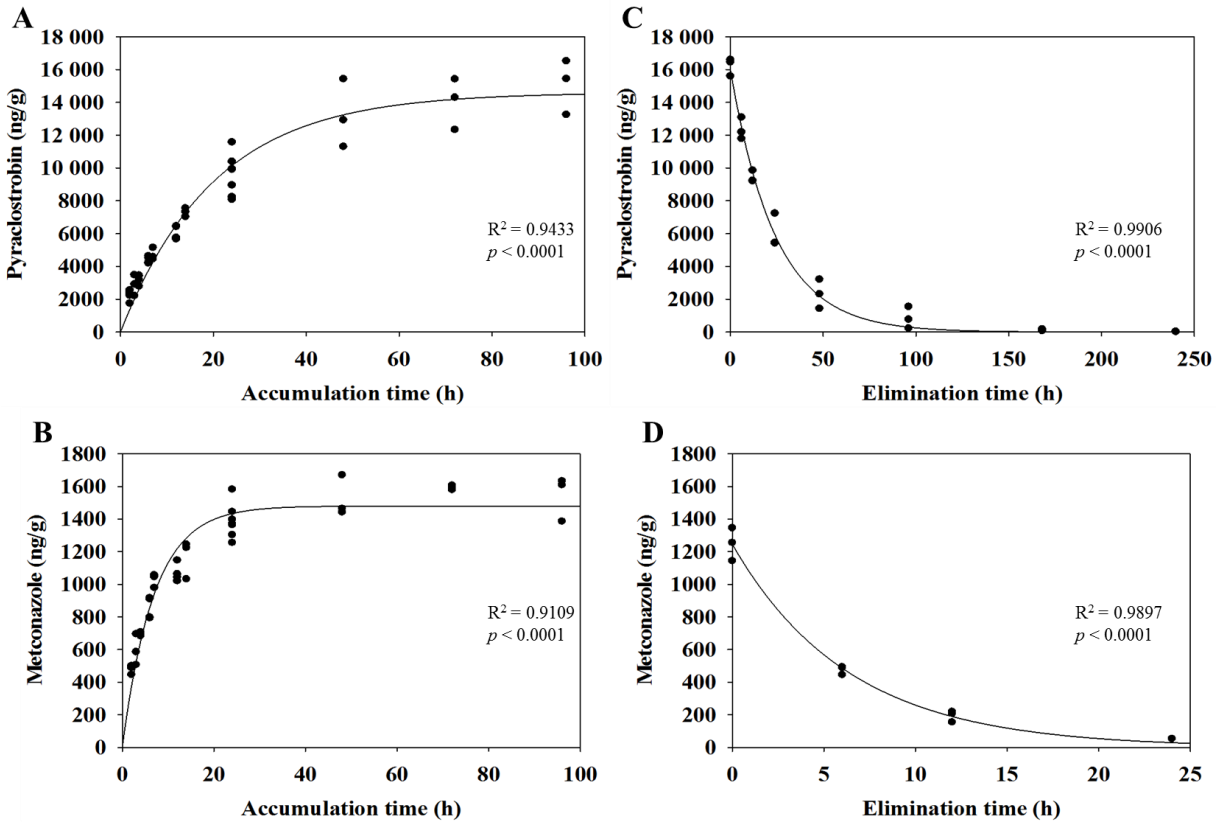

Figure 2. Tissue concentrations of pyraclostrobin (**A**, **C**) and metconazole (**B**, **D**) in *Helisoma trivolvis* pond snails during accumulation (**A**, **B**) and elimination (**C**, **D**) studies. Toxicokinetic data was fit with SigmaPlot using Equation 2 for accumulation and Equation 4 for elimination. Parameter estimates obtained during model fitting are provided in Table 1. Each data point corresponds to residues obtained from snails (n=9-10) collected from a single experimental unit. During elimination, metconazole residues fell below quantitation limits by 24 h. Measured tissue concentrations used for modeling accumulation and elimination data are reported in Supporting Information (Table S4 and Table S5).

Table 1. Toxicokinetic parameters (± standard error) for pyraclostrobin and metconazole fungicides in *Helisoma trivolvis* pond snails estimated from fitting accumulation and elimination data in SigmaPlot using Equations 2 and 4, respectively. Additional parameters were calculated from model-estimated parameters using Equation 3. Measured tissue concentrations used for modeling accumulation and elimination data are reported in Supporting Information (Table S4 and Table S5). Tissue concentration at steady-state (C_{SS}), elimination (k_e) and accumulation (k_u) coefficients, bioconcentration factor (BCF).

Parameter estimates from accumulation studies					
Fungicide	R-squared	C_{SS} (ng/g) [a]	k_e (h^{-1}) [a]	k_u (mL/g/h) [b]	BCF (mL/g) [b]
Pyraclostrobin	0.9433	14605 (± 452)	0.049 (± 0.004)	7.60	154
Metconazole	0.9131	1480 (± 31)	0.144 (± 0.009)	6.44	44.6
Parameter estimates from elimination study					
Fungicide	R-squared	C_{SS} (ng/g) [a]	k_e (h^{-1}) [a]	k_u (mL/g/h) [b]	BCF (mL/g) [b]
Pyraclostrobin	0.9906	16005 (± 288)	0.041 (± 0.002)	6.38	154
Metconazole	0.9897	1246 (± 30)	0.157 (± 0.008)	6.86	43.7

[a] Model estimated parameter ± standard error ($p < 0.0001$).

[b] Calculated from modeled parameters estimates using Equation 3.

No significant differences ($p > 0.05$) were observed between measured BCFs across all experiments (Table 2) or BCFs modeled from parameter estimates (Table 1) for either pyraclostrobin or metconazole. Mean (± SD) measured BCFs across all studies were 173.6 ± 21 mL/g (range: 136.8-210.8 mL/g) and 49.5 ± 7 mL/g (range: 39.4-59.1 mL/g) for pyraclostrobin and metconazole, respectively. Estimated BCF values are magnitudes less than would be predicted from methods proposed by Veith et al. [48] based on partitioning coefficients (log P) for organic compounds in fish. Specifically, BCFs for pyraclostrobin (log P = 3.99) [38] were 27% of predicted (634 mL/g). Similarly, BCFs for metconazole (log P = 3.85) [39] were 10% of predicted (497 mL/g). This disparity in BCF values can be expected due to the magnitude of difference between lipid content in snails (i.e. 7.0 ± 2 µg/mg or 0.8 ± 0.3% lipid by weight) compared to the whole body lipid content of fish (i.e. 10% by weight) used by Veith et al. [48]. Similar trends have been reported in literature where BCFs for snail species are lower than fish species. For instance, *H. trivolvis* are reported to have lower BCFs than fathead minnows (*Pimephales promelas*) following exposure to permethrin [47]. Legierse et al. [49] also reported lower BCFs in snails (*Lymnaea stagnalis*) as compared to guppies (*Poecilia reticulate*) for

chlorobenzenes. Regardless, data present herein combined with literature data suggest hydrophobic pesticides (log P ranges 3.8-7.6) accumulate in *H. trivolvis*. Specifically, *H. trivolvis* accumulate triclocarban and triclosan with bioaccumulation factors of 1,600 and 500, respectively [14], fenvalerate with a BCF range of 356-1,167 mL/g [46], and permethrin with BCFs ranging from 700-1,000 mL/g [47].

Table 2. Measured water concentrations and mean (± 95% CI) bioconcentration factor (BCF) in *Helisoma trivolvis* pond snails during all experiments following exposure to Headline AMP fungicides (i.e. pyraclostrobin and metconazole). Time-weighted water concentrations (Equation 1) are reported for exposure-response experiment and were used for calculation of measured BCFs (Equation 3). Average (± standard deviation) water concentrations are reported for 96-h accumulation and 0-h elimination as measured from samples taken prior to daily static renewals. Measured BCFs were calculated using measured water concentrations and correspond to 96-h takedown points (toxicity and accumulation studies) or the 0-h takedown of elimination.

Experiment	Pyraclostrobin		Metconazole	
	Measured water concentration (µg/L)	Measured BCF (mL/g)	Measured water concentration (µg/L)	Measured BCF (mL/g)
Exposure 1	83.9	164 (137 – 190)	22.5	43.8 (37.5 – 50.1)
Exposure 2	153	189 (162 – 216)	39.4	50.3 (44.4 – 56.2)
Exposure 3	284	180 (154 – 207)	78.0	47.2 (41.3 – 53.1)
Exposure 4	629	169 (142 – 195)	160	43.9 (37.9 – 49.8)
Exposure 5	842	181 (154 – 208)	280	56.7 (50.8 – 62.7)
Accumulation	94.9 ± 14.2	159 (132 – 186)	33.2 ± 4.9	46.5 (40.6 – 52.4)
Elimination	103.9 ± 13.8	156 (131 – 181)	28.5 ± 1.3	43.8 (38.1 – 49.4)

Results from the mass balance elimination study (where daily water exchanges were not performed), may suggest differential fungicide metabolism by *H. trivolvis*. Based on measured water and tissue concentrations across elimination takedown points, the mass balance for pyraclostrobin remained within 20% of initial (0 h) throughout the 168 h elimination, and water concentrations came to equilibrium within 12 h of elimination commencement (Figure 3A, Table S6). Based on this mass balance stability, *H. trivolvis* do not appear capable of breaking down or

biotransformation of pyraclostrobin. In contrast, the metconazole mass balance gradually declined to 38% of initial after 168 h and water concentrations never reached equilibrium (Figure 3B, Table S6). Although we did not monitor for metabolites, this could suggest that *H. trivolvis* is capable of breaking down or biotransformation of metconazole. Mass balance water concentration trends were consistent with measurements obtained during the 96-h static toxicity test where recoveries for pyraclostrobin were within 20% of nominal and metconazole recoveries dropped to 30-40% of nominal (Table S2). Measured body residues from the mass balance elimination study are reported in Supporting Information (Table S7).

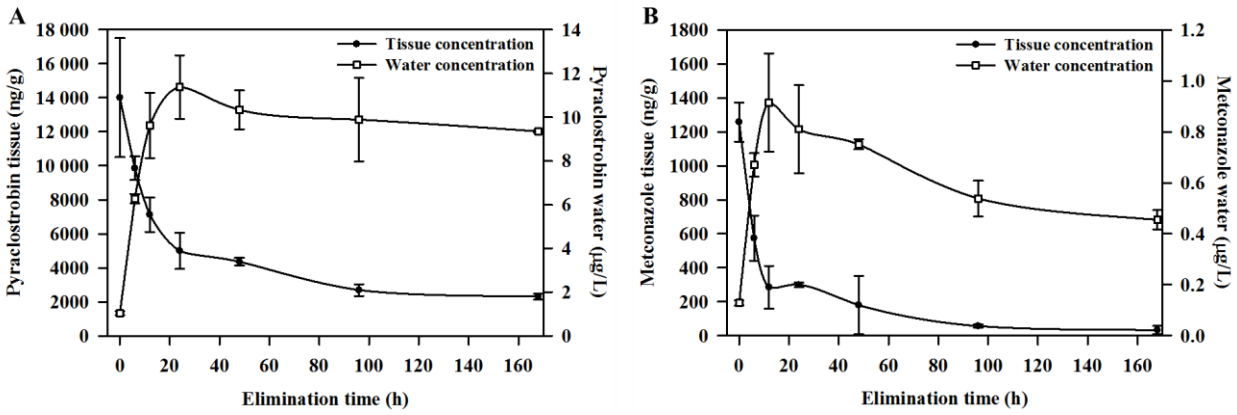

Figure 3. Mean (±SD) tissue and water concentrations of pyraclostrobin (**A**) and metconazole (**B**) from the mass balance elimination study conducted *without* water exchanges. Each takedown point consisted of three replicate (n=3) experimental units with ten (n=10) snails per replicate unit.

Previous studies have successfully demonstrated environmental exposure to current-use pesticides using residues in amphibians [18, 50] and land snails [15]. Although, these studies provide data indicative of exposure, toxicokinetic parameters were not measured; therefore, preventing any estimate of exposure extent. Progressing investigations by describing toxicokinetic parameters of organisms is the foundation towards using biomonitoring to predict water concentrations. The combination of broad pesticide tolerance and toxicokinetic data demonstrating the capacity of *H. trivolvis* to accumulate and retain tissue concentrations over time (Figure 2), snail tissue residues can be, at the very least, an indication that exposure has

occurred. Additionally, our results show that the QuEChERS method provides accurate recoveries from snail tissues over multiple fortification levels and yields low detection limits.

Using the toxicokinetic parameters characterized in this study in conjunction with data from an environmentally-relevant pulsed exposure scenario in sediment-water microcosms [3], provided an environmental context to quantify the accuracy of snail tissue residues to predict water concentrations. Analysis of pyraclostrobin water concentrations (Table S1) obtained from sediment-microcosm exposures yielded a mean (± SD) dissipation rate (k_d) from water of 0.232 ± 0.03 h^{-1} (range: 0.174-0.276 h^{-1}) and corresponding to a mean (± SD) water half-life of 3.0 ± 0.5 h (range: 2.5-4 h) [3]. As such, the elimination rate of pyraclostrobin from snail tissues (0.041-0.049 h^{-1} or t_{50} = 15 h; Table 1) was approximately 80% slower than dissipation from water within a sediment-microcosm. Under this pulsed exposure scenario, the peak and 12-h time-weighted average water values (TWA; Equation 1) were 150 µg/L and 45.4 µg/L, respectively. Modeled water values quickly declined to 30% of the initial exposure concentration within four hours (Figure 4). This rapid loss would present a challenge for analyzing water samples; however, a 2,000x concentration using one liter of surface water would yield measurable concentrations up to 40 h after the initial pulse, especially with lower detection limits of tandem mass spectrometry (MS/MS) technologies. Modeled snail tissues reached 3400 ng/g within six hours of initial exposure and remained above detection limits (assuming no extract concentration) for 126 h (5.3 d) post exposure (Figure 4). This is significantly shorter than the observed 240 h observed during elimination studies; however, this should be expected due to the shorter exposure duration that resulted in tissue residues below steady-state concentrations.

Modeled tissue values (Figure 4) can be expressed as water concentrations using dimensional analysis by dividing the modeled tissue values by snail-pyraclostrobin BCF (154 mL/g; Table 1). For instance, six hours after the initial pulse, modeled snail values resulted in predictions that were 14% and 48% of the actual peak and 12-h TWA average concentrations, respectively (Table 3). These predictions were similar to discrete water values that were 21% and

68% of the peak and 12-h TWA average concentration, respectively (Table 3). Although, discrete water values provided better predictions initially, modeled tissue values provided more reliable predictions of the actual peak and 12-h TWA average water values over time (Table 3). Accurate exposure predictions are important because biological effects are related to peak concentrations rather than peak duration [3, 7, 51]. These short-term measurements are important because significant mortality can occur before bioavailable fractions partition from surface waters [3, 29, 51]. Due to rapid accumulation and slow tissue elimination rates, as compared to dissipation from water, snail tissues provide better predictions of exposure concentrations than discrete water values.

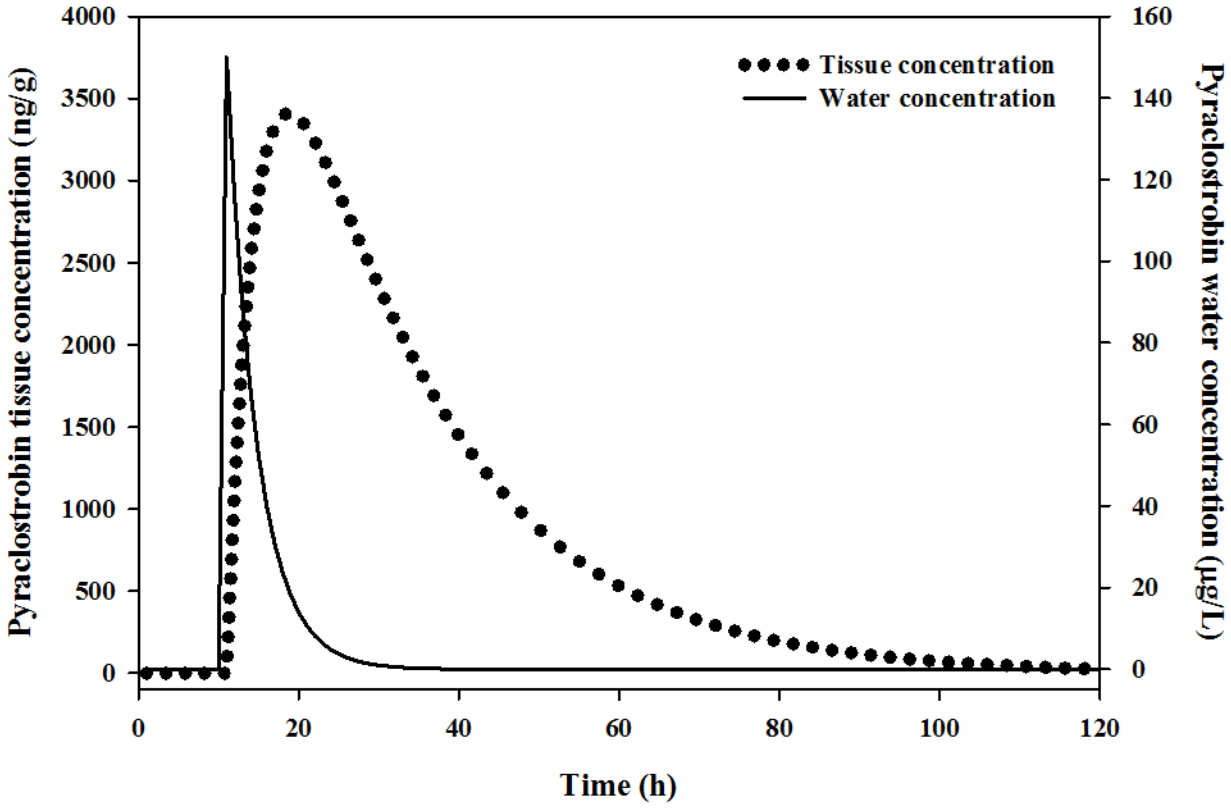

Figure 4. Modeled pyraclostrobin tissue (ng/g) and water concentrations (µg/L) based on toxicokinetic parameters (Table 1), water concentration trends in sediment-water microcosm studies reported by Morrison et al. [3], and maximum environmentally-relevant pyraclostrobin concentration (150 µg/L) using Headline EC [29]. Water and tissue concentrations were modeled using Equation 6 and Equation 7, respectively.

Table 3. Model prediction analysis evaluating the capacity of using either single time point water (i.e. Modeled water concentration) or predicted water concentrations (generated from Modeled tissue values) to accurately predict both the peak and 12-h time-weighted average (TWA) water concentrations. The peak and 12-h TWA concentrations were 150 µg/L and 45.4 µg/L, respectively.

Time Post Exposure (h)	Modeled water (µg/L)[a]	Percentage of (%)		Modeled tissue (ng/g)[b]	Predicted water concentration (µg/L)[c]	Percentage of (%)	
		Peak water concentration	12-h TWA water concentration			Peak water concentration	12-h TWA water concentration
6	31	21	68	3330	21.6	14.4	48
12	6.3	4.2	14	3147	20.4	13.6	45
24	0.27	0.18	0.59	1854	12.0	8.0	27
36	0.011	0.0075	0.025	1020	6.6	4.4	15

[a] Modeled water values calculated using Equation 6.
[b] Modeled tissue values calculated using Equation 7.
[c] Generated by dividing Modeled tissue concentration by bioconcentration factor (154 mL/g; Table 1).

For the purposes of monitoring water concentrations, deployment of passive sampling devices (PSDs) may be preferred over measuring organism residues due to simplistic extraction, well-defined analyte accumulation, and integration [1, 9, 10]. However, suitable organisms are often already present within aquatic systems and their tissue residues represent bioavailable contaminant fractions. Collectively, results demonstrate many analogous characteristics between analyte accumulation in *H. trivolvis* and PSDs. Although many PSD variations are available, analyte accumulation is always described as occurring either kinetically, (i.e. linearly) or based on thermodynamic equilibrium [52]. Our results show that snails are best described as equilibrium samplers due to the short linear accumulation phase as defined by the compartmental half-lives (t_{50}) of approximately 15 h and 5 h for pyraclostrobin and metconazole, respectively (Figure 2). As such, snail tissues provide a larger window in which water exposure concentrations can be predicted as compared to discrete water values (Figure 4, Table 3).

Although sediments readily accumulate pesticides and tissue residues are easily detected, concentrations often provide little information pertaining to bioavailable water concentrations responsible for adverse effects to non-target organisms [3, 6]. For instance, Morrison et al. [3] showed significant toxicity to *H. azteca* amphipods following a simulated overspray in a sediment-water microcosm. Significant mortality was observed following the application of 300 µg/L pyraclostrobin to the overlying water, which corresponded to a sediment concentration of 1,550 µg/kg seven days after application. However, direct sediment application of the same pyraclostrobin mass 24 h prior to water inundation did not induce mortality, despite sediment concentrations of 2,090 µg/kg. Although pesticide partitioning from the water can ameliorate pesticide toxicity [2, 3, 5], caution should be used when trying to predict exposure concentrations from sediment residues.

H. trivolvis (and other) snails have other benefits to consider for biomonitoring. For instance, their ubiquitous distribution across ephemeral water bodies (e.g. depressional wetlands,

headwater streams, or other drainage areas) allows for easy collection at many field locations. Although BCF models predict fish to accumulate pesticides to greater levels than snails [47, 49], ephemeral wetlands are frequently colonized by snail species but not fish species [53]. This study demonstrates that *H. trivolvis* snails are tolerant to fungicides at environmentally-relevant concentrations and are more tolerant than other species reported in literature. Moreover, results from other investigations suggest that tolerance and accumulation potential extends to other current-use pesticides [14, 37, 45-47]. The data also shows accumulation potential where one gram of snail tissue is equivalent to 140-200 mL of water when based on the BCF of pyraclostrobin for snails (Table 1 and Table 2). Toxicokinetic parameters in combination with pyraclostrobin water concentrations obtained from a sediment-water microcosms exposures show that snails can accumulate bioavailable fractions to significant levels even under pulsed exposures (Figure 3). Collectively, our results and literature data suggest that further research is warranted to develop freshwater snails as biomonitoring tools for current-use pesticides. Future investigations should focus on testing snail accumulation in more complex sediment-water systems including mesocosms and field sites. Despite most of the discussion herein relating to ephemeral wetlands, snails may be useful biomonitoring tools in other aquatic systems due to their ubiquitous presence. Snails may provide an important link to demonstrating exposure and subsequent risk of current-use pesticides to other (more sensitive) non-target organisms due to relatively high tolerance, accumulation, and retention of bioavailable fractions. As such, *H. trivolvis* body burdens may be useful for assessing exposure for more sensitive species that could be absent from affected areas.

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Supporting Information

Additional methodology information is available in the Supporting Information including description of test chemicals, lipid analysis, detailed procedures for tissue and water extractions, instrumental parameters, and quality control. Select values are also provided for measured tissue and water concentrations as they pertain to reported modeling exercises.

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Appendix

Supporting Information:

DEVELOPMENT OF *HELISOMA TRIVOLVIS* POND SNAILS AS BIOLOGICAL SAMPLERS FOR BIOMONITORING OF CURRENT-USE PESTICIDES

Summary of Contents:

Additional Experimental Detail

Test Chemicals

Headline AMP fungicide formulation (US EPA Reg. 7969-291; BASF) was purchased from a local distributor. Pyraclostrobin and metconazole are the two active fungicidal ingredients of this formulation and make up 13.64% and 5.14% of the formulation, respectively [1]. Formulation dosing suspensions were mixed with deionized water to achieve expected environmental concentrations based on maximum label application rates. Based on a shallow wetland system (approximately 16 cm deep), the maximum application rate for North American corn, and assuming full water incorporation, the maximum environmental concentrations of Headline AMP fungicides would be 86 µg/L and 32 µg/L for pyraclostrobin and metconazole, respectively. Analytical-grade pyraclostrobin (99.9% purity) and metconazole (99.5% purity) were purchased from Sigma-Aldrich (St. Louis, MO, USA) for calibration standards. Analytical-grade deuterated polycyclic aromatic hydrocarbons (chrysene-d_{12} and perylene-d_{12}) were purchased from Accustandard (New Haven, CT, USA) as internal standards. All solvents used in reagent make-up or sample preparation were at least pesticide grade. Dechlorinated water was obtained by carbon filtration of Oklahoma State University tap water (pH: 7.5-7.7; hardness: 190-220 mg/L as calcium carbonate; dissolved oxygen: 6.7-9.0 mg/L) and was used to carry out all experiments.

Lipid analysis

Analysis for percent lipid was performed using a modified method by Van Handel [2]. Individual snails (n=4) were thawed, weighed (approximately 130 mg), and placed in 12 mL test tubes. Lipids were extracted from tissues by adding 500 µL chloroform: methanol (1:1 v/v) solution to each vessel and gently prodding with a glass rod. Sample extracts were quantitatively transferred to clean tubes and evaporated to dryness under a gentle stream of nitrogen. A short acid digestion was performed by adding 200 µL of concentrated sulfuric acid to each tube and heating at 95 °C for 10 min then allowed to cool to room temperature. After reaching room

temperature, 5 mL of vanillin-phosphoric acid reagent (600 mg vanillin dissolved in 100 mL of hot deionized water and 400 mL concentrated phosphoric acid) was added to each vessel and left to stand for 8 min. When a red color was established, sample extracts were transferred to a 96 well plate and analyzed immediately using SPECTRAmax 190 Microplate Spectrophotometer (Molecular Devices Coup., Sunnyvale, CA, USA) at 522 nm. Calibration and blank standards were prepared and analyzed concurrently with samples. Calibrations standards were prepared from canola oil using the same method. The linear range ($R^2 = 0.995$) of lipid standards was as follows 31.25, 62.5, 125, 250, 500, and 1000 µg. An initial recovery study was performed using canola oil (n=6) as a lipid surrogate. Recoveries ranged from 95-105 % with a mean (± standard deviation) of 102% (± 5). Percent lipid was gravimetrically confirmed using a modified method from Hara and Radin [3]. Lipids were extracted from tissues (~6.0 g wet weight, n=3) using 30 mL of a 3:2 hexane/isopropanol (v/v) solvent mixture, homogenized with a T10 Ultra-Turrax homogenizer (IKA, Wilmington, NC, USA), and allowed to incubate at room temperature for 15 minutes. To facilitate adequate separation of lipid extract from excess tissue water, approximately 4.0 g of magnesium sulfate and 1.0 g of sodium chloride salts were added to each sample. Samples were immediately vortexed for two minutes followed by centrifugation at 3,000x *g* for eight minutes. Gravimetric analysis was performed in pre-weighed aluminum weigh pans using 25 mL aliquots of lipid suspensions. Extracts were slowly evaporated on a hot plate and reweighed after reaching room temperature. Percent lipid for each sample was determined by dividing the lipid weight for each sample (adjusted by 16.7% to account for the 5 mL of unevaporated lipid suspension) by the initial weight of each individual sample. An initial study (n=4) using canola oil as a lipid surrogate resulted in mean (± standard deviation) recoveries of 93% (± 0.3).

Analytical Determination of Fungicides in Water and Snail Tissues

Acquired water samples were passed through SampliQ solid phase extraction (SPE) cartridges (Agilent Technologies) immediately following collection. Small water volumes (15-20

mL) were extracted onto 100 mg C18 cartridges whereas larger water volumes (400 mL) were extracted onto 500 mg C8 cartridges. Cartridges were conditioned with 6 mL methanol and 10 mL deionized water and samples were extracted at a rate of approximately 2.5 mL/min. Loaded cartridges were centrifuged at 3,000x *g* for 5 min to remove excess water. Analytes were eluted with 8 mL ethyl acetate, dried with approximately 1.0 g sodium sulfate, and evaporated to 500 µL under a gentle stream of nitrogen. Individual spiking solutions used for treating experimental units were analyzed following each dosing event to confirm that proper spike delivery was maintained throughout all experiments. For ease of calibration, dosing solutions were diluted to approximately the same concentration based on the amount of formulation added and the resulting concentration expected.

To simplify body residue analysis, extraction and cleanup steps utilized the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method developed by Anastassiades et al. [4], which has been demonstrated to be effective for analysis of pesticides in tissue [5]. Snails collected from individual experimental units at designated takedown points were combined and stored frozen at -30 °C until analysis. As such, combined snails from any one experimental unit (i.e. snails remaining at a designated takedown point) constituted one sample. Frozen snails were thawed, de-shelled, weighed (wet weight ranged from 340-1347 mg), and placed in 50 mL centrifuge tubes. Prior to homogenization, internal standards including chrysene-d_{12} and perylene-d_{12} were added to each sample to correct for analyte recoveries and partial volume transfers. Snails were then homogenized into a suspension with 3 mL acetonitrile using an ULTRA-TURRAX homogenizer. Approximately 2.0 g magnesium sulfate and 500 mg of sodium chloride was added to each sample and the samples were immediately vortexed on high for 2 min to prevent clumping. Following this salting out procedure, samples were centrifuged for 5 min at 3,000x *g* to further facilitate recovery of acetonitrile extracts. Extract cleanup was achieved by transferring a 1 mL aliquot from the centrifuged sample to a preassembled QuEChERS tube (Restek Corporation, Bellefonte, PA, USA) containing 150 mg magnesium sulfate, 50 mg

primary secondary amine, and 50 mg C18. Samples were vortexed on high for 1 min, followed by 5 min centrifugation at 7,200x *g*. Finally, 500 µL of each sample extract was evaporated to dryness under a gentle stream of nitrogen, reconstituted with 500 µL of ethyl acetate, and analyzed via GC/MS.

Gas Chromatography Analysis

Analysis of chemical residues was performed using gas chromatography / mass spectrometry (GC/MS; Agilent 5975c, Santa Clara, CA, USA) using electron ionization (70 eV). The GC inlet temperature was 260 °C and the oven program started at 130 °C and increased over 16 min to 295 °C. Ultra inert, splitless, single taper inlets with glass wool packing were used (Agilent). Separation was achieved using a 15 m × 250 µm × 0.25 µm HP-5 capillary column (Agilent). Quantitation was performed using select ion monitoring with the following ions (Quantitation: Qualification): pyraclostrobin (132:164, 325) and metconazole (125:250, 319). The two qualitative ions had to be within 20% of expected ratio, relative to the quantitative ions response, to report the analyte without flagging the data as qualitatively uncertain. Internal standards (chrysene-d_{12} and perylene-d_{12}) were added to all samples and calibration standards. To correct for matrix effects during analysis of QuEChERS body residue extracts, matrix-matched standards were made by subjecting previously unexposed snail tissues to the QuEChERS method. Resulting extracts were spiked with analytical grade fungicide ingredients made from neat material to create a standard curve. Continuous calibration verification was performed during each analytical run by randomly reanalyzing low, intermediate, and high ranges of calibration standards.

Quality Control

SPE water extraction was verified by fortifying deionized water with fungicide concentrations of 25 ng/mL (n=4). The QuEChERS technique was verified by analyzing previously unexposed snail tissues fortified with individual fungicide ingredients at 15,000 (n=8), 6,000 (n=8) and 600 (n=8) ng/g. An additional MDL study was conducted (n=8) at a spike level

of 60 ng/g, which resulted in a final matrix concentration approximately 30% lower than the lowest calibration standard. All unexposed snail tissues were less than the detection limit of 11 ng/g (wet weight) and 14 ng/g (wet weight) for pyraclostrobin and metconazole, respectively. Data from replicate method spikes were averaged and reported with standard deviations (SD) for both pyraclostrobin and metconazole.

Supplemental References

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[3] Hara A, Radin NS. 1978. Lipid extraction of tissues with a low-toxicity solvent. *Anal Biochem* 90:420-426.

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Table S1: Pyraclostrobin water concentrations obtained from Morrison et al. [6] used for modeling snail accumulation during a simplistic, but environmentally-relevant pulsed exposure. Water measurements obtained from sediment-water microcosm exposures during fate and toxicity investigations. N/A = no measurements taken; QL = quantitation limit

Time (h)	Pyraclostrobin water concentration during sediment-water microcosm investigations (ng/mL)					
	Fungicide fate investigation	Fungicide toxicity test				
0	300	5	14	39	107	284
0	300	5	14	39	107	284
0	300	N/A	N/A	N/A	N/A	N/A
5	110	1.3	4.8	9.4	36.5	76.9
5	101	1.7	3.5	9.7	23.9	89.9
5	115	N/A	N/A	N/A	N/A	N/A
24	80.1	1.2	4.9	7.8	28.6	68.7
24	79.6	1.1	4.2	11.4	26.4	64.2
24	104	N/A	N/A	N/A	N/A	N/A
48	N/A	< QL	3.1	7.4	24.7	65.3
48	N/A	< QL	3.8	7.4	21.8	66.2
96	33.6	< QL	2.0	7.0	16.6	49.9
96	59.9	< QL	2.2	6.1	14.6	59.8
96	41.6	< QL	N/A	N/A	N/A	N/A
168	34.0	< QL	1.1	3.0	13.8	42.6
168	33.0	< QL	1.8	3.5	10.4	43.3
168	34.8	< QL	N/A	N/A	N/A	N/A

Table S2: Quality control results obtained from analysis of experimental unit water samples taken during accumulation phases of each experiment. Time-weighted water concentrations (Equation 1) are reported for dose-response experiment and were used for calculation of measured BCFs (Equation 3). Average water concentrations are reported for 96 h accumulation and 0 h elimination as measured from samples taken prior to daily static renewals.

Treatment/ Experiment	Pyraclostrobin			Metconazole		
	Target (µg/L)	Measured (µg/L)	Percent of target (%)	Target (µg/L)	Measured (µg/L)	Percent of target (%)
Exposure 1	86	84	98	32	23	72
Exposure 2	160	153	96	60	39	66
Exposure 3	300	284	95	111	78	70
Exposure 4	700	629	90	260	160	61
Exposure 5	1000	842	84	372	280	75
Accumulation	86	95	110	32	33	104
Elimination	86	104	121	32	28	89

Table S3: Fungicide tissue residues in *Helisoma trivolvis* snails following a 96 h toxicity test. Nominal (i.e. target), and time-weighted average (i.e. measured) water concentrations of pyraclostrobin and metconazole at each treatment level are provided in Table S2.

Treatment Level	Replicate	Snail wet weight (mg)	Pyraclostrobin (ng/g)	Metconazole (ng/g)
Exposure 1	A	376	11331	889
Exposure 1	B	423	15307	924
Exposure 1	C	653	14537	1149
Exposure 2	A	636	27844	1870
Exposure 2	B	558	27088	1929
Exposure 2	C	500	31802	2136
Exposure 3	A	390	50848	3467
Exposure 3	B	505	43556	3207
Exposure 3	C	502	59401	4371
Exposure 4	A	340	106511	6649
Exposure 4	B	385	118908	7997
Exposure 4	C	584	92894	6374
Exposure 5	A	561	160783	16677
Exposure 5	B	491	159579	15421
Exposure 5	C	719	137079	15617

Table S4: Fungicide tissue residues in *Helisoma trivolvis* snails throughout accumulation studies. Takedown points with four replicates (A-D) originated from 24 h accumulation study whereas takedown points with three replicates (A-C) originated from the 96 h study. Asterisked replicates differentiate replicates for the 24 h point that occurred during both studies. Measured residues were used within toxicokinetic models.

Takedown point (h)	Replicate	Snail wet weight (mg)	Pyraclostrobin (ng/g)	Metconazole (ng/g)
2	A	766	1756	497
2	B	586	2558	488
2	C	621	2242	446
2	D	778	2382	500
3	A	762	3487	696
3	B	870	2217	507
3	C	669	2920	586
4	A	882	2795	696
4	B	614	3433	694
4	C	818	3460	708
4	D	628	3201	684
6	A	790	4223	794
6	B	649	4211	799
6	C	790	4531	918
6	D	777	4636	911
7	A	598	5154	1046
7	B	727	4459	1058
7	C	885	4584	980
12	A	654	6463	1044
12	B	782	5760	1021
12	C	760	6433	1148
12	D	874	5677	1064
14	A	821	7039	1225
14	B	806	7553	1033
14	C	808	7336	1245
24	A	738	8240	1365
24	B	637	8083	1256
24	C	678	8955	1399
24	D	673	9950	1583
24	A*	751	11577	1446
24	B*	905	9914	1372
24	C*	963	10398	1304
48	A	878	15431	1443
48	B	1018	12931	1671

48	C	784	11307	1465
72	A	797	15425	1608
72	B	721	12333	1592
72	C	1030	14307	1580
96	A	929	13256	1387
96	B	762	16529	1610
96	C	872	15445	1634

Table S5: Fungicide tissue residues in *Helisoma trivolvis* snails measured throughout elimination study conducted *with* daily water exchanges to ensure minimal analyte water concentrations. Measured residues were used within toxicokinetic models.
Quantitation limits = QL.

Takedown point (h)	Replicate	Snail wet weight (mg)	Pyraclostrobin (ng/g)	Metconazole (ng/g)
0	A	724	15603	1144
0	B	958	16454	1346
0	C	613	16607	1256
6	A	754	11791	489
6	B	734	12190	494
6	C	409	13095	446
12	A	822	9221	155
12	B	820	9861	207
12	C	918	9237	220
24	A	783	5452	54
24	B	840	5419	53
24	C	780	7242	54
48	A	982	3218	< QL
48	B	690	2321	< QL
48	C	694	1435	< QL
96	A	814	774	< QL
96	B	643	235	< QL
96	C	735	1551	< QL
168	A	914	104	< QL
168	B	993	81	< QL
168	C	1206	183	< QL
240	A	1011	26	< QL
240	B	1144	25	< QL
240	C	1347	34	< QL
240	D	1056	38	< QL

Table S6: Mean fungicide recovery during the mass balance elimination study conducted *without* water exchanges. Each takedown point consisted of three replicate (n=3) experimental units. The amount of fungicide entering the elimination system corresponded to steady-state concentration obtained from the 0 h tissue assessment. QL = Quantitation limit

Sampling period (h)	Pyraclostrobin water and tissue concentrations during elimination			Metconazole water and tissue concentrations during elimination		
	Water (ng/mL)	Tissue (ng/g)	Mass balance [1] (%)	Water (ng/mL)	Tissue (ng/g)	Mass balance [1] (%)
0	< QL	14000	100	< QL	1258	100
6	6.3	9851	117	0.7	574	98
12	9.6	7125	127	0.9	285	98
24	11.4	5000	121	0.8	299	89
48	10.3	4353	102	0.8	179	72
96	9.9	2690	94	0.5	56	47
168	9.3	2313	85	0.4	33	38

[1] $\text{Mass balance} = \frac{([\text{water cocentration} \times 0.8\ \text{L}] + [\text{tissue concentration} \times \text{snail mass}])}{\text{steady-state snail concentration}} \times 100$

Table S7: Fungicide tissue residues in *Helisoma trivolvis* snails throughout the mass balance elimination study conducted *without* daily water exchanges. Due to differences in toxicokinetics, provided body residues were only used for the mass balance investigation and were not utilized during any toxicokinetic modeling procedures. QL = Quantitation limit

Takedown point (h)	Replicate	Snail wet weight (mg)	Pyraclostrobin (ng/g)	Metconazole (ng/g)
0	A	856	11424	1214
0	B	773	12597	1390
0	C	549	8990	1171
6	A	800	9069	513
6	B	804	10124	483
6	C	792	10359	727
12	A	894	7367	217
12	B	947	8006	430
12	C	831	6001	208
24	A	989	5040	302
24	B	842	6043	284
24	C	722	3915	310
48	A	854	4135	89
48	B	602	2596	378
48	C	634	4335	72
96	A	903	2193	64
96	B	894	2455	46
96	C	871	3091	58
168	A	847	2841	47
168	B	788	2838	< QL
168	C	721	2348	46

CHAPTER IV

CALIBRATION OF NYLON ORGANIC CHEMICAL INTEGRATIVE SAMPLERS AND SENTINEL SAMPLERS FOR QUANTITATIVE MEASUREMENT OF PULSED AQUATIC EXPOSURES

The following chapter appears as submitted to the *Journal of Chromatography A*:

Morrison, S.A. and J.B. Belden (2016). Calibration of nylon organic chemical integrative samplers and Sentinel samplers for quantitative measurement of pulsed aquatic exposures. *J Chromatogr A* (Submitted).

Abstract

Environmental exposures often occur through short, pulsed events; therefore, the ability to accurately measure these toxicologically relevant concentrations is important. Three different integrative passive sampler configurations were evaluated under different flow and pulsed exposure conditions for measurement of current-use pesticides (n=19), polyaromatic hydrocarbons (n=10), and personal care products (n=5) spanning a broad range of hydrophobicities (log *Kow* 1.5-7.6). Two modified POCIS-style samplers were investigated using macroporous nylon mesh membranes (35 µm pores) and two different sorbent materials (i.e. Oasis HLB and Dowex Optipore L-493). A recently developed design, the Sentinel Sampler (ABS Materials) utilizing Osorb media enclosed within stainless steel mesh (145 µm pores), was also investigated. Relatively high sampling rates (*Rs*) were achieved for all sampler configurations during the short eight-day exposure (4,300 – 27 mL/d). Under flow conditions,

median *Rs* were approximately 5 to 10 times higher for POCIS-style samplers and 27 times higher for Sentinel Samplers as compared to static conditions. The ability of samplers to rapidly measure hydrophobic contaminants may be a trade off with increased flow dependence. Analyte accumulation was integrative under pulsed and continuous exposures for POCIS-style samplers with mean difference between treatments of 11% and 33%; however, accumulation into Sentinel Samplers was more variable. Collectively, results show that reducing membrane limitations allows for rapid, integrative accumulation of a broad range of analytes even under pulsed exposures. As such, these sampler designs may be suitable for monitoring environmental substances that have short aquatic half-lives.

Key Words: Calibration, Passive Sampling, Pesticides, PAHs, Personal Care Products, Pulsed Exposure

1. Introduction

Toxicological responses are driven by the magnitude and frequency of exposure to the bioavailable fraction of contaminants (i.e. freely dissolved in water). This basic principle becomes complicated in aquatic systems because contaminant concentrations are continuously in flux due to intermittent inputs, contaminant dissipation, and flow variations [1-3]. In the case of many current-use pesticides, possessing short aquatic half-lives, the major complicating factor is dissipation rate from water due to sorption to sediments [4, 5] and other organic matter [6, 7]. These complicating factors also extend to other anthropogenic contaminants of concern with intermittent inputs such as polyaromatic hydrocarbons (PAHs), and personal care products (PCPs) which can be flushed into aquatic systems from rainwater runoff or municipal discharge. Regardless of short exposure periods, intermittent exposures can elicit significant toxicological responses [1, 5]. Therefore, developing effective sampling technologies capable of measuring short-term exposures (eight days or less) is important for providing toxicologically relevant exposure concentrations for contaminants that rapidly pulse through aquatic systems.

Current integrative passive samplers such as polar organic chemical integrative samplers (POCIS) continuously accumulate freely dissolved contaminants throughout their deployment and have been demonstrated to be a reliable technique for providing time-weighted average (TWA) concentrations of some organic contaminants [8, 9]. Continuous sampling allows for better detection and identification of contaminants while providing TWA concentrations, which is particularly important for contaminants that rapidly dissipate or degrade following release into the environment [8, 10]. Typically, exchange kinetics follow a first-order, one-compartmental model consisting of an initial linear (or kinetic) integrative phase followed by a curvilinear phase indicative of chemical equilibrium [8, 9]. Assuming an infinite sink, two processes primarily regulate uptake of an integrative sampler: 1) Diffusion through the aqueous boundary layer (ABL) and 2) Permeation through the sampler membrane. The thickness of the ABL changes with flow across the sampler thus sampling rates can be highly influenced by variations in flow [9, 11]. Ultimately, whichever process has the slowest kinetics determines the sampling rate [12].

The classical POCIS configuration utilizes microporous polyethersulfone (PES) membranes (0.1 μm pore size) which Alvarez et al. [8] selected over several other membranes based on high sampling rates of polar substances, minimal biofouling, and durability. This membrane has helped establish POCIS as a valuable tool for providing TWA concentrations of polar contaminants; however, limitations of diffusion limiting membrane cause inherent problems for accumulation of hydrophobic contaminants. First, the properties of the PES membranes generally restricts accumulation to hydrophilic chemicals with octanol-water partitioning coefficients (log *Kow*) less than four due to low accumulation coefficients for more hydrophobic substances (i.e. log *Kow*> 4) [8, 13-15]. Although some hydrophobic chemicals accumulate in POCIS, the diffusion limiting membrane generally causes a lag-effect in the initial accumulation kinetics [15]. This is particularly problematic for hydrophobic chemicals because they have the greatest potential to pulse through the water column and partition to sediment and biota. As such, the classical POCIS configuration may under predict actual exposure concentrations for more

hydrophobic contaminants due to limited or delayed accumulation. Second, POCIS are typically deployed for 30 (or more) days and have long integration times with linear accumulation of many polar contaminants greater than 28 d [8, 16]. Although this is beneficial for polar contaminants that tend to stay in the water column, the opposite is true for contaminants that are acutely toxic and quickly pulse through aquatic systems. For these compounds, 4-8 d TWA concentrations would likely be more comparable to toxicological endpoints. Collectively, the lag-effect phenomenon and long deployment times present major challenges for predicting water concentrations of hydrophobic substances using the classical POCIS configuration.

Previous research has suggested that adjustment of sampler design to reduce diffusion membrane limitations is possible [15]. Specifically, replacing the microporous-PES membrane with nylon mesh screens with 30 µm openings allows greater flow through the sampler and reduced diffusion membrane limitations [15]. Belles et al. [15] called this sampler configuration the "Nylon POCIS"; however we will refer to this design as the Nylon Organic Chemical Integrative Sampler (NOCIS) to emphasize the obvious advantage of this modification for increased sampling rates for hydrophobic contaminants. As opposed to the classical POCIS configuration, nylon configurations could be used for integrative measurement of hydrophobic contaminants pulsing through the water column; ultimately expanding the utility of the sampler by providing toxicologically relevant exposure data and better risk assessments for more hydrophobic substances.

However, there are some notable issues with the nylon configuration that should be addressed. First, the increased pore size increases membrane permeation; resulting in increased sampling rates and may reduce linear accumulation phases to weeks or days as compared to the long integration times of the classical POCIS configuration for most compounds. This potential weakness may not be problematic for most applications as shorter-term exposures (8 d or less) are typically a more useful measure of toxicity as it relates better to exposure scenarios used in standard 96-h acute toxicity tests. Regardless, linear accumulation should be confirmed for short-

term exposures. Second, removal of the diffusion-limiting PES membranes may increase flow sensitivity of these samplers thus affecting contaminant-sampling rates. As such, accumulation in NOCIS designs may be more flow dependent and require more calibrations under different flow conditions or use of other flow correction mechanisms.

Investigating new sampler configurations, whether it be modifications of previously described samplers or designs entirely new to literature, is important for expanding our knowledge base and utility passive samplers for environmental monitoring. For instance, Oasis HLB is well established as the classical POCIS receiving phase; however, other sorbents (e.g. Dowex Optipore L-493) have been incorporated in ceramic passive samplers and could provide a comparable performance within POICS-style samplers [17]. Moreover, a new sampler design was recently developed called the Sentinel Sampler (ABSMaterials, Inc., Wooster, OH) which encapsulates Osorb media (ABSMaterials, Inc.) within stainless steel mesh. The combination of the high capacity Osorb media and 145-μm stainless steel mesh openings suggests that this sampler configuration could be conducive for rapid sampling of a broad range of contaminants and warrants investigation.

The overall objective of this research was to evaluate options for optimization of integrative passive samplers with the goal of maintaining integrative accumulation over toxicologically relevant periods (i.e. 8 d) and obtaining high sampling rates for organic contaminants with a broad range of hydrophobicities (log *Kow* range: 1.5-7.6). During our investigations, we used two NOCIS configurations incorporating either Oasis HLB (Waters Corp., Milford, MA) or Dowex Optipore L-493 (The Dow Chemical Co., Midland, MI) which are both water-wettable sorbents. Sentinel Samplers (ABSMaterials, Inc., Wooster, OH) were also deployed through all experiments; however, the Osorb media is not water-wettable and requires pretreatment with polar organic solvent. The first step in evaluating the performance of these sampler configurations was to perform a controlled laboratory calibration using an eight-day deployment period and two flow conditions. The second investigation evaluated the integrative

nature of these samplers by comparing accumulation under different exposure scenarios. Specifically, we evaluated eight-day sampling rates of sampler configurations continuously exposed compared to samplers that experienced a four-day pulse immediately followed by four days in clean water.

2. Experimental

2.1 Test chemicals

Selected analytes included current-use pesticides (i.e. fungicides, herbicides, and insecticides), polyaromatic hydrocarbons (PAHs), and personal care products (PCPs). Relevant chemical properties of all analytes of interest (n=34) are listed in Table 1. Analytical-grade standards of current-use pesticides and PCPs were of the highest available purity (>98%) from Sigma-Aldrich (St. Louis, MO, USA). An expanded PAH mixture was purchased from Accustandard (New Haven, CT) and was used for analytical standards and spiking solutions. Atrazine-d_5 and benzo(a)anthracene-d_{16} were used as surrogate standards and a semi-volatile internal standard mixture, containing deuterated PAHs, was purchased from Accustandard. All solvents (acetone, dichloromethane, ethyl acetate, and methanol) were analytical grade or better. The tap water source at Oklahoma State University is Lake Carl Blackwell (Stillwater, OK) and was used for all experiments. Hardness ranged from 180-200 mg/L as calcium carbonate, pH ranged from 6.9 – 7.3, and experimental unit temperature was maintained at 20 °C (±1 °C).

Table 1. Selected analytes (n=34) and relevant physicochemical properties.

Analyte	Type [a]	CAS Number [b]	Log *Kow* [b]	Water Solubility (mg/L) [b]
Acetochlor	H	34256-82-1	3.05	700
Atrazine	H	1912-24-9	2.64	69
Azoxystrobin	F	131860-33-8	5.68	0.25
Benzo(a)anthracene	PAH	56-55-3	5.73	0.015
Benzo(a)pyrene	PAH	50-32-8	6.19	0.0019
Benzo(b)fluoranthene	PAH	205-99-2	6.19	0.005
Benzo(g,h,i)perylene	PAH	191-24-2	6.65	0.0016
Benzo(k)fluoranthene	PAH	207-08-9	6.19	0.0033
Bifenthrin	I	82657-04-3	7.31	0.076
Chlorothalonil	F	1897-45-6	3.04	1.6
Chlorpyrifos	I	2921-88-2	5.00	1.6
Chrysene	PAH	218-01-9	5.73	0.0096
Cis-permethrin	I	61949-76-6	7.64	0.038
Cyfluthrin	I	68359-37-5	6.42	0.017
λ-Cyhalothrin	I	68085-85-8	6.00	0.054
DEET	PCP	134-62-3	2.41	7500
Desethyl atrazine	M	6190-65-4	1.51	660
Dibenz(a,h)anthracene	PAH	53-70-3	6.91	0.0007
Fluoranthene	PAH	206-44-0	5.00	0.13
Galaxolide	PCP	1222-05-5	5.04	24
Indeno(1,2,3-cd)pyrene	PAH	193-39-5	6.65	0.0019
Metconazole	F	125116-23-6	3.72	45
Metolachlor	H	51218-45-2	3.03	510
Musk ketone	PCP	81-14-1	2.51	14
Musk tonalid	PCP	21145-77-7	5.06	18
Octinoxate	PCP	5466-77-3	5.92	6.4
Pendimethalin	H	40487-42-1	5.20	1.8
Propiconazole	F	60207-90-1	3.65	10
Pyraclostrobin	F	175013-18-0	3.14	12
Pyrene	PAH	129-00-0	5.00	0.044
Tebuconazole	F	107534-96-3	3.77	30
Trans-permethrin	I	61949-77-7	7.64	0.038
Trifloxystrobin	F	141517-21-7	4.78	0.65
Trifluralin	H	1582-09-8	4.56	1.1

[a] F = fungicide, H = herbicide, I = insecticide, M = metabolite, PAH = polyaromatic hydrocarbon, PCP = personal care product.

[b] Values obtained from Scifinder (http://scifinder.cas.org; accessed 2015 September 8) and calculated using Advanced Chemistry Development Software V11.02 (ACD/Labs). Conditions were modeled at 25 °C and pH 7.

2.2 Sampler design and assembly

Three passive sampler configurations were investigated and each configuration contained a different type of sorbent media. The nylon organic chemical integrative sampler (NOCIS) configurations were constructed in house containing 200 mg (± 5 mg) of either Oasis HLB or Dowex Optipore L-493 sorbents, held between porous nylon mesh screening (35 μm openings; Pentair Aquatic Ecosystems) which were secured between two stainless steel washers. Similar to classical POCIS configurations, the stainless steel washers contained circular openings with 5.5 cm internal diameter, resulting in approximately 48 cm^2 of exposed surface area per sampler. Oasis HLB sorbent was removed from Oasis solid phase extraction (SPE) cartridges and the Dowex sorbent was graciously provided by Dow Water and Process Solutions (The Dow Chemical Company, Midland, MI). Prior to use, sorbents were cleaned with methanol, dried under a vacuum, and baked at 40 °C overnight. The Sentinel Sampler, graciously provided by ABSMaterials, Inc. (Wooster, OH), was the third sampler configuration tested. The Sentinel Samplers are maintained in ethanol prior to deployment and its construction utilizes stainless steel mesh (145 μm openings) to encapsulate Osorb media (ABSMaterials, Inc.), swellable organically modified silica particles, with strong affinity for a wide range of organic contaminants. Ethanol absorption leads to a 1.6x increase in the particle diameter and a 4x increase in pore volume. After deployment, the ethanol in the pore structure is rapidly exchanged for aqueous solution preserving expanded swollen state of the sorbent [18]. Details pertaining to passive sampler designs and physical properties of the three passive sampling sorbents are provided in Table 2.

Table 2. Sorbent physical properties as reported by manufacturers for media contained within each integrative passive sampler configuration.

Sorbent	Dowex Optipore L-493	Oasis HLB	Osorb media
Passive sampler configuration	Nylon Organic Chemical Integrative Sampler	Nylon Organic Chemical Integrative Sampler	Sentinel Passive Sampler
Matrix structure	Macroporous styrenic polymer	Hydrophilic-lipophilic balanced copolymer	Swellable organically modified silica
Membrane material	Nylon screen	Nylon screen	Stainless steel
Membrane pore size	35 µm	35 µm	145 µm
Sorbent mass per sampler	200 mg	200 mg	500 mg
Particle size	20-50 µm	60 µm	250-500 µm (dry)
Specific surface area	1,100 m^2/g	800 m^2/g	736 m^2/g
Specific pore volume	1.16 mL/g	1.3 mL/g	0.65 mL/g
Average pore diameter	46 Å	80 Å	60 Å (dry)

2.3 General design of experimental unit

Passive samplers were exposed to analytes of interest using experimental units consisting of stainless steel pots (inner diameter: 40 cm, height: 30.5 cm) containing 30 L of tap water (Figure 1). To achieve laminar flow, smaller stainless steel pots (inner diameter: 15 cm, height 17.7 cm) were secured to an aluminum crossbar and suspended in the center of each unit. The bottoms were removed from the small pots, resulting in a stainless steel cylinder projecting into the upper portion of the water column. Flow was generated by securing circulation pumps (water pumping portion of Aqueon Model AT10) to stainless steel threaded rods so that the pump head was 7.6 cm below the water surface and 3.8 cm above the bottom of the central cylindrical insert. The mean (± standard deviation) of flow achieved in flowing experimental units was 9.33 (± 0.99) cm/s measured by timing the movement of a float in replicate experimental units.

Experimental units with static flow were similarly constructed except the aquarium pump head was excluded. Each experimental unit contained three passive samplers (i.e. one sampler of each configuration). Nylon samplers were secured to stainless steel threaded rods horizontally in the water column with 3.1 cm of clearance between the top and the bottom sampler to ensure adequate and even flow across the nylon membranes. Sentinel Samplers were suspended at the same depth in the water column with nylon fishing line as suggested by the packaging instructions.

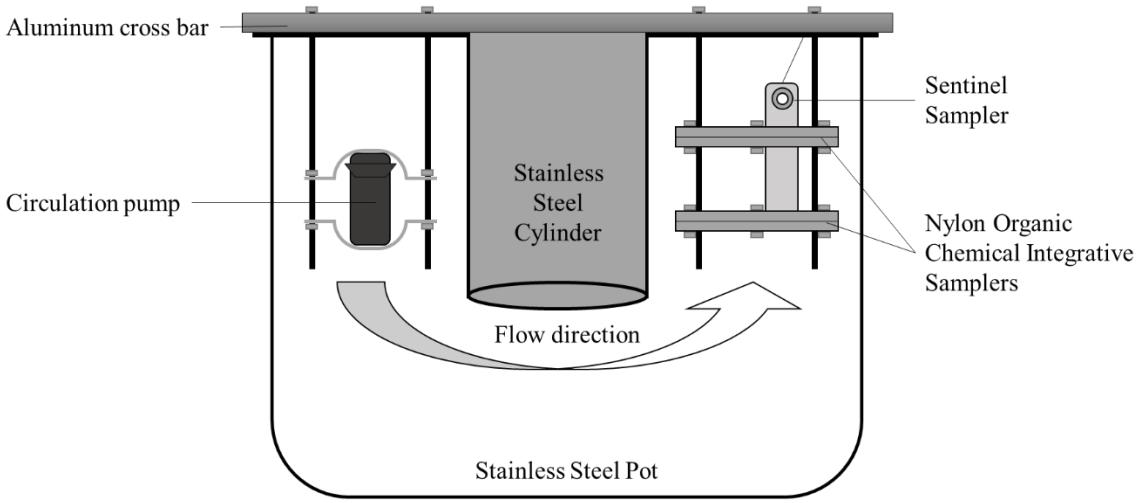

Fig. 1. Schematic of experimental unit with flow treatment. The circulation pump was omitted from static experimental units. To eliminate galvanic reactions within the experimental units, all fastening/structural components (i.e. pots, threaded bar, fastening nuts, washes, and clamps) were stainless steel.

2.4 Sampler calibration

The first experiment was designed to determine analyte sampling rates for the three sampler configurations under two flow conditions and an eight-day continuous exposure. For most analytes target water concentrations were 1 ng/mL; however, nominal concentrations for PAHs and insecticides were 0.2 ng/mL due to lower water solubility. Target analytes concentrations were achieved by adding 1 mL of the spiking solution to the center of the cylindrical sleeve protruding into the depths of the experimental unit. Experimental units with

static flow were stirred with a solvent rinsed stainless steel spoon for 2 min after fortification to facilitate analyte diffusion. To maintain water concentrations throughout the study, a 100% static renewal regimen was conducted daily. Experimental units (containing one of each sampler type) were destructively taken down for both flow treatments at 2, 4, and 8 d to determine sampling rates. Three replicate samplers were analyzed for each time point and both flow treatment (15 samplers of each configuration). After removal from exposure units samplers were stored frozen at -80°C until further analysis. To monitor water concentrations throughout, one-liter aliquots were obtained daily before (n=8 per treatment) and 5 h after (n=8 treatment) water exchanges from duplicate experimental units for each flow condition.

2.5 Influence of pulsed exposure

The second experiment was designed to evaluate integrative nature of the passive sampler configurations by measuring the accumulation and retention of target analytes under a short term pulsed exposure. Two exposure scenarios (i.e. continuous and pulsed) were established, each with four replicate (n=4) experimental units containing three passive samplers (i.e. n=1 for each configuration) in each replicate exposure tank. Based on the results of the first experiment, the 9.33 (± 0.99) cm/s flow treatment was chosen for all experimental units. The continuous exposure scenario replicated the eight-day exposure from the initial calibration experiment; however, target analyte concentrations were 0.5 ng/mL or 0.1 ng/mL depending on analyte water solubility. The pulsed exposure scenario entailed a four-day continuous exposure immediately followed by four days in contaminant free water. Target analyte concentrations during the initial pulsed exposure were 1 ng/mL or 0.2 ng/mL depending on analyte water solubility. During the uncontaminated depuration period, activated carbon water filters were placed in the bottom of the experimental units to help maintain minimal water concentrations. Daily static renewals were conducted as described above; however, the experimental units were not refortified during the second half of the eight-day exposure. Passive samplers were removed from experimental units at the end of the eight-day exposure and stored frozen at -80°C until further analysis. To confirm water

concentrations throughout, 2 L of water were obtained prior to water exchanges at 2, 4, 6, and 8 d by combining aliquots of 500 mL from each of the replicate experimental units (n=4) for each exposure scenario.

2.6 Passive sampler and water extractions

Passive samplers were removed from the freezer and allowed to thaw on aluminum foil at room temperature. Sorbents were removed from passive samplers by cutting the membranes with a solvent rinsed stainless steel scalpel (nylon membranes) or solvent rinsed stainless steel scissors (Sentinel Samplers) and rinsing the sorbents into empty SPE cartridges with deionized water. Water samples obtained throughout both experiments and were spiked with surrogate standards, mixed on a stir plate for one hour, and extracted via Oasis HLB solid phase extraction (SPE) 500 mg cartridges containing HLB at approximately 15 mL/min. Prior to extraction, cartridges were conditioned with 8 mL ethyl acetate, 8 mL of methanol, and 15 mL of deionized water. Loaded passive samplers and water cartridges were dried via a vacuum for five minutes and centrifuged at 3,000x *g* for 8 min to remove excess water. Cartridges containing recovered media from passive samplers were spiked with surrogate standards prior to elution. Analytes were eluted with 1.5 mL acetone followed by 10 mL dichloromethane, dried with anhydrous sodium sulfate, and evaporated under a gentle stream of nitrogen. During evaporation, all samples were solvent exchanged to ethyl acetate. Sample final volumes ranged from 500 µL to 2,000 µL with few samples needing additional dilutions to ensure that analyte responses fell within the calibration range.

2.7 Gas chromatography analysis

Analysis of analyte residues will be performed using gas chromatography / mass spectrometry (GC/MS; Agilent 5975c, Santa Clara, CA, USA) using electron ionization (70 eV). Separation was achieved using a 15 m × 250 µm × 0.25 µm HP-5 capillary column (Agilent). The GC inlet was configured with an ultra inert, splitless single taper inlet with glass wool packing and the temperature was set at 260 °C. To accommodate the large analyte list and reduce

degradation of analytes sensitive to cool starting temperatures and lengthy chromatographic runs, two different oven programs were utilized. The oven programming for the first method was held at the initial temperature of 80 °C for 1 min, ramped at 11 °C/min to 170 °C, ramped at 7 °C/min to 190 °C, ramped at 10 °C/min to 255 °C, ramped at 12 °C/min to 295 °C, and held for 2 min. The oven programming for the second method was held at the initial temperature of 140 °C for 1 min, ramped at 11 °C/min to 170 °C, ramped at 7 °C/min to 190 °C, ramped at 7 °C/min to 255 °C, ramped at 12 °C/min to 295 °C, and held for 2 min. Analyte quantitation was achieved using three ion select ion monitoring (SIM; Table S1). The two qualitative ions had to be within 20% of expected ratio, relative to the quantitative ion response, to report the analyte without flagging the data as qualitatively uncertain. Semi-volatile internal standards were added to all samples and calibration standards. Analytes were paired to internal standards based on nearest retention time. Continuous calibration verification was performed during each analytical run by reanalyzing low, intermediate, and high ranges of calibration standards throughout.

2.8 Data analysis

Integrative samplers are typically calibrated in controlled laboratory studies [11] to accurately predict water concentrations (C_w) as described by Equation 1

$$C_W = \frac{N}{R_S \times t} \quad \text{EQ 1}$$

where, N is the amount of the chemical accumulated by the sampler (ng), R_S is the sampling rate (mL/day), and t is the exposure time (day). To confirm accumulation linearity, linear regressions were performed by plotting N as a function of time while forcing the intercept at the origin for each sampler configuration and flow treatment. Sampling rates were calculated for each sampler configuration using the mean measured water concentration for each flow treatment. Sampling rates were determined pointwise using Equation 1 are reported with standard deviations for each sampler configuration and flow treatment based on statistical analysis. Comparisons in *Rs* values were conducted in IBM SPSS Statistics Data Editor Version 21 (IBM Corp., NY, USA) using a

univariate general linear model for analysis of variance and a Tukey's Honestly Significant Differences test (α=0.05) was used for post hoc analysis. Results obtained from sampler calibration tests used flow verses static as fixed effects; whereas, results from comparing the influence of a pulsed exposure used continuous verses pulsed as fixed effects.

3. Results and Discussion

3.1 Quality control

Mean (± standard deviation; SD) percentage of analyte recoveries from sorbents and water are reported in Table S2. Expected recoveries are based on analysis of spiking solutions (n=9 per experiment) conducted throughout. Recovery of analytes from fortified sorbent quality control samples (n=4 per sorbent) averaged 90% (range: 66-127%), 91% (range: 66-135%), and 96% (range: 63-127%), respectively for Dowex Optipore L-493, Oasis HLB, and Osorb media. Mean (SD) percentage of surrogate standard recovery from water and sorbents was 100% (8) and 69% (9) for atrazine-d5 and benzo(a)anthracene-d_{16}, respectively. Recoveries of PAHs were corrected by benzo(a)anthracene-d_{16} recoveries for each sample. Analyte residues in blank sorbent extract concentrations (n=5 per sorbent) were below quantitation limits for all analytes; therefore, the limit of detection was set at the lowest calibration standard within the linear range for each analyte. Trace levels of ten target analytes (atrazine, DEET, desethyl atrazine, fluoranthene, galaxolide, metolachlor, musk ketone, octinoxate, pendimethalin, and pyrene) were detected in blank water samplers (n=8) due to presence in the water source. The median background concentration was 15 ng/L (range: 6-81 ng/L) with atrazine being the highest. Mean recovery of analytes directly from fortified water samples (n=15) was 102% (range: 45-148%; Table S2).

Measured experimental unit water concentrations were consistent throughout calibration and the pulsed studies (Table S3 and Table S4) and were not blank corrected as background concentrations were in the water source. On average, measured water concentrations were approximately 70% of expected based on analysis of spiking solutions (n=18). Furthermore,

measured concentrations decreased as log *Kow* increased ($R^2 = 0.35$; $p < 0.001$; Table S3 and Table S4) where the more hydrophobic insecticides and PAHs had lower than expected concentrations. Although measurements were below expected concentrations, these data likely represent actual experimental concentrations based on the overall success of laboratory quality control spikes (Table S2). Since daily water renewals were performed in an attempt to maintain stable water concentrations, the most probable explanation for analyte loss in the system is adsorption to the plastic circulation pumps used to generate flow (Fig. 1) or volatility for a few analytes such as galaxolide, musk tonalid, and trifluralin.

3.2 Sampler calibration

All sampler configurations readily accumulated measureable levels target analytes with the exception of benzo(a)pyrene and cyfluthrin in Sentinel Samplers (Table 3). These analytes were excluded from Sentinel Samplers due to matrix interferences at the GC/MS detector, which were not present in standards, water extracts, or other sorbent extracts. Linear regression analysis of sampler accumulation as a function of exposure time provided good correlations with the data where R-square ranged from 0.7722 – 0.9968 across all sampler configurations and flow treatments (Table 3). For NOCIS configurations, 129 of 136 calibration regressions had R-square greater than 0.900; whereas, regression fitting for Sentinel Samplers was more variable with 31of 68 R-square values greater than 0.900 (Table 3). As such, each passive sampler configuration displayed linear (kinetic) accumulation of target analytes under both flow and static conditions (Table 3, Fig. S1, Fig. S2). As previously discussed, analyte uptake follows a first-order, one-compartmental model with an initial linear (or kinetic) phase followed by a curvilinear equilibrium partitioning phase [8, 9]. Despite increased sampling rates (*Rs*) for even some of the more polar compounds, uptake remained within the kinetic accumulation range for all sampler configurations. These accumulation trends are most significant for measuring pulsed exposures where substances quickly pulse through aquatic systems.

Table 3. Statistical results from linear regression (i.e. ng/sampler verses time) of the calibration study. Slope (standard error, SE) and R-square fittings are provided with the y-intercept set at the origin. N.D. = non-detect

Analyte	Dowex Optipore L-493				Oasis HLB				Osorb media			
	Slope (SE) (ng/sampler/d)		R-square		Slope (SE) (ng/sampler/d)		R-square		Slope (SE) (ng/sampler/d)		R-square	
	Flow	Static	Flow	Static	Flow	Static	Flow	Static	Flow	Static	Flow	Static
Acetochlor	719 (34)	77 (4)	0.9841	0.9810	988 (31)	154 (5)	0.9931	0.9915	2109 (282)	60 (4)	0.8890	0.9717
Atrazine	748 (39)	78 (4)	0.9814	0.9832	937 (26)	148 (4)	0.9945	0.9950	1715 (252)	62 (3)	0.8683	0.9810
Azoxystrobin	971 (54)	84 (5)	0.9786	0.9780	1327 (43)	180 (6)	0.9928	0.9931	2569 (491)	64 (5)	0.7966	0.9522
Benzo(a)anthrancene	60 (2)	5 (1)	0.9918	0.9359	90 (7)	17 (1)	0.9542	0.9816	203 (25)	10 (1)	0.9072	0.9137
Benzo(a)pyrene	9 (0)	2 (0)	0.9860	0.9283	13 (2)	5 (0)	0.8763	0.9614	N.D.	N.D.	N.D.	N.D.
Benzo(b)fluoranthene	52 (2)	3 (0)	0.9925	0.8924	77 (9)	8 (0)	0.9118	0.9761	220 (25)	10 (1)	0.9168	0.9260
Benzo(g,h,i)perylene	14 (1)	3 (0)	0.9677	0.9333	23 (4)	7 (1)	0.8344	0.9407	103 (15)	7 (1)	0.8682	0.8681
Benzo(k)fluoranthene	32 (1)	3 (0)	0.9850	0.9196	50 (8)	8 (1)	0.8558	0.9471	159 (23)	8 (1)	0.8706	0.8749
Bifenthrin	27 (1)	16 (2)	0.9893	0.9373	38 (3)	29 (1)	0.9622	0.9862	65 (7)	7 (1)	0.9158	0.7775
Chlorothalonil	1025 (49)	111 (9)	0.9840	0.9546	1420 (44)	252 (6)	0.9933	0.9955	3035 (390)	55 (8)	0.8965	0.8815
Chlorpyrifos	122 (7)	26 (2)	0.9762	0.9727	184 (6)	55 (4)	0.9916	0.9661	330 (41)	10 (1)	0.9028	0.9474
Chrysene	74 (3)	5 (1)	0.9895	0.9112	112 (10)	16 (1)	0.9472	0.9769	246 (31)	10 (1)	0.8989	0.9072
Cis-permethrin	48 (1)	11 (0)	0.9935	0.9878	75 (4)	26 (1)	0.9768	0.9933	153 (20)	8 (1)	0.8906	0.8383
Cyfluthrin	47 (2)	16 (1)	0.9873	0.9772	68 (5)	36 (1)	0.9673	0.9932	N.D.	N.D.	N.D.	N.D.
λ-Cyhalothrin	38 (1)	17 (1)	0.9928	0.9658	60 (5)	34 (2)	0.9570	0.9752	109 (14)	7 (1)	0.8938	0.7787
DEET	732 (39)	79 (4)	0.9807	0.9846	820 (23)	144 (5)	0.9943	0.9929	1771 (238)	62 (4)	0.8874	0.9704
Desethyl atrazine	534 (40)	77 (4)	0.9616	0.9839	356 (28)	118 (3)	0.9579	0.9947	210 (33)	28 (3)	0.8511	0.9304
Dibenz(a,h)anthracene	11 (1)	4 (0)	0.9428	0.9305	15 (2)	8 (1)	0.8722	0.9291	65 (10)	8 (1)	0.8650	0.8385
Fluoranthene	138 (8)	21 (1)	0.9741	0.9692	201 (9)	57 (6)	0.9871	0.9287	367 (41)	16 (2)	0.9209	0.9255
Galaxolide	491 (24)	130 (6)	0.9835	0.9834	714 (26)	251 (19)	0.9907	0.9625	1287 (144)	49 (6)	0.9196	0.9096
Indeno(1,2,3-cd)pyrene	15 (1)	5 (1)	0.9544	0.9376	19 (3)	10 (1)	0.8557	0.9338	71 (10)	6 (1)	0.8701	0.8682
Metconazole	768 (39)	61 (4)	0.9826	0.9737	1144 (43)	148 (4)	0.9902	0.9941	2015 (260)	47 (4)	0.8955	0.9551

Metolachlor	706 (33)	75 (4)	0.9847	0.9793	986 (31)	151 (5)	0.9931	0.9933	2053 (289)	57 (3)	0.8784	0.9742
Musk ketone	736 (35)	135 (5)	0.9848	0.9888	1083 (38)	280 (11)	0.9914	0.9901	2120 (279)	66 (6)	0.8920	0.9476
Musk tonalid	550 (25)	120 (5)	0.9853	0.9892	817 (32)	250 (10)	0.9893	0.9878	1563 (203)	52 (5)	0.8947	0.9289
Octinoxate	316 (41)	57 (7)	0.8965	0.9040	468 (40)	142 (14)	0.9520	0.9390	822 (169)	34 (6)	0.7722	0.7969
Pendamethalin	737 (33)	144 (6)	0.9861	0.9887	1088 (38)	307 (13)	0.9915	0.9879	1997 (254)	60 (5)	0.8984	0.9465
Propiconazole	749 (35)	71 (5)	0.9853	0.9712	1098 (43)	152 (6)	0.9894	0.9887	2037 (256)	50 (4)	0.9002	0.9601
Pyraclostrobin	985 (35)	141 (5)	0.9913	0.9896	1384 (51)	307 (7)	0.9907	0.9968	2576 (426)	80 (7)	0.8392	0.9514
Pyrene	122 (7)	17 (1)	0.9775	0.9706	180 (9)	46 (3)	0.9842	0.9775	335 (37)	15 (2)	0.9198	0.9202
Tebuconazole	815 (43)	74 (6)	0.9810	0.9547	1172 (46)	160 (6)	0.9892	0.9905	1947 (336)	43 (6)	0.8273	0.8937
Trans-permethrin	55 (1)	15 (1)	0.9958	0.9852	87 (7)	31 (1)	0.9515	0.9893	182 (26)	10 (2)	0.8795	0.8439
Trifloxystrobin	700 (28)	129 (5)	0.9887	0.9878	1019 (45)	265 (12)	0.9867	0.9869	1882 (233)	55 (5)	0.9032	0.9417
Trifluralin	393 (25)	93 (2)	0.9728	0.9962	577 (28)	213 (8)	0.9835	0.9900	1051 (124)	38 (4)	0.9118	0.9186

Relatively high *Rs* were achieved for all sampler configurations during the short eight-day exposure (Table 4, Fig. 2), especially under flowing conditions. Under flow conditions, median *Rs* were highest for Sentinel Samplers (2300 mL/d) followed by NOCIS containing Oasis HLB (1100 mL/d) and Dowex Optipore L-493 (970 mL/d; Fig. 2). All analytes followed this trend with the exception of desethyl atrazine, which had the opposite trend (Figure S3). However, normalizing *Rs* by sorbent weight (200 mg for NOCIS and 500 mg for Sentinel Samplers), the median *Rs* are similar between all three sampler configurations under flow conditions (Fig. 2). Under static conditions, median *Rs* were highest for nylon samplers containing Oasis HLB (220 mL/d) followed by Dowex Optipore L-493 (100 mL/d) and the Sentinel Sampler (85 mL/d; Fig. 2). Due to relatively low *Rs* during static conditions, this trend was not affected when normalized by sorbent weights (Fig. 2).

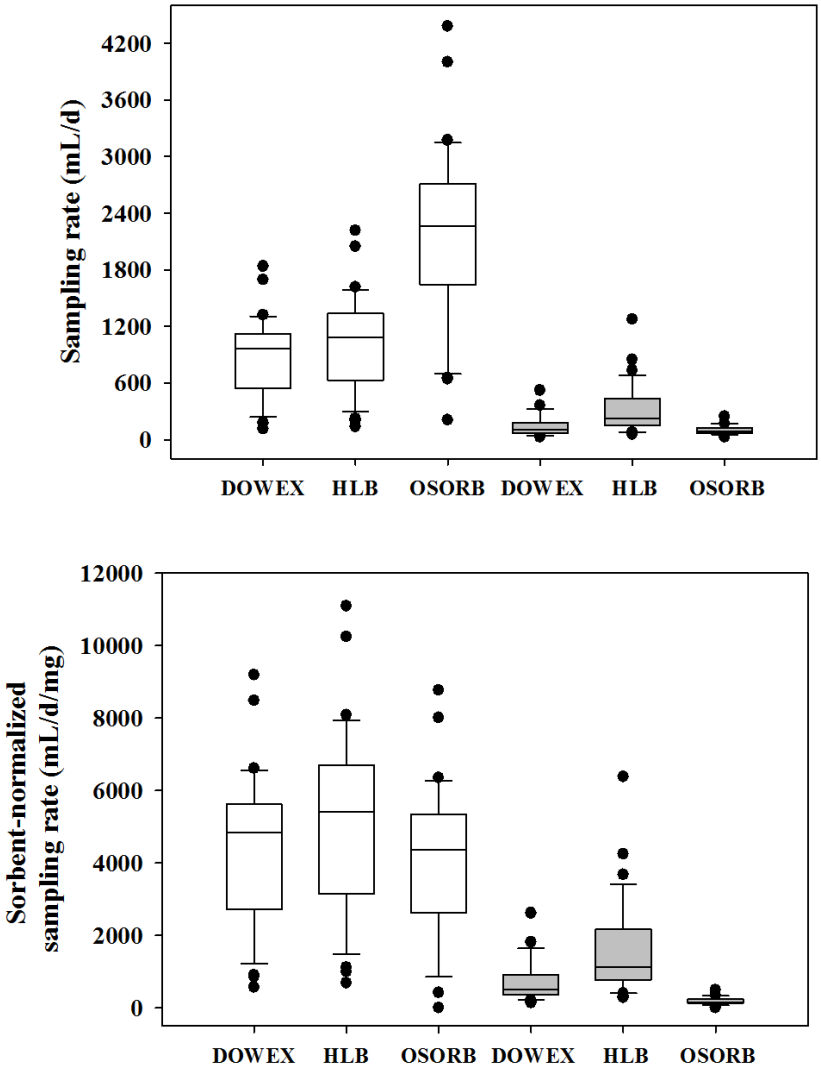

Fig. 2. Box-whisker-plots summarizing analyte sampling rates for each passive sampler configuration during flow (white) and static (grey) exposure scenarios. The top chart corresponds to sampling rates listed in Table 4 and the bottom chart represents sampling rates normalized for sorbent weights (i.e. 200 mg for DOWEX and HLB and 500 mg for OSORB).

Table 4. Mean (standard deviation, SD) sampling rates obtained during the initial calibration experiment. Summary results are depicted in Figure 3 and full for ANOVA comparisons are supplied in the Supporting Information. N.D. = non-detect

Sorbent	Dowex Optipore L-493		Oasis HLB		Osorb media	
	Sampling rate (SD) (mL/d)		Sampling rate (SD) (mL/d)		Sampling rate (SD) (mL/d)	
Analyte	Flow	Static	Flow	Static	Flow	Static
Acetochlor	959 (180)	93 (15)	1088 (224)	179 (22)	2294 (1019)	71 (20)
Atrazine	960 (193)	91 (18)	1001 (198)	163 (15)	1820 (845)	68 (18)
Azoxystrobin	949 (156)	71 (15)	1077 (243)	144 (19)	1836 (1067)	53 (19)
Benzo(a)anthrancene	988 (168)	83 (25)	1147 (434)	220 (31)	2872 (1101)	159 (69)
Benzo(a)pyrene	351 (70)	64 (18)	420 (270)	151 (32)	N.D.	N.D.
Benzo(b)fluoranthene	652 (86)	43 (16)	720 (384)	81 (17)	2561 (946)	107 (46)
Benzo(g,h,i)perylene	180 (59)	27 (9)	223 (174)	56 (20)	1191 (659)	76 (42)
Benzo(k)fluoranthene	409 (75)	45 (16)	475 (316)	79 (18)	1772 (811)	96 (43)
Bifenthrin	305 (34)	175 (45)	370 (120)	280 (54)	656 (264)	94 (54)
Chlorothalonil	1149 (192)	106 (22)	1349 (273)	230 (26)	3086 (1400)	39 (24)
Chlorpyrifos	1212 (249)	292 (74)	1516 (312)	608 (173)	2640 (1029)	111 (36)
Chrysene	903 (122)	67 (26)	1053 (416)	152 (25)	2614 (1034)	133 (60)
Cis-permethrin	614 (59)	117 (40)	775 (230)	231 (41)	1610 (663)	87 (49)
Cyfluthrin	563 (87)	152 (39)	700 (190)	301 (47)	N.D.	N.D.
λ-Cyhalothrin	445 (64)	148 (36)	598 (239)	285 (78)	1126 (551)	67 (44)
DEET	979 (203)	96 (22)	936 (180)	169 (20)	1953 (842)	72 (26)
Desethyl atrazine	647 (174)	77 (11)	426 (132)	125 (16)	209 (130)	27 (10)
Dibenz(a,h)anthracene	113 (52)	35 (14)	137 (92)	63 (22)	643 (391)	71 (38)
Fluoranthene	1057 (200)	179 (59)	1266 (262)	476 (139)	2338 (874)	136 (45)
Galaxolide	1295 (180)	523 (317)	1554 (343)	848 (202)	2920 (1177)	173 (68)
Indeno(1,2,3-cd)pyrene	172 (68)	49 (17)	198 (137)	86 (27)	798 (437)	75 (36)
Metconazole	1026 (201)	73 (16)	1245 (295)	161 (14)	2117 (877)	58 (20)
Metolachlor	996 (187)	96 (16)	1144 (239)	183 (20)	2373 (1076)	70 (19)

Musk ketone	1162 (203)	208 (26)	1392 (298)	432 (59)	2737 (1166)	109 (37)
Musk tonalid	1321 (182)	362 (65)	1616 (378)	735 (120)	3174 (1365)	166 (60)
Octinoxate	1838 (608)	284 (112)	2218 (769)	627 (195)	4382 (2148)	172 (97)
Pendimethalin	1044 (168)	195 (20)	1263 (287)	440 (68)	2275 (914)	83 (29)
Propiconazole	1044 (198)	97 (27)	1250 (299)	190 (28)	2255 (932)	64 (20)
Pyraclostrobin	870 (103)	118 (14)	1023 (234)	254 (22)	1739 (870)	65 (22)
Pyrene	1221 (216)	176 (40)	1480 (339)	444 (76)	2839 (1090)	156 (54)
Tebuconazole	1007 (209)	76 (17)	1198 (287)	163 (19)	2037 (923)	59 (31)
Trans-permethrin	494 (32)	126 (30)	643 (241)	230 (42)	1353 (639)	103 (57)
Trifloxystrobin	1113 (189)	216 (50)	1337 (350)	431 (82)	2441 (983)	95 (38)
Trifluralin	1695 (313)	521 (33)	2048 (607)	1276 (202)	4002 (1755)	246 (104)

Direct comparisons with previous studies are limited as Belles et al. [15] is the only previous publication to investigate nylon samplers and analyte accumulation by Sentinel Samplers have yet to be described in peer review literature. Belles et al. [15] compared POCIS-nylon and classic POCIS configurations (i.e. PES membranes) and found that *Rs* were approximately 2.6 times faster in nylon samplers. Moreover, nylon membranes allowed for accumulation of more hydrophobic substances without a lag effect that normally occurs for classical POCIS configurations. For instance, using classical POCIS configurations, Ahrens et al. [14] reported no accumulation of cyfluthrin and limited sampling (50 mL/d) of chlorpyrifos. Similarly, Belles et al. [15] reported lag effects (no appreciable analyte accumulation) for bifenthrin and chlorpyrifos. Although the target analyte list presented herein covered a broad range of hydrophobicities (log *Kow* range: 1.5-7.6; Table 1), the primary interest was hydrophobic substances. Physiochemical properties of hydrophobic substances make integrative sampling challenging due to rapid dissipation from the water column and poor accumulation in integrative passive samplers [14, 15]. As such, the classical POCIS configuration (i.e. PES membranes encapsulating Oasis HLB) was not investigated in the present study. Instead, investigations focused on new sampler configurations (Sentinel Sampler) and integrative sampler designs shown to accumulate hydrophobic analytes and have increased *Rs* compared to classical POCIS configurations [15]. Sampling rates reported herein were within range of *Rs* reported by Belles et al. [15] for POCIS-nylon samplers when comparing overlapping chemicals (n=9). Specifically, similar *Rs* values were reported for atrazine (1100 mL/d), azoxystrobin (870 mL/d), and propiconazole (2700 mL/d) [15]. Results reported herein suggest *Rs* approximately ten times greater for bifenthrin (30 mL/d), chlorpyrifos (150 mL/d), and tebuconazole (380 mL/d); however, Belles et al. [15] report higher sampling rates for acetochlor (2800 mL/d), desethyl atrazine (1200 mL/d), and metolachlor (1800 mL/d). Unfortunately, direct comparisons are still challenging because flow across samplers was not explicitly explained in previous work and data herein suggests that the flow can significantly influence *Rs* for these sampler configurations.

3.2.1 Influence of flow

Median *Rs* were higher under flow conditions for each sampler configuration (Fig. 2). Median *Rs* values were 9.5 times greater under flow conditions for NOCIS containing Dowex Optipore L-493 and 4.8 times greater for samplers containing Oasis HLB (Fig. 2). The difference was even greater for Sentinel Samplers with median *Rs* values 26.6 times higher under flow conditions as compared to static conditions (Fig. 2). This significant treatment effect for flow was evident for most analytes with higher *Rs* under flowing conditions as compared to static conditions ($p < 0.05$; Table 4, Fig. 2, Fig. S3). Specifically, there was a significant flow effect on *Rs* for all analytes detected in Sentinel Samplers (n=32). Flow also affected *Rs* for most analytes within NOCIS configurations with Dowex Optipore L-493 (n=27) and Oasis HLB (n=26); however, the affect was much lower resulting in statically similar ($p > 0.05$) *Rs* for a few analytes (Table 4, Fig. S3). The magnitude of the flow affect suggests that diffusion through and thickness of the aqueous boundary layer is the rate-limiting step of accumulation as opposed to permeation/diffusion through the macroporous nylon and stainless steel membrane. Simplistically, increased flow decreases the aqueous boundary layer thickness, resulting in increased analyte mass transfer and ultimately sampling rates. Similar influence of variable hydrodynamic conditions on *Rs* have been previously observed for several passive sampler configurations [9, 11, 19-25]. For instance, two- to three-fold differences in *Rs* have been reported classical POCIS configurations when comparing flow and static exposures and field evaluations spanning 2.6 – 37 cm/s [19, 20]. Similarly, Charlestra et al. [21] reported approximately two-fold differences in *Rs* for several pesticides under static, mixed, and flow (i.e. 0.32 cm/s) systems using the classical POCIS configuration. Even greater *Rs* flow dependencies have been observed for some Chemcatcher configurations with five-fold differences reported for pharmaceuticals experiencing 30 – 370 cm/s flow scenarios [24, 25]. Comparison of our NOCIS-HLB flow dependency (median difference of 5x) to the 2-3 fold affect in magnitude for classical

POCIS configurations [19, 20] suggests that the ability to measure hydrophobic contaminants is a trade off with increased flow dependence [9, 11].

Recently, the use of performance reference compounds (PRCs) for in-situ flow calibrations has been presented as viable method to improve calibration for POCIS-style samplers [10, 11, 15, 26]. Performance reference compounds have been effective for equilibrium-based passive samplers as stable isotope compounds can be spiked into samplers prior to deployment and dissipation occurs proportional with uptake [27]. However, the use of PRCs is more problematic for POCIS-style samplers because the integrative nature restricts fugacity out of stable isotope compounds [10, 11]. Regardless, several analytes have been suggested to have high fugacity out of classical POCIS samplers including desisopropyl atrazine-d_5 [10, 15, 26] and caffeine-C_{13} [15, 16]. These PRCs are especially important for adjusting *Rs* when analyte accumulation controlled by the aqueous boundary layer rather than diffusion through the membrane [15]. Further investigation using several hydrodynamic conditions is warranted to increase the utility of these PRCs with the NOCIS configurations investigated herein; however, the Osorb media contained within Sentinel Samplers complicates PRC incorporation because the samplers are stored in ethanol prior to deployment and the media is not water-wettable. Regardless, Sentinel Samplers were the most affected by flow variations and would therefore benefit from *in-situ* flow calibrations.

3.2.2 Influence of physiochemical properties

Data suggests two distinct trends of analyte accumulation based on physiochemical properties (i.e. log *Kow*). Specifically, log *Rs* increased as log *Kow* increased for fungicides, herbicides, and personal care products (log *Kow* < 6.0); however, log *Rs* for insecticides and PAHs (log *Kow*: 5-7.6), decreased as log *Kow* increased (Fig. 3). Similar trends were observed in static systems; however, regressions for insecticides and PAHs were not significant ($p > 0.05$) for NOCIS samplers (Fig. S4). Regressions are provided to better understand observed *Rs* trends but

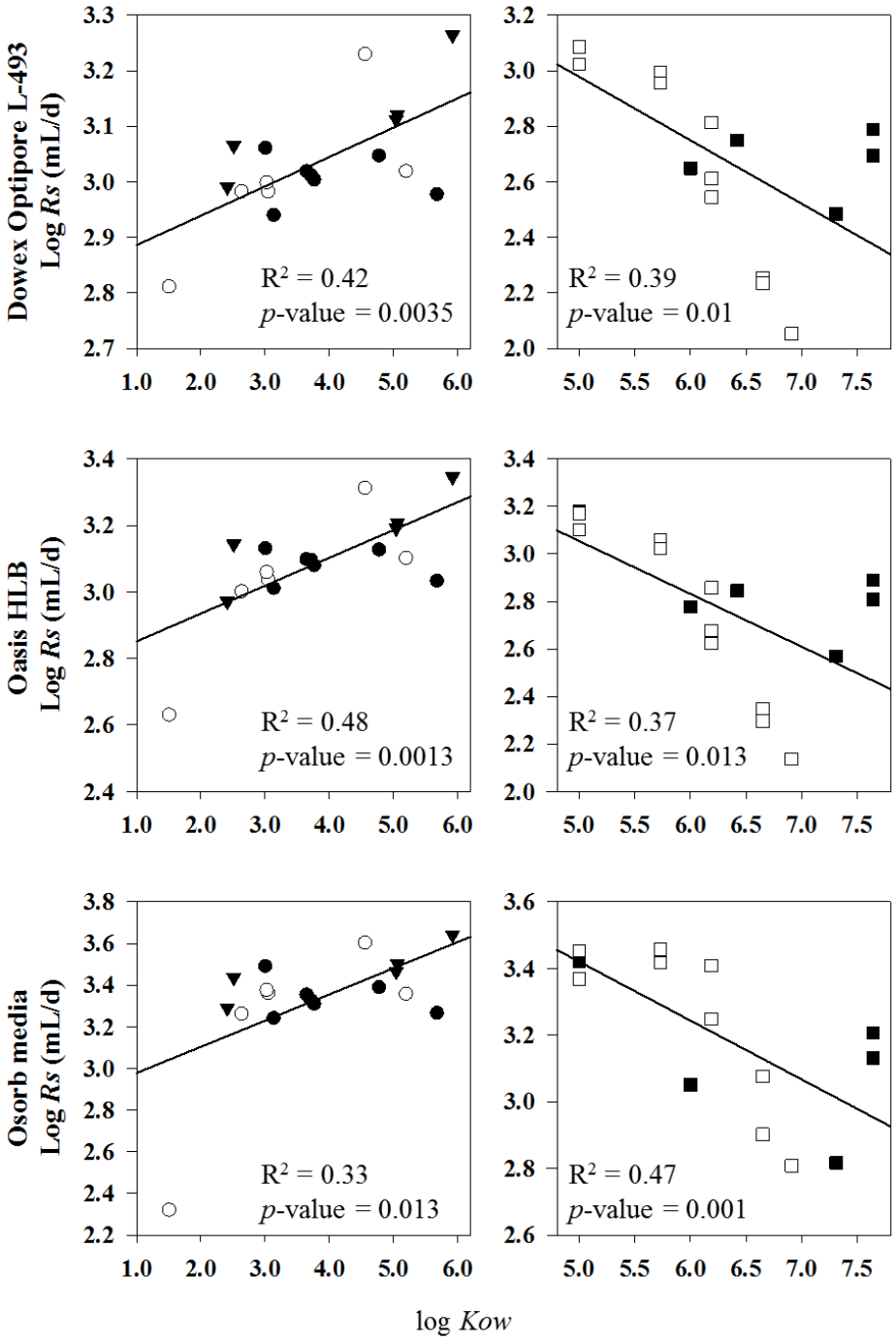

Fig. 3. Linear regression analysis between log *Rs* with log *Kow* for each passive sampler sorbent under flow conditions. Results for fungicides (●), herbicides (○), and personal care products (▼) are displayed in the left panel while insecticides (■) and PAHs (□) are in the right panel.

are not meant to provide a predictive model to estimate analyte *Rs* based on log *Kow*. Despite widespread use, contaminant uptake mechanisms for POCIS remains poorly understood [28]. Although nylon membranes should offer simpler transport mechanisms compared to PES membranes, uptake is likely more complicated than simple diffusion and should be researched further and compared with other sampler types. For instances, although our results suggest two distinct trends, Ahrens et al. [14] reported a single linear trend for silicone rubber and an analyte list with log *Kow* range from -2.6 to 7.0 where log *Rs* values where directly proportional to hydrophobicity. Therefore, it remains important to conduct laboratory calibration experiments to determine analyte *Rs* due to data gaps in literature describing predictive accumulation models for POICS-style passive samplers based on analyte physiochemical properties [29].

3.3 Influence of pulsed exposure

Analyte accumulation into passive samplers was determined at 8 d for each treatment under flowing conditions, including 8 d continuous exposure, and the 4 d pulsed exposure followed by 4 d in clean water. Accumulation is expressed as sampling rates for both exposure scenarios (Fig. 4, Table S5). Comparison of *Rs* values under the two exposure conditions provides an assessment tool to determine if integrative sampling is occurring. Samplers should yield similar *Rs* under various exposure conditions to be considered integrative. Median *Rs* for NOCIS containing Dowex Optipore L-493 were 908 mL/d and 870 mL/d under continuous and pulsed exposures, respectively. Similarly, median *Rs* for NOCIS containing Oasis HLB were 992 mL/d and 1028 mL/d under continuous and pulsed exposures, respectively. These *Rs* values were also consistent with *Rs* values from the flow treatment of the calibration study (Fig. 2). Moreover, ANOVA results determined accumulation was similar ($p > 0.05$) for all analytes under both continuous and pulsed exposure scenarios for NOCIS containing Dowex Optipore L-493 and Oasis HLB (Table S4). Although no statistical differences in *Rs* were observed on an analyte basis, analysis of percent differences between analyte *Rs* obtained under continuous and pulsed scenarios suggest slight variations occurred (Table S4). On an analyte basis, the mean (±SD)

percent difference of *Rs* between continuous and pulsed exposures (calculated by %diff = [continuous – pulse]/continuous) were -11 ± 2% (range: -69% to 35 %) and -33 ± 46% (range: -186% to 11%) for NOCIS samplers containing Dowex Optipore L-493 and Oasis HLB, respectively (Table S4, Fig. 4). Negative values indicate higher *Rs* during the pulsed exposure scenario. Linear regression analysis plotting pulsed exposure *Rs* as a function of continuous exposure *Rs* values yielded slopes of m = 0.96 and m = 1.01 for Dowex Optipore L-493 (R^2 = 0.95, $p < 0.0001$) and Oasis HLB (R^2 = 1.01, $p < 0.0001$), respectively (Fig. 4). In contrast, Sentinel Samplers demonstrated differences in *Rs* values between exposure scenarios with median *Rs* values of 1300 mL/d and 740 mL/d for continuous and pulsed exposures, respectively ($p < 0.05$; Table S4, Fig. 4). Moreover, median sampling rates were reduced approximately 50% from median *Rs* of the flow treatment calibration study (Fig. 2). Analysis of variance showed statistically similar *Rs* ($p > 0.05$) between exposure scenarios for twelve analytes that consisted of octinoxate, pyrethroid insecticides (n=5) and heavier PAHs (n= 6; Table S4). On an analyte basis, the mean (±SD) percent difference of *Rs* between continuous and pulsed exposures for Sentinel Samplers (calculated by %diff = [continuous – pulse]/continuous) was 38 ± 21% (range: -17% to 63%; Table S4). Positive values indicate higher *Rs* during the continuous exposure scenario. For Sentinel Samplers with Osorb media, linear regression analysis plotting pulsed exposure *Rs* as a function of continuous exposure *Rs* values yielded a slope of m = 0.51 (R^2 = 0.84, $p < 0.0001$; Fig. 4).

Despite some variability between experiments, ANOVA results and regression analysis with slopes approximating one suggest that analyte accumulation for both NOCIS configurations will be integrative during pulsed exposures, provided that analyte accumulation occurs linearly and assuming the pulse duration does not exceed the kinetic phase of the sampler. Although this concept is theoretically understood, few studies have investigated pulsed exposures with integrative samplers [16, 30, 31]. Overall analyte accumulation in NOCIS configurations under both continuous and pulsed exposures were in good agreement between treatments (i.e. ± 30%).

Collectively, data suggests *Rs* values for Sentinel Samplers were the most sensitive to flow variations (Table 4, Fig. 2), which could account for differences in *Rs* values between experiments. It is plausible that subtle variations in deployment depth and/or sampler orientation between and throughout experiments could result in flow variations across the sampler and ultimately higher variation in *Rs* values. As such, observed variations could be an artifact of deployment method of these samplers based on the a priori decision to follow directions provided by the manufacturer and suspend samplers using a nylon tether rather than securely bolt the samplers in place (as done for NOCIS configurations). As a result, Sentinel Samplers were subject to subtle movement within experimental units that could result in subtle changes in flow across the sampler throughout all experiments.

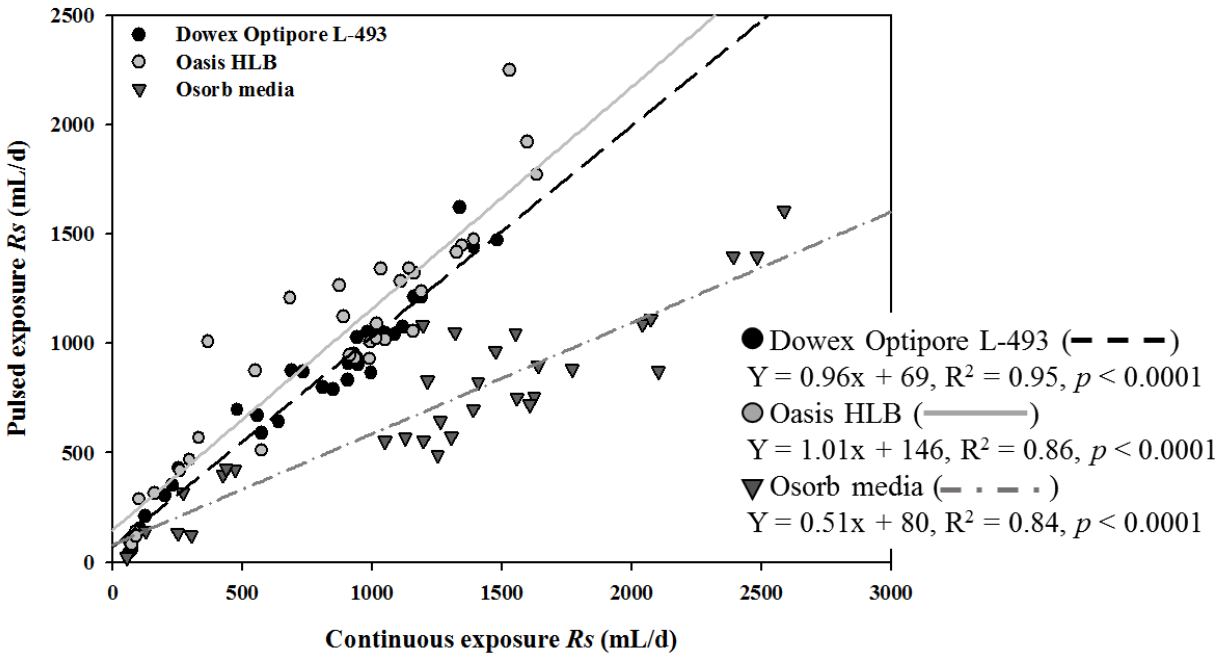

Fig. 4. Linear regression comparing analyte sampling rates (Rs) obtained from continuous 8 d exposure verses pulsed exposure (i.e. 4 d contamination + 4 d clean water). A slope of m = 1 indicates agreement between experiments and suggests analyte accumulation is integrative.

4. Conclusions

The three integrative sampler configurations investigated integratively accumulated fungicides, herbicides, insecticides, PAHs, and PCPs that spanned a broad range of hydrophobicities (log *Kow* range: 1.5-7.6). Moreover, integrative sampling occurred under two hydrodynamic conditions and during 4 d pulsed exposure followed by 4 d in clean water. The highly porous membranes increased sampling rates and capacity to integratively measure hydrophobic contaminants; however, these characteristics translated to flow dependent sampling rates for each sampler configuration investigated. Due to the high degree of flow dependence observed for most analytes sampling rates, future investigation should focus on the use of PRCs or other flow adjustment strategies under several hydrodynamic conditions. Regardless, this tradeoff seems reasonable to achieve integrative measurements of pulsed exposures of hydrophobic contaminants typically missed by classical POCIS configurations. Moreover, obtaining time-weighted average concentrations over shorter durations may be more comparable when comparing data obtained from field investigations to thresholds of concern established in standard 96-h and 10 d toxicity tests.

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Appendix

Supporting Information:

CALIBRATION OF NYLON ORGANIC CHEMICAL INTEGRATIVE PASSIVE SAMPLERS AND SENTINEL PASSIVE SAMPLERS FOR QUANTITATIVE MEASUREMENT OF PULSED AQUATIC EXPOSURES

Summary of Contents:

Table S1. Analyte analysis method, retention time (RT), select ions monitored (SIM). The two qualitative (Qual) ions had to be within 20% of expected ratio, relative to the quantitative (Quant) ion response, to report the analyte without flagging the data as qualitatively uncertain.

Analyte	GC Method	RT (min)	SIM ions Quantitative: Qualitative (m/z)
DEET	1	6.85	119:190, 191
Desethyl atrazine	1	7.76	172:174, 187
Trifluralin	1	7.98	306:264, 290
Atrazine-d_5 (surrogate)	1	8.69	205:178, 220
Atrazine	1	8.72	200:173, 215
Chlorothalonil	1	8.99	266:264, 268
Galaxolide	1	9.56	243:258, 213
Musk tonalid	1	9.66	243:258, 187
Acetochlor	1	9.89	146:162, 223
Metolachlor	1	10.79	162:238, 240
Musk ketone	1	10.82	279:128, 294
Chlorpyrifos	1	10.88	197:258, 314
Pendimethalin	1	11.58	252:253, 281
Fluoranthene	1	11.68	202:200, 203
Pyrene	1	12.22	202:200, 203
Octinoxate	1	14.50	178:161,179
Tebuconazole	1	14.91	125:163, 250
Propiconazole	2	9.75	259:173, 191
Trifloxystrobin	2	9.94	116:131, 222
Benzo(a)anthracene-d_{16} (surrogate)	2	10.82	240
Benzo(a)anthracene	2	10.75	228:226
Chrysene	2	10.84	228:226
Bifenthrin	2	11.27	181:165, 166
Metconazole	2	11.41	125:250, 319
λ-Cyhalothrin	2	12.66	181:141, 197
Trans-permethrin	2	13.58	183:163, 164
Cis-permethrin	2	13.78	183:163, 165
Benzo(b)fluoranthene	2	13.88	252:253
Benzo(k)fluoranthene	2	13.96	252:254
Cyfluthrin	2	14.71	163:165, 226
Benzo(a)pyrene	2	14.83	252:250
Pyraclostrobin	2	16.06	132:164, 325
Azoxystrobin	2	17.14	344:388, 403
Benzo(g,h,i)perylene	2	17.14	276:277
Dibenz(a,h)anthracene	2	17.72	278:276

Indeno(1,2,3-cd)pyrene	2	18.02	276:277

Table S2. Mean (standard deviation) analyte extraction recoveries from fortified quality control samples for the three sorbent medias and water. Expected recoveries are based on analysis of spiking solutions (n=18) conducted throughout all experiments.

Analyte	Percentage of expected (SD)			
	Dowex Optipore L-493 (n=4)	Oasis HLB (n=4)	Osorb media (n=4)	Water (n=15)
Acetochlor	94 (10)	88 (12)	80 (2)	88 (11)
Atrazine	96 (9)	90 (12)	82 (2)	105 (9)
Azoxystrobin	96 (27)	94 (9)	110 (17)	137 (14)
Benzo(a)anthracene	85 (9)	100 (11)	109 (16)	105 (9)
Benzo(a)pyrene	83 (8)	109 (9)	nd	89 (13)
Benzo(b)fluoranthene	97 (15)	121 (9)	123 (11)	69 (10)
Benzo(g,h,i)perylene	87 (19)	119 (11)	118 (21)	117 (15)
Benzo(k)fluoranthene	74 (3)	100 (14)	90 (16)	99 (14)
Bifenthrin	83 (8)	77 (15)	108 (11)	94 (14)
Chlorothalonil	127 (14)	114 (16)	103 (11)	148 (11)
Chlorpyrifos	85 (7)	80 (13)	92 (3)	93 (15)
Chrysene	82 (6)	98 (9)	93 (26)	91 (12)
Cis-permethrin	94 (15)	89 (12)	105 (19)	122 (12)
Cyfluthrin	96 (17)	87 (13)	nd	123 (11)
λ-Cyhalothrin	103 (16)	89 (14)	112 (14)	111 (13)
DEET	100 (14)	82 (14)	86 (7)	105 (19)
Desethyl atrazine	97 (9)	89 (16)	63 (1)	123 (11)
Dibenz(a,h)anthracene	115 (18)	135 (18)	127 (21)	119 (10)
Fluoranthene	95 (7)	99 (13)	107 (7)	92 (15)
Galaxolide	91 (13)	81 (12)	80 (2)	45 (7)
Indeno(1,2,3-cd)pyrene	75 (9)	109 (11)	103 (21)	114 (14)
Metconazole	68 (7)	71 (12)	73 (20)	114 (14)

Metolachlor	94 (10)	86 (13)	77 (3)	106 (16)
Musk ketone	91 (10)	85 (13)	78 (2)	103 (14)
Musk Tonalid	88 (12)	80 (12)	74 (2)	69 (11)
Octinoxate	85 (7)	83 (10)	99 (6)	75 (12)
Pendimethalin	83 (8)	79 (13)	89 (4)	85 (11)
Propiconazole	76 (5)	77 (12)	99 (9)	96 (13)
Pyraclostrobin	95 (16)	81 (17)	101 (15)	144 (14)
Pyrene	93 (6)	100 (13)	108 (7)	93 (10)
Tebuconazole	66 (7)	66 (9)	76 (8)	118 (13)
Trans-permethrin	102 (18)	96 (11)	115 (12)	126 (12)
Trifloxystrobin	77 (4)	74 (14)	96 (8)	100 (13)
Trifluralin	92 (15)	80 (13)	88 (4)	46 (8)

Table S3. Mean (standard error) measured and percentage of expected of water concentrations in experimental units during the calibration study for both flow and static treatments. For each flow treatment, water samples were obtained daily before water exchanges (n=8) and after 5-h after exchanges (n=8). Expected recoveries are based on analysis of spiking solutions (n=18) conducted throughout all experiments.

Treatment	Flow (n=16)		Static (n=16)	
Analyte	Measured (ng/mL)	Percent of expected	Measured (ng/mL)	Percent of expected
Acetochlor	0.876 (0.023)	96.6 (2.5)	0.922 (0.010)	101.7 (1.1)
Atrazine	0.921 (0.019)	107.5 (2.3)	0.954 (0.011)	111.4 (1.3)
Azoxystrobin	1.157 (0.038)	122.3 (4.0)	1.204 (0.038)	127.4 (4.1)
Benzo(a)anthracene	0.066 (0.005)	37.6 (2.8)	0.074 (0.007)	42.4 (4.0)
Benzo(a)pyrene	0.021 (0.003)	13.3 (2.1)	0.031 (0.007)	19.2 (4.2)
Benzo(b)fluoranthene	0.079 (0.005)	45.7 (3.0)	0.093 (0.007)	54.3 (4.1)
Benzo(g,h,i)perylene	0.063 (0.007)	34.3 (3.8)	0.092 (0.011)	50.0 (6.1)
Benzo(k)fluoranthene	0.072 (0.005)	40.2 (2.7)	0.091 (0.008)	50.4 (4.2)
Bifenthrin	0.084 (0.005)	44.6 (2.7)	0.101 (0.010)	53.9 (5.1)

Chlorothalonil	1.022 (0.047)	155.4 (7.2)	1.086 (0.074)	165.1 (11.3)
Chlorpyrifos	0.121 (0.004)	64.7 (2.3)	0.111 (0.005)	59.7 (2.6)
Chrysene	0.088 (0.004)	51.3 (2.5)	0.098 (0.006)	57.3 (3.5)
Cis-permethrin	0.085 (0.006)	44.9 (3.4)	0.112 (0.011)	59.2 (5.6)
Cyfluthrin	0.090 (0.006)	48.1 (3.1)	0.121 (0.012)	64.2 (6.4)
λ-Cyhalothrin	0.076 (0.006)	42.4 (3.2)	0.101 (0.012)	55.9 (6.6)
DEET	0.891 (0.022)	97.9 (2.4)	0.923 (0.013)	101.4 (1.5)
Desethyl atrazine	1.045 (0.015)	121.3 (1.8)	1.034 (0.011)	120.0 (1.3)
Dibenz(a,h)anthracene	0.069 (0.008)	39.7 (4.7)	0.107 (0.014)	61.4 (8.1)
Fluoranthene	0.155 (0.004)	88.4 (2.5)	0.149 (0.006)	85.0 (3.7)
Galaxolide	0.423 (0.024)	47.9 (2.8)	0.357 (0.010)	40.4 (1.2)
Indeno(1,2,3-cd)pyrene	0.064 (0.007)	35.7 (4.0)	0.095 (0.012)	53.3 (6.7)
Metconazole	0.882 (0.020)	92.9 (2.1)	0.918 (0.015)	96.7 (1.6)
Metolachlor	0.827 (0.019)	94.8 (2.2)	0.870 (0.011)	99.7 (1.3)
Musk ketone	0.731 (0.021)	83.0 (2.4)	0.714 (0.026)	81.1 (2.9)
Musk Tonalid	0.459 (0.023)	48.8 (2.4)	0.381 (0.015)	40.6 (1.6)
Octinoxate	0.241 (0.041)	21.6 (3.7)	0.299 (0.036)	26.9 (3.2)
Pendimethalin	0.804 (0.029)	78.3 (2.8)	0.776 (0.048)	75.6 (4.7)
Propiconazole	0.835 (0.021)	85.2 (2.2)	0.874 (0.013)	89.1 (1.4)
Pyraclostrobin	1.231 (0.032)	123.9 (3.2)	1.187 (0.035)	119.5 (3.5)
Pyrene	0.116 (0.005)	67.6 (3.0)	0.115 (0.006)	67.2 (3.4)
Tebuconazole	0.951 (0.033)	94.1 (3.3)	1.031 (0.025)	102.0 (2.5)
Trans-permethrin	0.106 (0.007)	55.4 (3.5)	0.124 (0.012)	64.8 (6.0)
Trifloxystrobin	0.715 (0.030)	70.2 (3.0)	0.692 (0.029)	68.0 (2.8)
Trifluralin	0.251 (0.023)	28.6 (2.7)	0.183 (0.009)	20.9 (1.1)

Table S4. Mean measured and percentage of expected of water concentrations in experimental units during integration study (i.e. pulsed and continuous exposures) for both flow and static treatments. Values are provided with standard error. During the depuration period of the pulsed exposure, water concentrations were less than 10% of fortified concentrations (n=3). Expected recoveries are based on analysis of spiking solutions (n=18) conducted throughout all experiments.

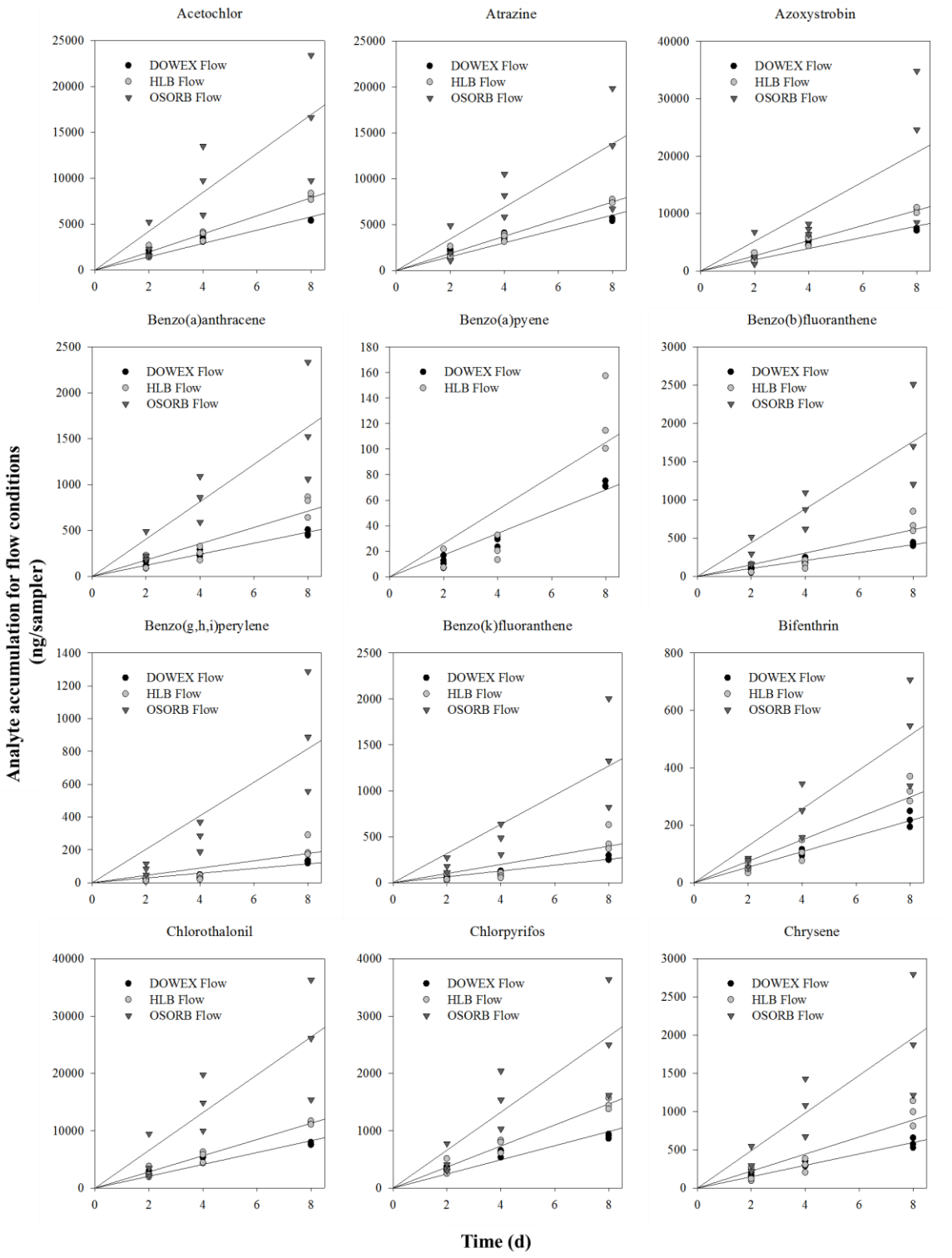
Treatment	Continuous (8 d, n=4)		Pulsed (4 d, n=3)	
Analyte	Measured (ng/mL)	Percent of expected	Measured (ng/mL)	Percent of expected
Acetochlor	0.358 (0.018)	80.9 (4.2)	0.776 (0.066)	87.7 (7.5)
Atrazine	0.464 (0.025)	98.8 (5.4)	0.949 (0.055)	100.9 (5.9)
Azoxystrobin	0.531 (0.019)	111.2 (4.0)	1.091 (0.031)	114.4 (3.3)
Benzo(a)anthracene	0.016 (0.002)	18.9 (1.9)	0.030 (0.003)	17.5 (2.0)
Benzo(a)pyrene	0.004 (0.000)	5.2 (0.5)	0.008 (0.001)	4.9 (0.7)
Benzo(b)fluoranthene	0.037 (0.006)	49.7 (5.8)	0.046 (0.012)	39.1 (1.2)
Benzo(g,h,i)perylene	0.040 (0.005)	41.8 (6.7)	0.064 (0.002)	26.2 (6.9)
Benzo(k)fluoranthene	0.031 (0.004)	37.6 (4.5)	0.049 (0.003)	29.5 (1.7)
Bifenthrin	0.099 (0.024)	107.4 (26.4)	0.213 (0.018)	114.9 (9.7)
Chlorothalonil	0.518 (0.026)	146.6 (7.2)	1.116 (0.024)	157.8 (3.4)
Chlorpyrifos	0.054 (0.002)	57.1 (2.6)	0.118 (0.008)	62.7 (4.2)
Chrysene	0.042 (0.004)	50.9 (4.3)	0.073 (0.005)	44.1 (3.0)
Cis-permethrin	0.083 (0.014)	91.0 (14.9)	0.159 (0.000)	87.4 (0.2)
Cyfluthrin	0.076 (0.013)	77.3 (13.5)	0.136 (0.009)	68.6 (4.3)
λ-Cyhalothrin	0.090 (0.020)	97.6 (22.2)	0.162 (0.001)	88.1 (0.6)
DEET	0.383 (0.018)	86.5 (4.0)	0.807 (0.075)	91.1 (8.5)
Desethyl atrazine	0.485 (0.029)	102.8 (6.2)	1.017 (0.095)	107.7 (10.1)
Dibenz(a,h)anthracene	0.038 (0.007)	45.2 (8.5)	0.098 (0.025)	58.4 (15.1)
Fluoranthene	0.079 (0.012)	92.3 (14.5)	0.145 (0.034)	85.0 (20.1)
Galaxolide	0.200 (0.014)	44.8 (3.2)	0.484 (0.031)	54.1 (3.5)
Indeno(1,2,3-cd)pyrene	0.039 (0.008)	46.2 (9.0)	0.083 (0.028)	48.8 (16.5)
Metconazole	0.398 (0.025)	84.0 (5.3)	0.895 (0.115)	94.4 (12.1)

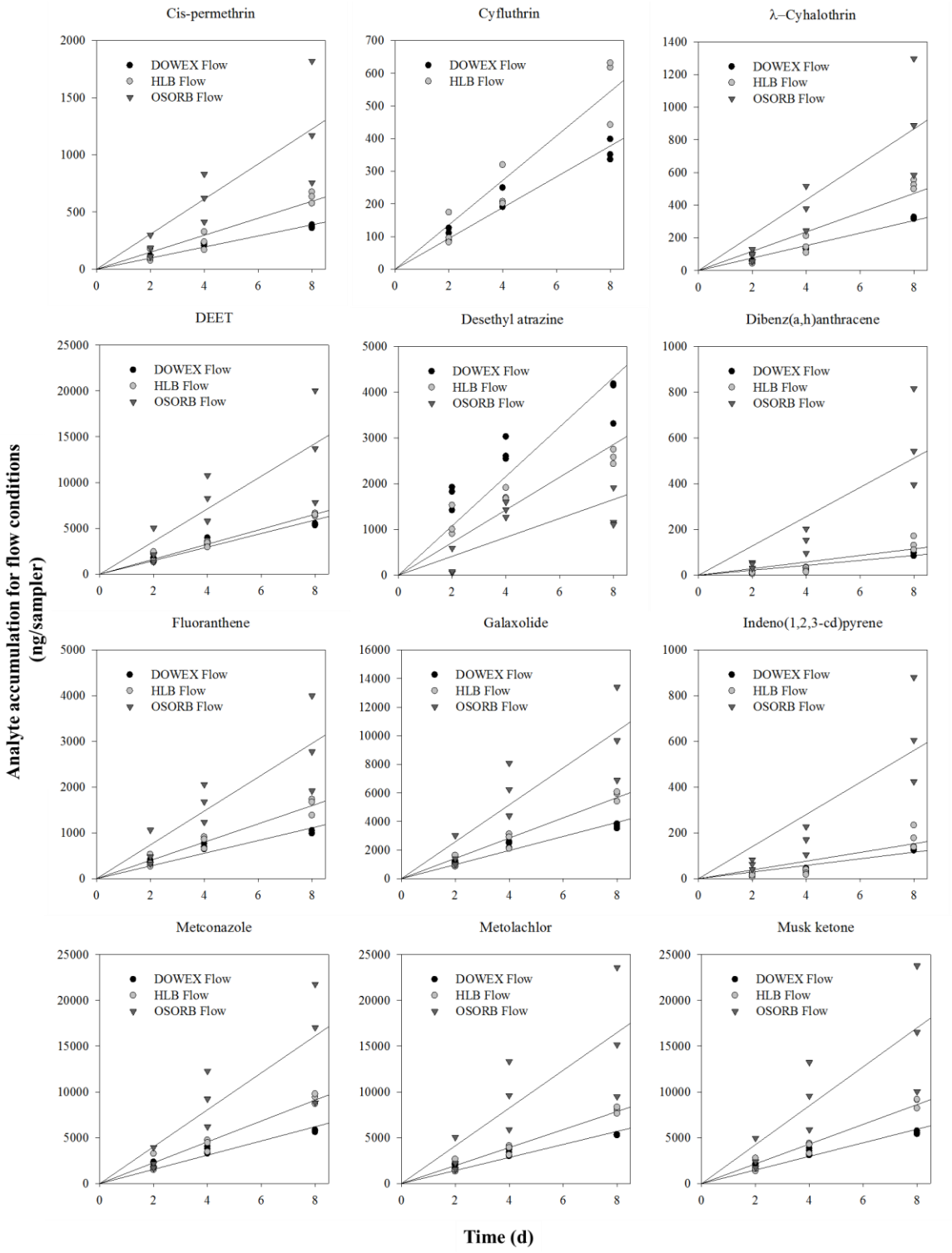
Metolachlor	0.359 (0.020)	79.1 (4.4)	0.800 (0.068)	88.3 (7.5)
Musk ketone	0.329 (0.020)	71.8 (4.3)	0.711 (0.048)	77.4 (5.2)
Musk tonalid	0.213 (0.011)	47.1 (2.5)	0.508 (0.045)	56.3 (5.0)
Octinoxate	0.236 (0.017)	45.3 (3.3)	0.501 (0.041)	48.0 (3.9)
Pendimethalin	0.298 (0.022)	62.7 (4.6)	0.620 (0.045)	65.3 (4.7)
Propiconazole	0.430 (0.027)	87.5 (5.4)	0.921 (0.169)	93.6 (17.2)
Pyraclostrobin	0.568 (0.006)	113.5 (1.2)	1.194 (0.021)	119.4 (2.1)
Pyrene	0.041 (0.003)	47.9 (3.8)	0.082 (0.014)	47.8 (7.9)
Tebuconazole	0.472 (0.031)	99.3 (6.4)	1.058 (0.196)	111.2 (20.6)
Trans-permethrin	0.089 (0.015)	98.0 (16.3)	0.167 (0.000)	92.0 (0.1)
Trifloxystrobin	0.361 (0.018)	75.8 (3.7)	0.774 (0.111)	81.2 (11.6)
Trifluralin	0.143 (0.017)	30.7 (3.6)	0.381 (0.023)	40.9 (2.4)

Table S5. Mean (SD) sampling rates (*Rs*) obtained from continuous and pulsed experiments. Average percentage of difference between the two studies is provided as an evaluation of agreement between exposure scenarios.

Analyte	Dowex Optipore L-493			Oasis HLB			Osorb media		
	Rs (SD) (mL/d)		Percent difference	*Rs* (SD) (mL/d)		Percent difference	*Rs* (SD) (mL/d)		Percent difference
	Continuous	Pulse	(%)	Continuous	Pulse	(%)	Continuous	Pulse	(%)
Acetochlor	944 (264)	931 (146)	1.4	1019 (110)	1034 (193)	-1.4	1625 (199)	756 (72)	53.5
Atrazine	929 (243)	954 (150)	-2.6	994 (110)	1009 (161)	-1.6	1049 (116)	553 (31)	47.3
Azoxystrobin	1120 (374)	1074 (187)	4.1	1110 (163)	1286 (330)	-15.9	1129 (120)	568 (87)	49.7
Benzo(a)anthrancene	1340 (436)	1622 (279)	-21.0	1530 (527)	2252 (720)	-47.2	2589 (1239)	1606 (212)	38.0
Benzo(a)pyrene	575 (348)	588 (64)	-2.2	684 (306)	1208 (612)	-76.6			
Benzo(b)fluoranthene	481 (263)	697 (169)	-45.1	368 (302)	1010 (388)	-174.3	1196 (630)	1083 (311)	9.4
Benzo(g,h,i)perylene	105 (68)	151 (53)	-43.7	101 (56)	288 (176)	-186.1	438 (297)	428 (176)	2.3
Benzo(k)fluoranthene	560 (370)	670 (162)	-19.7	550 (181)	877 (402)	-59.6	1320 (753)	1051 (303)	20.4
Bifenthrin	66 (53)	93 (18)	-41.1	88 (55)	137 (28)	-55.4	128 (94)	142 (41)	-11.2
Chlorothalonil	996 (338)	864 (171)	13.2	1158 (165)	1055 (243)	8.9	2105 (344)	873 (126)	58.5
Chlorpyrifos	1012 (232)	1061 (173)	-4.8	1328 (49)	1417 (346)	-6.7	1558 (299)	751 (142)	51.8
Chrysene	734 (99)	870 (148)	-18.6	875 (204)	1267 (468)	-44.8	1553 (475)	1045 (214)	32.7
Cis-permethrin	230 (153)	352 (60)	-53.1	295 (166)	468 (108)	-58.5	473 (311)	424 (91)	10.4
Cyfluthrin	254 (165)	431 (57)	-69.5	332 (174)	569 (132)	-71.5			
λ-Cyhalothrin	124 (85)	209 (34)	-67.8	163 (98)	313 (65)	-91.7	272 (151)	320 (68)	-17.7
DEET	912 (244)	922 (150)	-1.1	936 (99)	931 (139)	0.5	1264 (117)	645 (47)	49.0
Desethyl atrazine	640 (170)	641 (81)	0.0	574 (58)	511 (46)	11.0	55 (14)	20 (4)	63.0
Dibenz(a,h)anthracene	61 (38)	40 (10)	35.1	73 (36)	80 (40)	-9.6	303 (179)	122 (48)	59.6
Fluoranthene	941 (178)	1028 (189)	-9.2	1034 (93)	1341 (325)	-29.7	1475 (223)	967 (120)	34.5
Galaxolide	1162 (301)	1213 (189)	-4.3	1348 (24)	1448 (303)	-7.5	2043 (165)	1089 (86)	46.7
Indeno(1,2,3-cd)pyrene	74 (43)	57 (10)	23.6	89 (48)	120 (60)	-34.9	251 (158)	131 (54)	48.0
Metconazole	907 (280)	834 (159)	8.0	1016 (122)	1023 (221)	-0.7	1305 (163)	573 (44)	56.1
Metolachlor	947 (260)	906 (151)	4.4	1050 (114)	1020 (197)	2.9	1609 (192)	720 (64)	55.3

Musk ketone	1053 (268)	1046 (163)	0.7	1190 (104)	1236 (242)	-3.9	1771 (197)	882 (88)	50.2
Musk tonalid	1190 (309)	1213 (192)	-2.0	1393 (47)	1475 (312)	-5.9	2074 (158)	1111 (98)	46.4
Octinoxate	691 (263)	877 (120)	-26.8	890 (328)	1123 (280)	-26.1	1215 (523)	830 (119)	31.7
Pendimethalin	982 (232)	1053 (162)	-7.3	1162 (124)	1324 (308)	-13.9	1639 (263)	899 (97)	45.2
Propiconazole	809 (250)	800 (173)	1.1	916 (92)	946 (199)	-3.3	1198 (182)	556 (51)	53.5
Pyraclostrobin	1088 (359)	1041 (157)	4.3	1144 (161)	1343 (339)	-17.4	1409 (169)	823 (100)	41.6
Pyrene	1483 (298)	1472 (249)	0.8	1600 (186)	1921 (475)	-20.1	2392 (448)	1398 (147)	41.6
Tebuconazole	851 (212)	791 (154)	7.1	990 (122)	930 (178)	6.1	1253 (182)	489 (105)	61.0
Trans-permethrin	202 (146)	304 (57)	-50.7	259 (140)	420 (94)	-61.8	426 (276)	398 (91)	6.5
Trifloxystrobin	909 (272)	908 (156)	0.1	1018 (99)	1090 (242)	-7.0	1389 (233)	698 (61)	49.8
Trifluralin	1393 (374)	1442 (229)	-3.5	1634 (80)	1772 (402)	-8.4	2484 (185)	1396 (143)	43.8


Acetochlor
Atrazine
Azoxystrobin
Benzo(a)anthracene
Benzo(a)pyene
Benzo(b)fluoranthene
Benzo(g,h,i)perylene
Benzo(k)fluoranthene
Bifenthrin
Chlorothalonil
Chlorpyrifos
Chrysene
DOWEX Flow
HLB Flow
OSORB Flow
Analyte accumulation for flow conditions (ng/sampler)
Time (d)


Cis-permethrin
Cyfluthrin
λ–Cyhalothrin
DEET
Desethyl atrazine
Dibenz(a,h)anthracene
Fluoranthene
Galaxolide
Indeno(1,2,3-cd)pyrene
Metconazole
Metolachlor
Musk ketone
DOWEX Flow
HLB Flow
OSORB Flow
Analyte accumulation for flow conditions (ng/sampler)
Time (d)

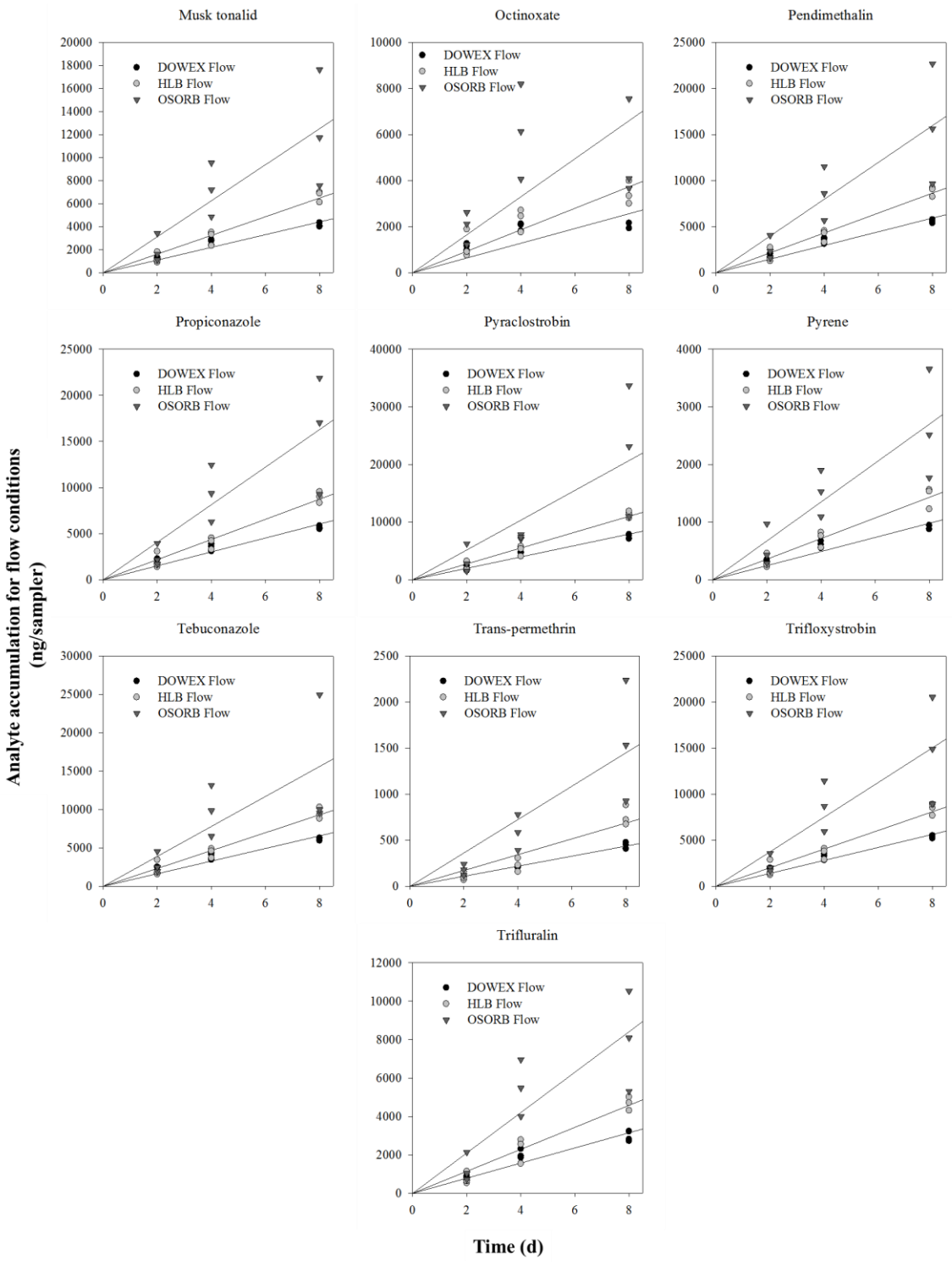
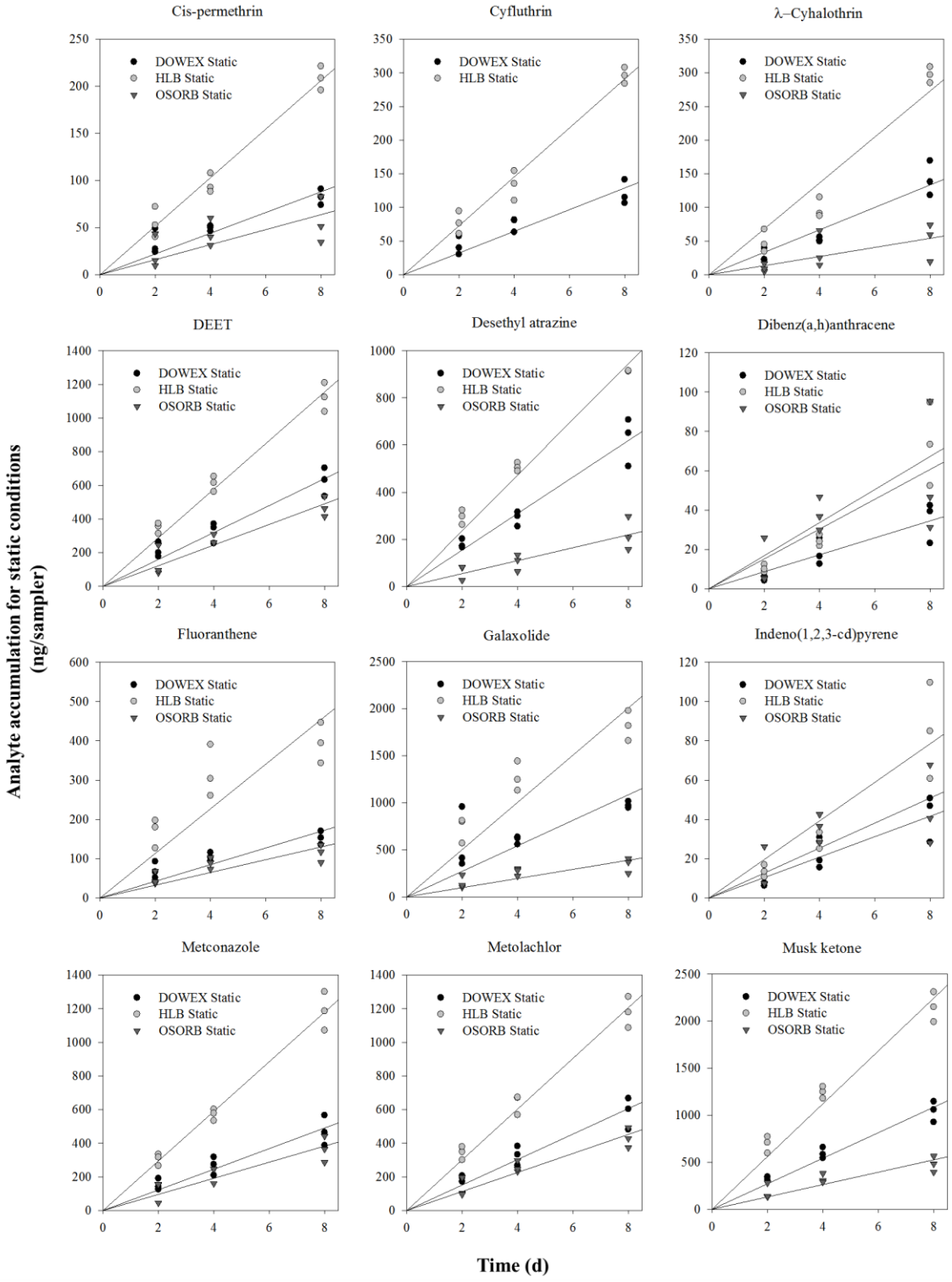
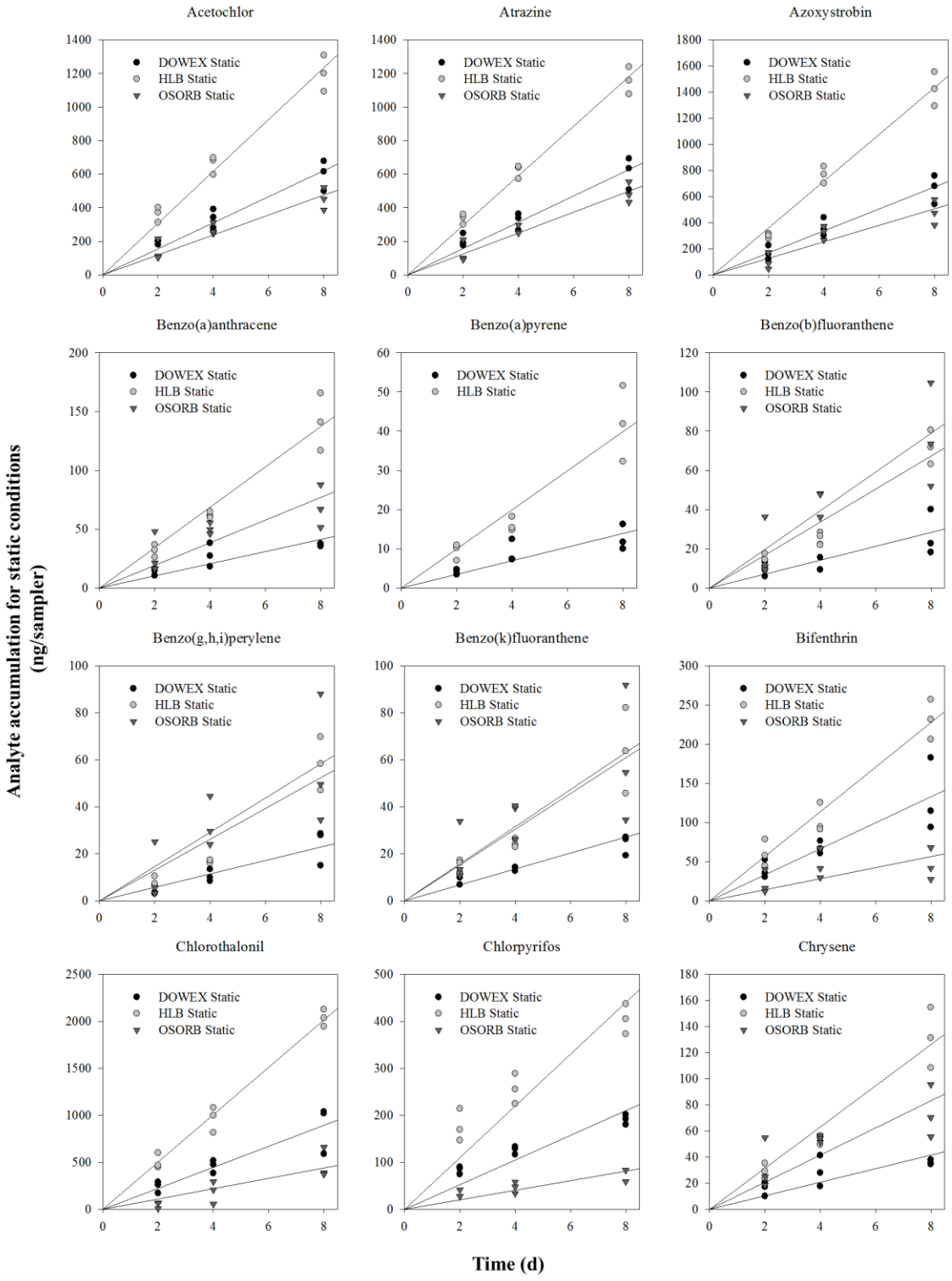


Fig. S1. Passive sampler linear regressions for each analyte under flow conditions. Regression fitting provided in Table 3.


Cis-permethrin
Cyfluthrin
λ–Cyhalothrin
DEET
Desethyl atrazine
Dibenz(a,h)anthracene
Fluoranthene
Galaxolide
Indeno(1,2,3-cd)pyrene
Metconazole
Metolachlor
Musk ketone
DOWEX Static
HLB Static
OSORB Static
Analyte accumulation for static conditions (ng/sampler)
Time (d)


Acetochlor
Atrazine
Azoxystrobin
Benzo(a)anthracene
Benzo(a)pyrene
Benzo(b)fluoranthene
Benzo(g,h,i)perylene
Benzo(k)fluoranthene
Bifenthrin
Chlorothalonil
Chlorpyrifos
Chrysene
DOWEX Static
HLB Static
OSORB Static
Analyte accumulation for static conditions (ng/sampler)
Time (d)

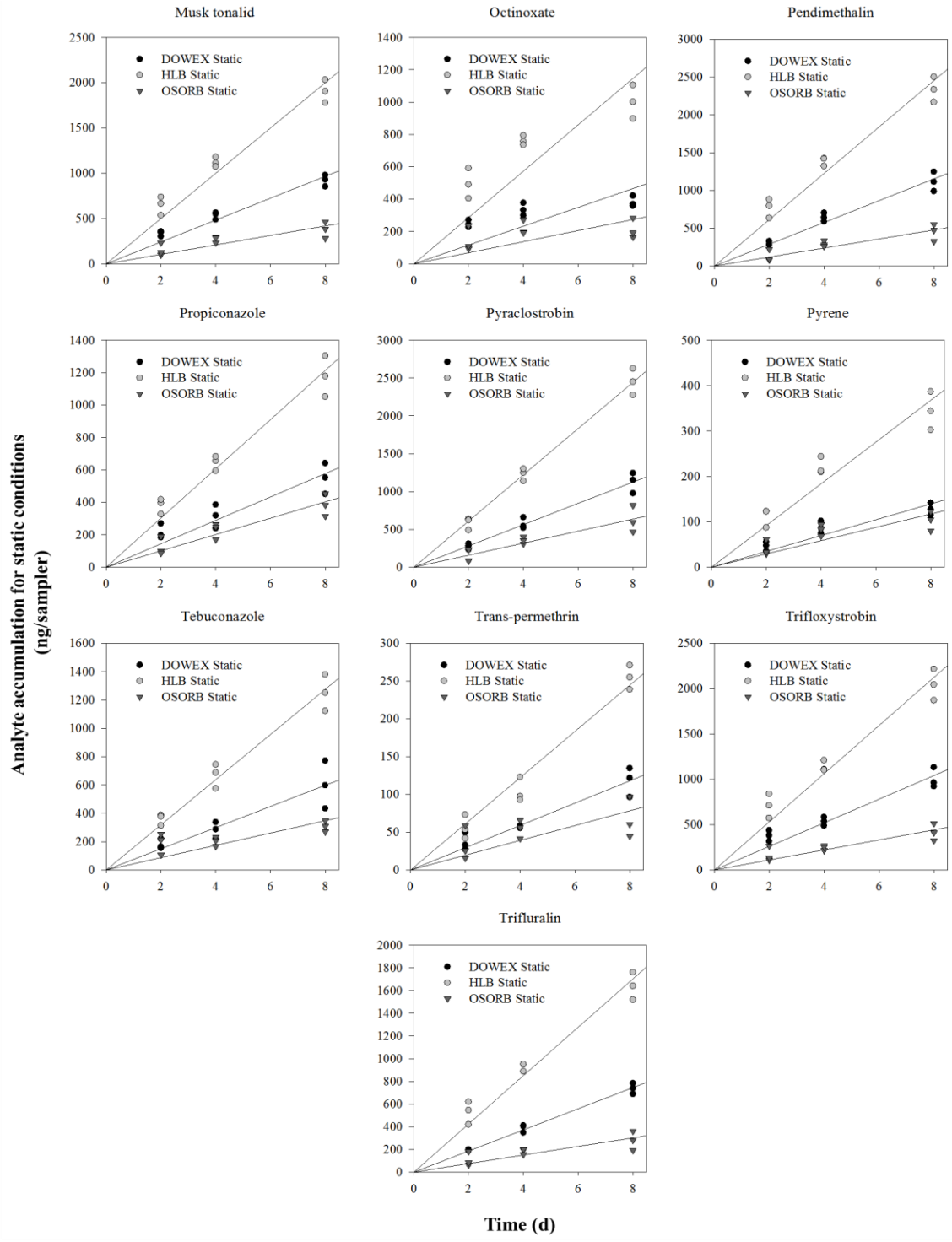
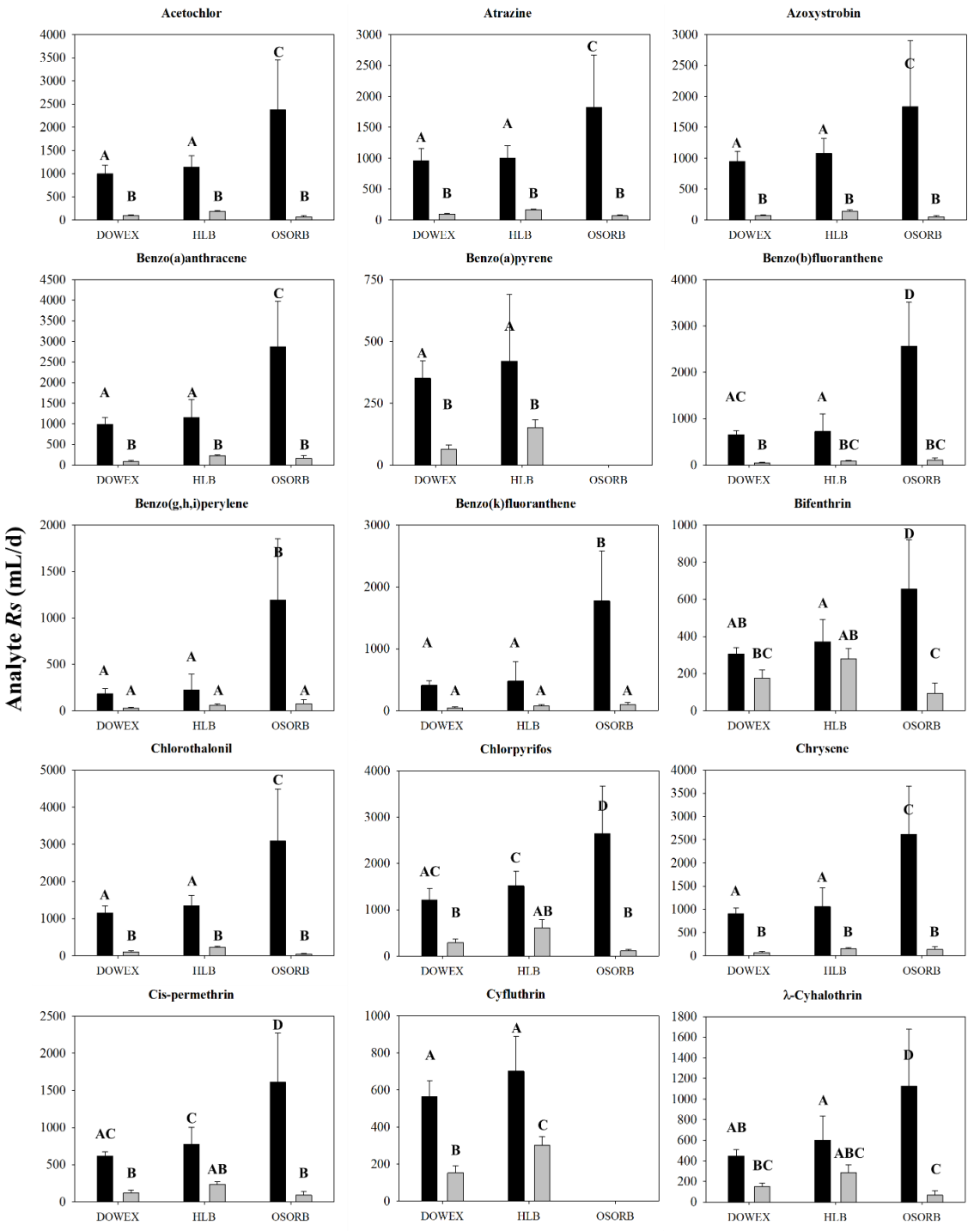
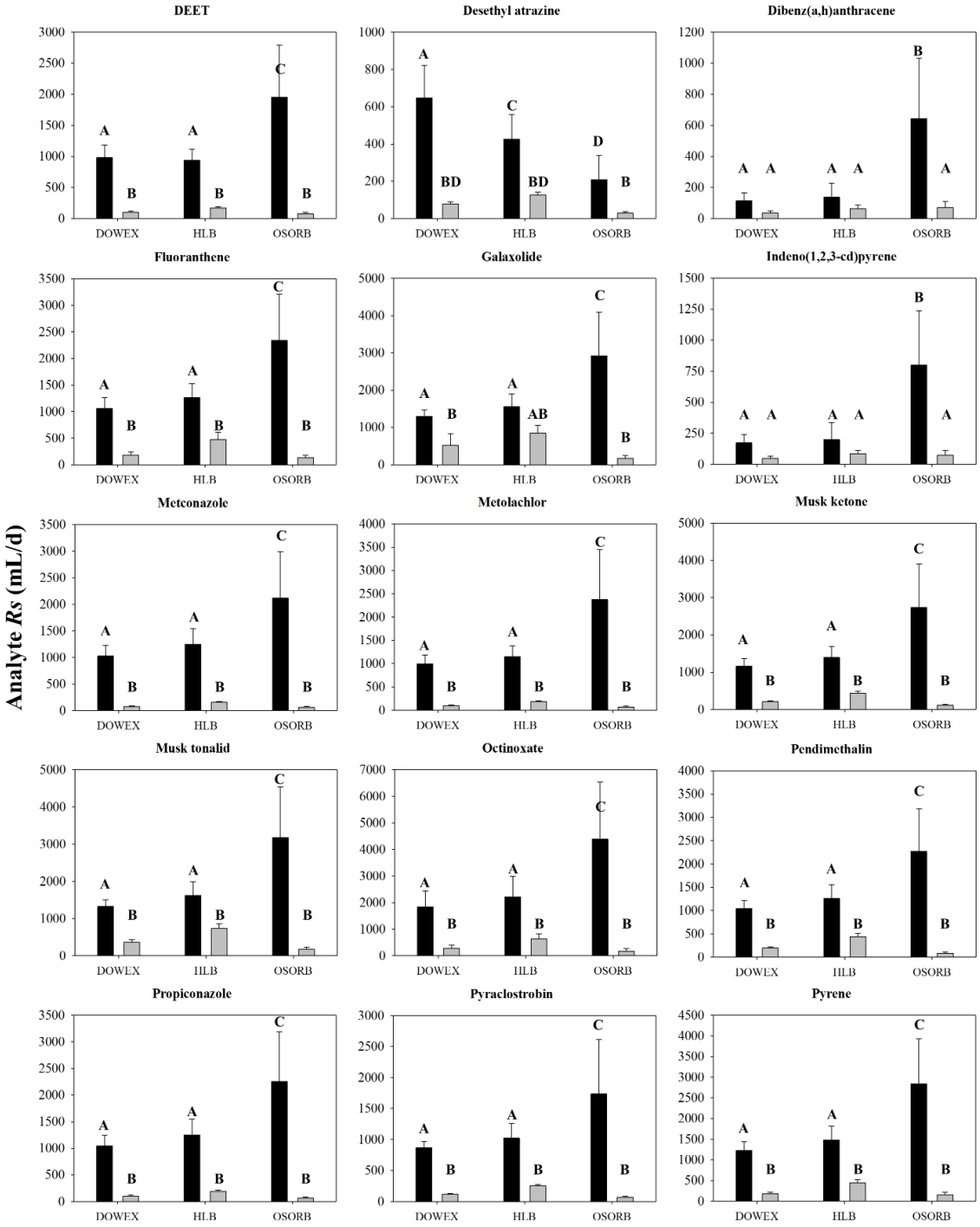


Fig S2. Passive sampler linear regression for each analyte under static conditions. Regression fitting provided in Table 3.


Acetochlor
Atrazine
Azoxystrobin
Benzo(a)anthracene
Benzo(a)pyrene
Benzo(b)fluoranthene
Benzo(g,h,i)perylene
Benzo(k)fluoranthene
Bifenthrin
Chlorothalonil
Chlorpyrifos
Chrysene
Cis-permethrin
Cyfluthrin
λ-Cyhalothrin
Analyte Rs (mL/d)
DOWEX
HLB
OSORB


DEET
Desethyl atrazine
Dibenz(a,h)anthracene
Fluoranthene
Galaxolide
Indeno(1,2,3-cd)pyrene
Metconazole
Metolachlor
Musk ketone
Musk tonalid
Octinoxate
Pendimethalin
Propiconazole
Pyraclostrobin
Pyrene
Analyte Rs (mL/d)
DOWEX
HLB
OSORB

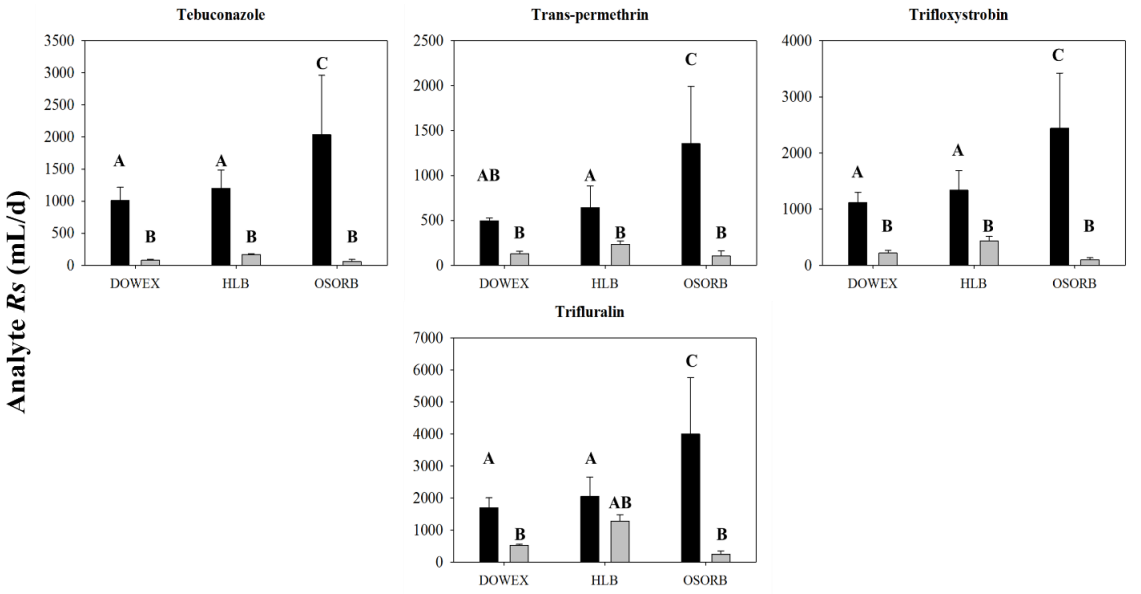


Fig S3. Mean (standard deviation, SD) sampling rates (*Rs*) obtained during sampler calibration experiments under both flowing (black) and static (grey) conditions. Categorical letters represent statistical differences in *Rs* for each sorbent and flow treatment combination ($p < 0.05$). Summary results are depicted in Fig. 3 and actual values are provided in Table 4.

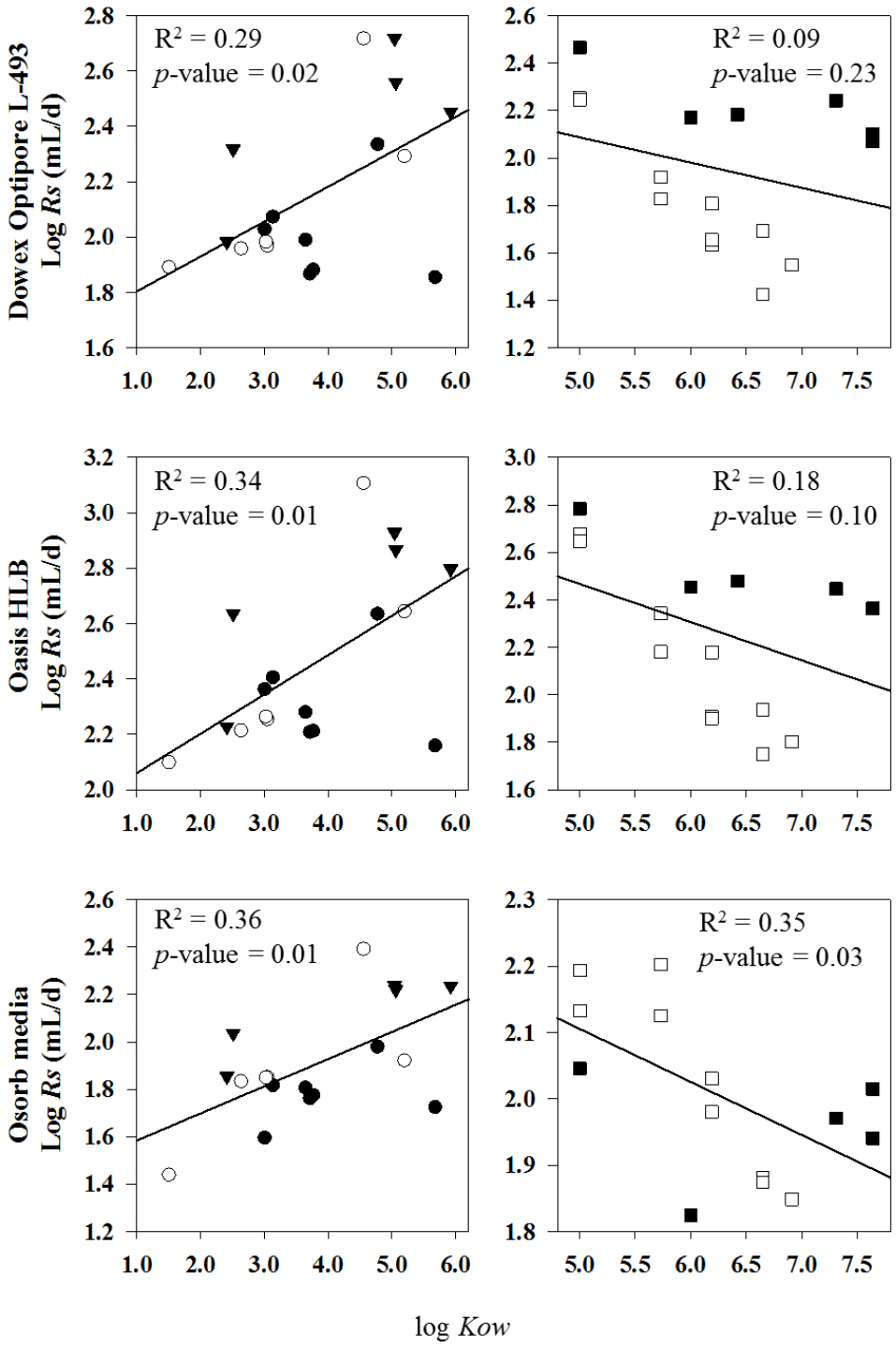


Fig. S4. Linear regression analysis between log *Rs* with log *Kow* for each passive sampler sorbent under static conditions. Results for fungicides (●), herbicides (○), and personal care products (▼) are displayed in the left panel while insecticides (■) and PAHs (□) are in the right panel.

CHAPTER V

CHARACTERIZATION OF PERFORMANCE REFERENCE COMPOUND KINETICS AND ANALYTE SAMPLING RATE CORRECTIONS UNDER THREE FLOW REGIMES USING POCIS-STYLE NYLON ORGANIC CHEMICAL INTEGRATIVE SAMPLERS

Abstract

Performance reference compounds (PRCs) can be spiked into passive samplers prior to deployment. If the dissipation kinetics of PRCs from the sampler corresponds to analyte accumulation kinetics, then PRCs can be used to estimate *in-situ* sampling rates. Under controlled laboratory conditions, the effectiveness of PRC corrections on prediction accuracy of water concentrations were evaluated using nylon organic chemical integrative samplers (NOCIS). Results from PRC calibrations suggest that PRC elimination occurs faster under higher flow conditions; however, minimal differences were observed for PRC elimination between fast flow (9.3 cm/s) and slow flow (5.0 cm/s) conditions. Moreover, minimal differences were observed for PRC elimination from Dowex Optipore L-493; therefore, PRC corrections did not improve results for NOCIS configurations containing Dowex Optipore L-493. Regardless, results suggest that PRC corrections were beneficial for NOCIS configurations containing Oasis HLB; however, due to differences in flow dependencies of analyte sampling rates and PRC elimination rates across the investigated flow regimes, the use of multiple PRC corrections was necessary. As such, a "Best-Fit PRC" approach was utilized for Oasis HLB corrections using caffeine-$^{13}C_3$, DIA-d_5, or no correction based on the relative flow dependencies of analytes and these PRCs. Although PRC

corrections reduced the variability when in-situ conditions differed from laboratory calibrations (e.g. static verses moderate flow), applying PRC corrections under similar flow conditions increases variability in estimated values.

1. Introduction

Passive sampling devices are promising environmental monitoring tools for the aquatic environment, capable of measuring trace and fluctuating contaminant concentrations under numerous environmental conditions. Analytes accumulate following first-order, one-compartmental models consisting of an initial linear (or kinetic) integrative phase followed by a curvilinear phase indicative of chemical equilibrium [1, 2]. During the initial linear phase, accumulation is integrative and time-weighted average concentrations can be obtained [3, 4]. Contaminant sampling rates (*Rs*) can be empirically determined under controlled laboratory calibrations [5]; however, environmental exposures rarely occur under stable conditions due to intermittent inputs, contaminant dissipation, and flow variations [6-9]. Fluctuating contaminant concentrations can be compensated for using integrative samplers such as Chemcatchers [10] or the Polar Organic Chemical Integrative Sampler (POCIS) [3, 11]; however, these samplers may accumulate target analytes at different rates when there is variation in flow across the sampler membrane [2, 5, 12-18]. Theoretically, the changes in flow across the sampler causes variations in the thickness of aqueous boundary layer (ABL); therefore, contaminant diffusion rates across this boundary layer is highly influenced by flow. This is especially true when the limiting step of accumulation is diffusion across the ABL rather than diffusion/permeation through the sampler membrane [2, 5, 19].

Performance reference compounds (PRCs) have been suggested as a viable method to improve calibration for POCIS-style samplers by providing in-situ flow calibrations [5, 19-21]. Equilibrium-based passive samplers have benefitted from PRCs as stable isotope compounds can be spiked into samplers prior to deployment and dissipation occurs proportional with uptake [22]. However, the integrative nature of POCIS-style samplers presents a challenge for PRCs due to

minimal fugacity out of stable isotope compounds [5, 21]. Regardless, several PRCs have been suggested to have sufficient fugacity out of classical POCIS devices including desisopropyl atrazine- d_5 [19-21, 23] and caffeine-C_{13} [3, 19]. These PRCs are especially important for adjusting *Rs* when analyte accumulation controlled by the aqueous boundary layer rather than diffusion through the membrane [19, 20].

Previous investigations have evaluated modified configurations of POCIS-style passive samplers, in attempt to increase analyte *Rs*, reduce lag-phase accumulation, and ultimately provide integrative sampling of hydrophobic contaminants [19](Chapter IV). Samplers were constructed similarly to classical POCIS specifications; however, polyethersulfone (PES) membranes (0.1 μm pore size) were replaced with high porosity nylon mesh screening (30-35 μm pore sizes). These POCIS-Nylon configurations are hereafter referred to as Nylon Organic Chemical Integrative Samplers (NOCIS) (Chapter IV). Although this modification successfully increased *Rs* and the ability to measure hydrophobic contaminants, analyte accumulation was governed by diffusion through the aqueous boundary layer resulting in increased flow sensitivity as compared to standard POCIS configurations (Chapter IV). Specifically, NOCIS configurations were shown to be highly flow sensitive with five- to ten-fold differences in median *Rs* between static and flow (9.3 cm/s) treatments (Chapter IV). In contrast, accumulation in classical POCIS configurations is generally limited by permeation through the diffusion membrane where POCIS only have a two- to three-fold differences in *Rs* under different flow conditions [12, 13]. These differences suggest that PRC dissipation should also be evaluated over a range of flow conditions to ensure satisfactory flow corrections. Moreover, these data suggest that finding a PRC approach that adequately corrects for in-situ flow differences may be more important for NOCIS configurations that experience greater flow sensitivities as compared to classical POCIS configurations.

Belles et al. [19] investigated PRC elimination from classical POCIS configurations and NOCIS configurations and suggested the approach was promising with three PRCs including

desisopropyl atrazine-d_5, caffeine-C_{13}, and salbutamol-d_3. All three PRCs demonstrated similar eliminate rates from samplers, thus illustrating the potential of relating PRC elimination to analyte accumulation. However, dissipation was only investigated under a single flow regime, thus not testing the PRC approach to the full capacity.

The overall objective of this research was to build from previous work to improve the utility of NOCIS configurations by evaluated PRC dissipation under several hydrodynamic conditions to provide *Rs* flow corrections for organic contaminants with a broad range of hydrophobicities (log *Kow* range: 1.5-7.6). This was accomplished using two experimental designs. First, to calibrate elimination rates under several flow conditions, PRC dissipation was evaluated as a function of time using fast (9.3 cm/s), slow (5.0 cm/s), static (0 cm/s) flow treatments. Second, analyte *Rs* and PRC dissipation were concurrently measured to quantitatively evaluate the prediction accuracy of known water concentrations based on PRC corrections and previously described analyte calibration studies (Chapter IV).

2. Experimental

2.1 Test chemicals

Selected analytes (n=34) included current-use pesticides (i.e. fungicides, herbicides, and insecticides), polyaromatic hydrocarbons (PAHs), and personal care products (PCPs). Relevant chemical properties of all analytes of interest are listed in Table 1. Analytical-grade standards of current-use pesticides and PCPs were of the highest available purity from Sigma-Aldrich (St. Louis, MO, USA). An expanded PAH mixture was purchased from Accustandard (New Haven, CT) and was used for analytical standards and spiking solutions. Performance reference compounds included atrazine-d_5, caffeine-$^{13}C_3$, cotinine-d_3, desisopropyl atrazine-d_5 (DIA-d_5; Santa Cruz Biotechnology, Dallas, TX), fluoranthene-d_{10}, and lindane. Benzo(a)anthracene-d_{16}, dibutyl chlorendate, and tetrachloro-m-xylene were used as surrogate standards and a semi-volatile internal standard mixture, containing deuterated PAHs, was purchased from Accustandard. All solvents (acetone, dichloromethane, ethyl acetate, and methanol) were

Table 1. Selected analytes (n=34) and relevant physicochemical properties.

Analyte	Type [a]	CAS Number [b]	Log *Kow* [b]	Water Solubility (mg/L) [b]
Acetochlor	H	34256-82-1	3.05	700
Atrazine	H	1912-24-9	2.64	69
Azoxystrobin	F	131860-33-8	5.68	0.25
Benzo(a)anthracene	PAH	56-55-3	5.73	0.015
Benzo(a)pyrene	PAH	50-32-8	6.19	0.0019
Benzo(b)fluoranthene	PAH	205-99-2	6.19	0.005
Benzo(g,h,i)perylene	PAH	191-24-2	6.65	0.0016
Benzo(k)fluoranthene	PAH	207-08-9	6.19	0.0033
Bifenthrin	I	82657-04-3	7.31	0.076
Chlorothalonil	F	1897-45-6	3.04	1.6
Chlorpyrifos	I	2921-88-2	5.00	1.6
Chrysene	PAH	218-01-9	5.73	0.0096
Cis-permethrin	I	61949-76-6	7.64	0.038
Cyfluthrin	I	68359-37-5	6.42	0.017
λ-Cyhalothrin	I	68085-85-8	6.00	0.054
DEET	PCP	134-62-3	2.41	7500
Desethyl atrazine	M	6190-65-4	1.51	660
Dibenz(a,h)anthracene	PAH	53-70-3	6.91	0.0007
Fluoranthene	PAH	206-44-0	5.00	0.13
Galaxolide	PCP	1222-05-5	5.04	24
Indeno(1,2,3-cd)pyrene	PAH	193-39-5	6.65	0.0019
Metconazole	F	125116-23-6	3.72	45
Metolachlor	H	51218-45-2	3.03	510
Musk ketone	PCP	81-14-1	2.51	14
Musk tonalid	PCP	21145-77-7	5.06	18
Octinoxate	PCP	5466-77-3	5.92	6.4
Pendimethalin	H	40487-42-1	5.20	1.8
Propiconazole	F	60207-90-1	3.65	10
Pyraclostrobin	F	175013-18-0	3.14	12
Pyrene	PAH	129-00-0	5.00	0.044
Tebuconazole	F	107534-96-3	3.77	30
Trans-permethrin	I	61949-77-7	7.64	0.038
Trifloxystrobin	F	141517-21-7	4.78	0.65
Trifluralin	H	1582-09-8	4.56	1.1

[a] F = fungicide, H = herbicide, I = insecticide, M = metabolite, PAH = polyaromatic hydrocarbon, PCP = personal care product.

[b] Values obtained from Scifinder (http://scifinder.cas.org; accessed 2015 September 8) and calculated using Advanced Chemistry Development Software V11.02 (ACD/Labs). Conditions were modeled at 25 °C and pH 7.

analytical grade or better. The tap water source at Oklahoma State University is Lake Carl Blackwell (Stillwater, OK) and was used for all experiments. Hardness ranged from 180-200 mg/L as calcium carbonate, pH ranged from 6.9-7.6, and experimental unit was maintained at 20 °C (±1 °C).

2.2 Sorbent fortification

Oasis HLB sorbent was removed from Oasis solid phase extraction (SPE) cartridges and the DOWEX sorbent was graciously provided by Dow Water and Process Solutions (The Dow Chemical Company, Midland, MI). Prior to use, sorbents were cleaned with methanol, dried under a vacuum, and baked at 40 °C overnight. Sorbents were fortified with performance reference compounds (PRCs) using modified methods proposed by Mazzella et al. [23]. Aliquots (10 g) of both sorbents were weighed out in 200 mL French Square glass bottles, covered with 30 mL of methanol, and fortified with PRC stock solutions. Fortified bottles were placed on a rotating table and shaken at 125 rpm for 4 h to evenly distribute PRCs across the sorbents. Solvent was allowed to evaporate by removing the lids and rotating overnight (~12 h) at 125 rpm at 30 °C. Four 200 mg aliquots were immediately analyzed to determine PRC fortification levels and homogeneity. The remaining fortified sorbents were used to construct samplers for PRC dissipation studies (n=27 per sorbent), in-situ analyte uptake and PRC loss studies (n=9 per sorbent), and additional quality control samples (n=6 per sorbent). Quality control samples were weighed into glass vials, stored frozen at -30 °C, and analyzed with passive samplers to determine PRC stability.

2.3 Sampler design and assembly

The nylon organic chemical integrative sampler (NOCIS) configurations were constructed in house containing 200 mg (± 3 mg) of either Oasis HLB or Dowex Optipore L-493 that were fortified with PRC as previously described. Similar to the classical POCIS configuration, stainless steel washers contained circular openings with 5.5 cm internal diameter, resulting in approximately 48 cm^2 of exposed surface area per sampler; however, the standard

polyethersulfone (PES) membranes (0.1 μm pore size) were replaced with nylon mesh screening (35 μm openings; Pentair Aquatic Ecosystems). Physical properties of sorbents and NOCIS configurations were previous described (Chapter IV).

2.4 General design/layout of experimental unit

Experimental units were construct as described in a previous investigation (Chapter IV); however, additional flows were investigated. Passive samplers were exposed to analytes of interest using experimental units consisting of stainless steel pots (inner diameter: 40 cm, height: 30.5 cm) containing 30 L of tap water (Figure 1). To achieve laminar flow, smaller stainless steel pots (inner diameter: 15 cm, height 17.7 cm) were secured to an aluminum crossbar and suspended in the center of each unit. The bottoms were removed from the small pots, resulting in a stainless steel cylinder projecting into the upper portion of the water column. Flow was generated by securing aquarium pump heads (Aqueon Model AT10) to stainless steel threaded rods so that the pump head was 7.6 cm below the water surface and 3.8 cm above the bottom of the central cylindrical insert. Flow rate was determined for fast and slow flows by timing the movement of a float in replicate experimental units with mean (± standard deviation) of 9.33 (± 0.99) cm/s and 5.01 (± 0.49), respectively. Experimental units with static flow were similarly constructed except the aquarium pump head was excluded. Each experimental unit contained two passive samplers (i.e. one sampler of each configuration). Nylon samplers were secured to stainless steel threaded rods horizontally in the water column with 3.1 cm of clearance between the top and the bottom sampler to ensure adequate and even flow across the nylon membranes.

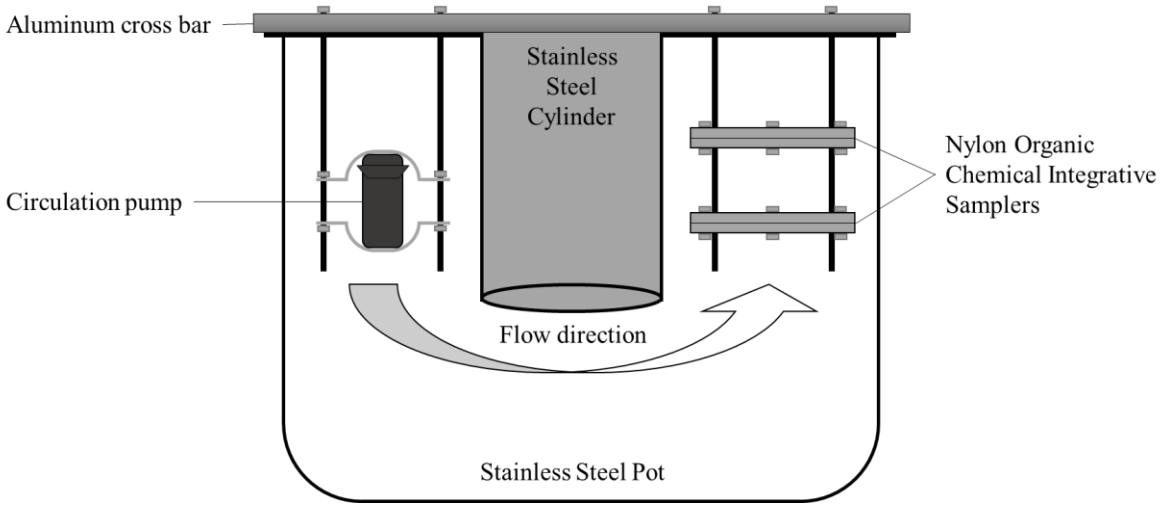


Fig. 1. Schematic of experimental unit with flow treatment. The circulation pump was omitted from static experimental units. To eliminate galvanic reactions within the experimental units, all fastening/structural components (i.e. pots, threaded bar, fastening nuts, washes, and clamps) were stainless steel. Adapted from Morrison and Belden (Chapter IV).

2.5 Calibration of performance reference compound (PRC) dissipation

The first experiment was designed to determine PRC dissipation rates from both NOCIS configurations under fast, slow, and static flow conditions. Complete static renewals were conducted daily to minimize PRC reuptake by completely draining the water from each experimental unit into reservoirs containing activated carbon, refilling with tap water. Calibration tests lasted eight days and triplicate (n=3) experimental units were destructively taken down for all flow treatments at 2, 4, and 8 d. Passive samplers were removed from experimental units at the designated periods and stored frozen at -80°C until further analysis. To confirm water concentrations throughout, one-liter aliquots were obtained prior to water exchanges every 24 h from replicate experimental units for each flow condition.

2.6 PRC validation study

The second experiment was conducted to evaluate if differential PRC dissipation across flow rates improved the accuracy of water concentration estimates when applying *Rs* obtained single flow regime across multiple flow regimes. Similar to the PRC calibration study, three replicate units (n=3) were constructed for each flow rate. To maintain stable analyte water

concentrations throughout the eight day study, a daily static renewal regimen was used by completely draining the water from each experimental unit into reservoirs containing activated carbon, refilling experimental units with tap water, and immediately fortifying with target analytes. Target analytes concentrations were achieved by adding 0.5 mL of spiking solution to the center of the cylindrical sleeve protruding into the depths of the experimental unit to achieve target analyte concentrations of 0.5 ng/mL or 0.1 ng/mL depending on analyte water solubility. Experimental units with static flow were stirred with a solvent rinsed stainless steel spoon for 2 min after fortification to facilitate analyte dilution. Target analyte concentrations during the initial pulsed exposure were 0.5 ng/mL or 0.1 ng/mL depending on analyte water solubility. Passive samplers were removed from experimental units at the end of the eight-day exposure and stored frozen at -80°C until further analysis. To confirm water concentrations throughout, 1 L of water was obtained from replicate experiments for each flow treatment. On alternate days, water was obtained either pre- or post- (5 h) water exchanges so that four measurements (n=4) were taken pre and post exchange.

2.7 Passive sampler and water extractions

Passive samplers were removed from the freezer and allowed to thaw on aluminum foil at room temperature. Sorbents were removed from passive samplers by cutting the membranes with a solvent rinsed stainless steel scalpel and rinsing the sorbents into empty SPE cartridges with deionized water. Water samples obtained throughout both experiments and were spiked with surrogate standards, mixed on a stir plate for one hour, and extracted via Oasis HLB solid phase extraction (SPE) 500 mg cartridges containing HLB at approximately 15 mL/min. Prior to extraction, cartridges were conditioned with 8 mL ethyl acetate, 8 mL of methanol, and 15 mL of deionized water. Loaded passive samplers and water cartridges were dried via a vacuum for five minutes and centrifuged at 3,000x *g* for 8 min to remove excess water. Cartridges containing recovered media from passive samplers were spiked with surrogate standards prior to elution. Analytes were eluted with 1.5 mL acetone followed by 10 mL dichloromethane, dried with

anhydrous sodium sulfate, and evaporated under a gentle stream of nitrogen. During evaporation, all samples were solvent exchanged to ethyl acetate. Water samples were concentrated 5,000x to achieve detection limits. Sample final volumes for water samples, sorbent-PRC, and sorbent-analytes ranged from 200 µL to 1,000 µL.

2.8 Gas chromatography analysis

Analysis of analyte residues will be performed using gas chromatography / mass spectrometry (GC/MS; Agilent 5975c, Santa Clara, CA, USA) using electron ionization (70 eV). Separation was achieved using a 15 m × 250 µm × 0.25 µm HP-5 capillary column (Agilent). The GC inlet was configured with an ultra inert, splitless single taper inlet with glass wool packing and the temperature was set at 260 °C. To accommodate the large analyte list, maintain sensitivity within analyte windows, and reduce degradation of analytes sensitive to cool starting temperatures and lengthy chromatographic runs, three different oven programs were utilized. Performance reference compounds were analyzed separately from contaminants to minimize the number of ions within quantitation windows. The oven programming for PRCs was held at the initial temperature of 90 °C for 1 min, ramped at 10 °C/min to 255 °C, ramped at 12 °C/min to 295 °C, and held for 2 min. The oven programing and GC/MS parameters for target analytes were previously described (Chapter IV). Analyte quantitation was achieved using three ion select ion monitoring (SIM) with the following ions (Table S1). The two qualitative ions had to be within 20% of expected ratio, relative to the quantitative ion response, to report the analyte without flagging the data as qualitatively uncertain. Semi-volatile internal standards were added to all samples and calibration standards. Analytes were paired to internal standards based on nearest retention time. Continuous calibration verification was performed during each analytical run by randomly reanalyzing low, intermediate, and high ranges of calibration standards.

2.9 Theory and modeling

Analyte accumulation in passive samplers generally follows a kinetic (i.e. linear) uptake period followed by a curvilinear equilibrium regime [1, 2] so that

$$C_{PSD} = C_{TWA} \times \left(\frac{k_u}{k_e}\right) \times \left(1 - \exp(-k_e \times t)\right) \quad \text{EQ 1}$$

where C_{PSD} is the sorbent analyte concentration (ng/g) within the passive sampling device (PSD), C_{TWA} is the time-weighted average concentration (TWA; ng/mL) of the water, and k_u and k_e are accumulation (mL/g/d) and elimination (d^{-1}) rate coefficients. However, POCIS-style samplers generally have long integration periods [1, 3] which simplifies Equation 1 so that

$$C_{PSD} = C_{TWA} \times R_s \times t \quad \text{EQ 2}$$

where R_S is the sampling rate (mL/d) and t is the exposure time (d); therefore, the TWA water concentrations can be estimated for each analyte by rearranging Equation 2 so that

$$C_{TWA} = \frac{C_{PSD}}{R_S \times t} \quad \text{EQ 3}$$

Sampling rates for the 34 target analytes were determined for static and fast flow conditions in a previous publication (Chapter IV). Sampling rates corresponding to fast flow (i.e. 9.3 cm/s) were used as the R_{Scal} throughout calculations present study.

Performance reference compound (PRC) desorption can be used to correct laboratory calibrated *Rs* values. Assuming exchange kinetics are similar between PRCs and target analytes, the PRC elimination rate constant k_{ePRC} can be derived using a first order elimination model

$$C_{PRC(t)} = C_{PRC0} \times \exp(-k_{ePRClab} \times t) \quad \text{EQ 4}$$

where C_{PRC0} is the initial fortified PRC concentration (ng/g) and $C_{PRC(t)}$ is the remaining PRC concentration after an exposure of time *(t*) [21]. Similar to analyte R_S, PRC elimination is determined in controlled laboratory conditions ($k_{ePRClab}$). Rearrangement of Equation 4 allows for determination of PRC elimination during in situ ($k_{ePRCval}$) deployments so that

$$k_{ePRCinsitu} = \frac{\ln(C_{PRC0}/C_{PRC(t)})}{t} \quad \text{EQ 5}$$

Once calibrated, $k_{ePRClab}$ can be used to normalize $k_{ePRCval}$, thus providing a means to correct laboratory derived analyte sampling rates (R_{Scal}) resulting in-situ corrected sampling rates (R_{Scorr})

$$R_{\mathrm{Scorr}} = R_{\mathrm{Scal}} \times \left(\frac{k_{e\mathrm{PRCval}}}{k_{e\mathrm{PRClab}}}\right) \quad \text{EQ 6}$$

2.10 Data analysis

All graphics were constructed using SigmaPlot 10.0 (Systat Software, CA, USA). Sampler PRC concentrations obtained during dissipation rate calibration were normalized to C_{PRC0} to provide a visual for the total percentage lost for each PRC investigated. Regression analysis of PRC loss was performed in SigmaPlot by fitting a first order exponential model (EQ 4) for the data plotted as percentage of C_{PRC0} as a function of exposure time. The initial quality control samples (n=4 per sorbent) were included in the regression analysis as day zero.

Flow dependencies of analyte *Rs* were determined individually for each analyte and sorbent. Analyte *Rs* for fast, slow, and static flow conditions were estimated from eight-day time point during the PRC validation experiment using Equation 3. Flow dependencies were determined for fast and slow conditions by normalizing estimated *Rs* to static conditions; therefore, calculating the magnitude of difference compared to static conditions. Flow dependencies were similarly calculated for PRC dissipation for compounds exhibiting first order release from NOCIS configurations. Using PRC elimination rates from the same experiment calculated using Equation 5, elimination rates from fast and slow conditions were normalized by static conditions to determine the magnitude of difference in PRC loss compared to static conditions. When considering multiple PRC corrections, the PRC flow dependencies from fast flow conditions were used to determine which PRC corrections should be applied to individual analytes.

Since different PRCs may more accurately correct the *Rs* of any given analyte, a process was developed to assign each analyte a PRC *a priori* based on flow dependencies. The "Best-Fit PRC" was thus selected by matching the analyte flow dependencies from fast flow conditions to the PRC that had the most similar flow dependency. If the analyte had limited flow dependency, no PRC correction was made.

To visualize differences R_{Scal} and R_{Scorr}, box and whisker plots were constructed with median (solid horizontal line), mean (dotted horizontal line), inner quartile range (25^{th} and 75^{th} percentiles; box), and the 10^{th} and 90^{th} percentiles (whiskers). In-situ sampling rates were calculated using Equation 6 and R_S previously published using fast flow conditions (Chapter IV). The fast flow condition (i.e. 9.3 cm/s) was chosen as the calibration flow rate due to higher R_S and more importantly higher PRC dissipation as compared to static flow conditions.

3. Results and Discussion

3.1 Quality control

Method accuracy and precision was determined based on analysis of laboratory fortified samples including both sorbents and tap water throughout chemical analysis procedures and were compared against measured values of spiking solutions (n=10) conducted throughout. Mean (± standard deviation; SD) percentage of analyte recoveries from fortified quality control samples are reported in Table S2. Recovery of analytes from fortified sorbent quality control samples (n=6 per sorbent) averaged 93% (range: 74-205%) and 93% (range: 77-143%), respectively for Dowex Optipore L-493 and Oasis HLB. Chlorothalonil recoveries were high in both sorbents. Similarly, recovery of PRCs from fortified sorbents (n=6 per sorbent) averaged 100% (range: 88-105) and 102% (range: 94-114%), respectively for Dowex Optipore L-493 and Oasis HLB (Table S3). Analysis of fortified water samples (n=4) yielded mean recoveries of 87% (range: 33-167%) and 98% (range: 71-115%), respectively for all contaminants (n=7; Table S2) and PRCs (n=4; Table S3).

Mean (± standard deviation; SD) initial PRC concentrations (C_{PRC0}) for Dowex Optipore L-493 (n=4) were 4.52 ± 0.34 µg/g (atrazine-d_5), 6.32 ± 0.62 µg/g (caffeine-$^{13}C_3$), 4.67 ± 0.39 µg/g (cotinine-d_3), 3.41 ± 0.13 µg/g (DIA-d_5), 6.60 ± 0.35 µg/g (fluoranthene-d_{10}), and 5.74 ± 0.30 µg/g (lindane). Similarly, initial PRC concentrations for Oasis HLB (n=4) were 4.51 ± 0.34 µg/g (atrazine-d_5), 4.62 ± 0.30 µg/g (caffeine-$^{13}C_3$), 4.01 ± 0.08 µg/g (cotinine-d_3), 3.87 ± 0.24 µg/g (DIA-d_5), 4.42 ± 0.16 µg/g (fluoranthene-d_{10}), and 4.65 ± 0.14 µg/g (lindane). These results

indicate that uniform sorbent fortification was achieved for all PRCs prior to sampler construction and exposures.

Performance reference compound concentrations in water obtained from experimental units and unfortified tap water were below quantitation limits. Analyte residues in blank sorbent extract concentrations (n=6 per sorbent) were below quantitation limits for all PRCs and target analytes; therefore, the limit of detection was set at the lowest calibration standard within the linear range for each analyte. Trace levels of ten target analytes (atrazine, DEET, desethyl atrazine, fluoranthene, galaxolide, metolachlor, musk ketone, octinoxate, pendimethalin, and pyrene) were detected in blank water samples (n=4) due to presence in the water source. The median background concentration was 4 ng/L (range: 1-21 ng/L) with atrazine being the highest. Laboratory method blanks (n=6) were below detection limits for all analytes and PRCs. Mean (SD) percentage of surrogate standard recovery from water and sorbents was 69% (12), 85% (6), and 84% (6) respectively for benzo(a)anthracene-d_{16}, dibutyl chlorendate, and tetrachloro-m-xylene. Recoveries of PAHs were corrected by benzo(a)anthracene-d_{16} recoveries for each sample.

During the PRC validation study, measured experimental unit water concentrations were consistent across all flow treatments for all target analytes (Table S4) and were not blank corrected as background concentrations were in the water source. On average, measured water concentrations were approximately 79.4% of expected based on analysis of spiking solutions (n=10). Expected concentrations of several analytes were consistently below 50% of expected (i.e. benzo(a)anthracene, benzo(a)pyrene, benzo(k)fluoranthene, octinoxate, and trifluralin; Table S4) across all flow regimes.

3.2 Calibration of performance reference compound (PRC) dissipation

First order elimination from NOCIS configurations was demonstrated for caffeine-$^{13}C_3$, cotinine-d_3, and DIA-d_5; however, no discernable loss was observed for atrazine-d_5, fluoranthene-d_{10}, or lindane (Fig. 2, Table 2). Therefore, sorbent residues of the three PRCs with no elimination

were monitored throughout as a metric of sorbent recovery with average recoveries within 30% of expected for both sorbents, suggesting stability of fortified analytes and good sorbent recoveries during sample processing. For PRCs exhibiting first order elimination, regression analysis of PRC elimination as a function of exposure time provided good correlations with the data where R-square averaged 0.7290 and 0.9507 for NOCIS configurations containing Dowex Optipore L-493 and Oasis HLB, respectively (Table 2). Lower R-square values were observed for PRC elimination from Dowex Optipore L-493 due to lower overall dissipation out as flow rate decreased (Table 2). R-square values for PRC elimination from NOCIS configurations containing Oasis HLB were more consistent across all flow conditions, despite lower overall elimination rates in static conditions as compared to flowing conditions (Table 2).

Table 2. Performance reference compound (PRC) parameter estimates and regression fitting results from the initial loss-out calibration study for the three PRCs that demonstrated first order release (EQ 4) from both sorbents.

PRC	Dowex Optipore L-493				Oasis HLB			
	Percent C_{PRC0} (%)	k_{PRClab} (d^{-1})	R^2	*p*-value	Percent C_{PRC0} (%)	k_{PRClab} (d^{-1})	R^2	*p*-value
	Fast flow conditions (9.3 cm/s)							
Caffeine-$^{13}C_3$	94.98	0.0827	0.7964	<0.0001	99.29	0.3504	0.9737	<0.0001
Cotinine-d_3	96.86	0.1359	0.9000	<0.0001	99.99	0.5974	0.9952	<0.0001
DIA-d_5	98.67	0.2061	0.9328	<0.0001	99.92	0.2449	0.9692	<0.0001
	Slow flow conditions (5.0 cm/s)							
Caffeine-$^{13}C_3$	97.47	0.0572	0.7596	0.0001	99.50	0.3262	0.9775	<0.0001
Cotinine-d_3	99.2	0.1020	0.8854	<0.0001	99.94	0.5275	0.9896	<0.0001
DIA-d_5	95.83	0.1475	0.8914	<0.0001	101.55	0.2342	0.9566	<0.0001
	Static flow conditions (0 cm/s)							
Caffeine-$^{13}C_3$	94.42	0.0293	0.3974	0.0209	99.39	0.0949	0.9471	<0.0001
Cotinine-d_3	93.61	0.0446	0.4879	0.0079	102.12	0.1451	0.9633	<0.0001
DIA-d_5	89.67	0.0697	0.5098	0.0061	103.88	0.0547	0.7837	<0.0001

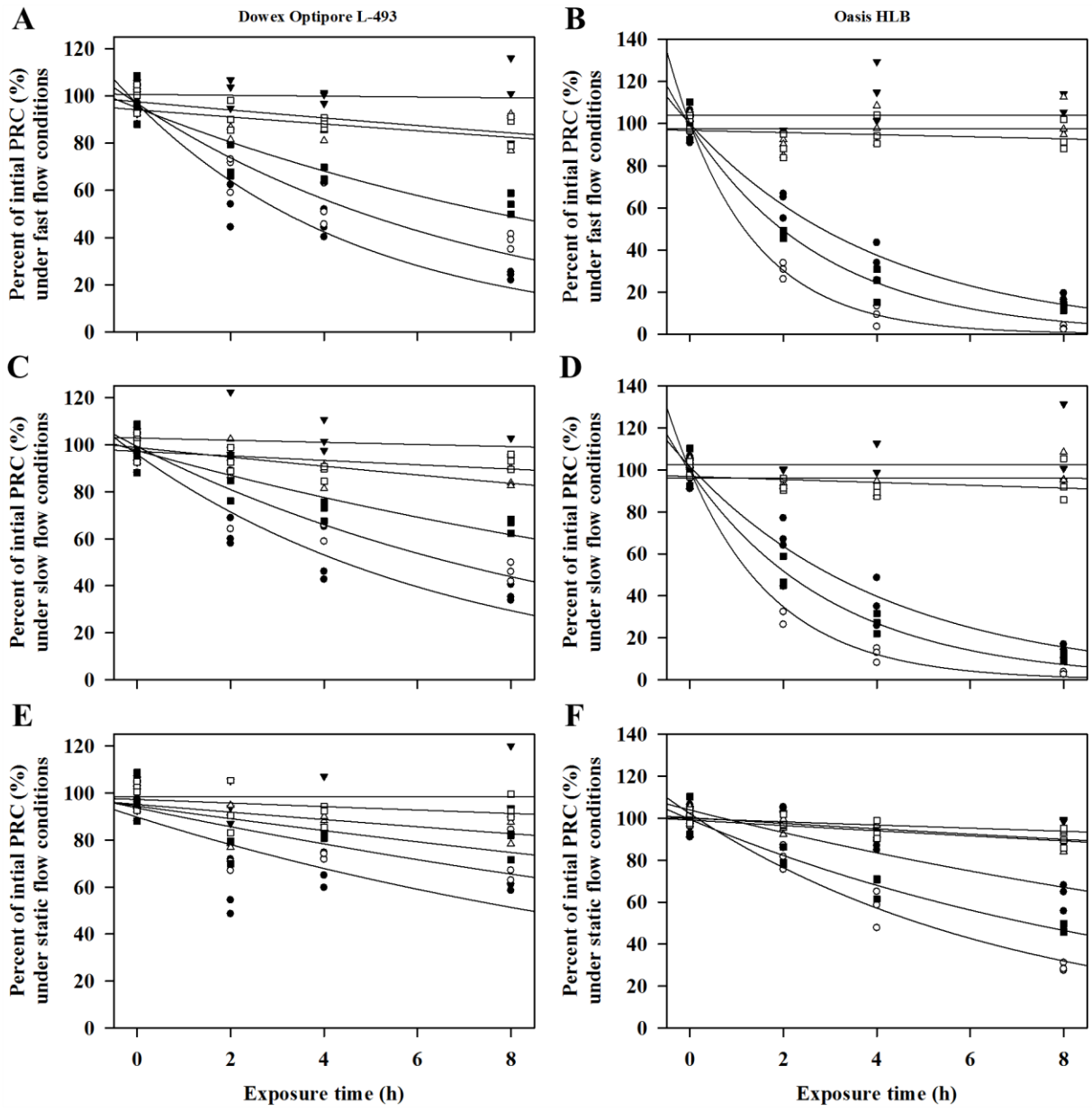


Fig. 2. Performance reference compound (PRC) elimination from Dowex Optipore L-493 (left column) and Oasis HLB (right column) under fast (**A**,**B**), slow (**C**,**D**), and static (**E**,**F**) flow conditions. Elimination rates were normalized to the initial sorbent fortification level to visually demonstrate the total percentage loss over the exposure time. Regression fitting values for PRCs demonstrating first order elimination are reported in Table 2. Symbol key: desisopropyl atrazine-d_5 (●), cotinine-d_3 (○), caffeine-$^{13}C_3$ (■), atrazine-d_5 (Δ), lindane (▼), and fluoranthene-d_{10} (□).

Dissipation from NOCIS devices differed between PRCs for each sorbent (Fig. 2, Table 2). Across all flow treatments, the order of PRC elimination (fastest to slowest) from Dowex Optipore L-493 was DIA-d_5 > cotinine-d_3 > caffeine-$^{13}C_3$ (Fig. 2, Table 2). Mean percentage of initial PRC concentrations in Dowex Optipore L-493 at the conclusion of the eight-day calibration was 38.9% (3.1), 49.4% (3.6), and 70.2% (6.4) for fast, slow, and static flow conditions, respectively (Fig. 2). In contrast, the order of PRC elimination (fastest to slowest) from Oasis HLB was cotinine-d_3 > caffeine-$^{13}C_3$ > DIA-d_5 (Fig. 2, Table 2). Mean percentage of initial PRC concentrations in Oasis HLB at the conclusion of the eight-day calibration was 10.8% (1.5), 9.2% (2.0), and 46.4% (3.6) for fast, slow, and static flow conditions, respectively (Fig. 2). Notably, PRC elimination was lower from Dowex Optipore L-493 as compared to Oasis HLB across all flow conditions (Fig. 2).

Direct comparisons with previous studies are limited as Belles et al. [19] is the only previous publication to investigate PRC release from nylon-POCIS configurations and this is the first investigation to evaluate PRC elimination from Dowex Optipore L-493. Regardless, DIA-d_5 [19-21, 23] and caffeine-$^{13}C_3$ [3, 19] have been suggested as promising PRCs for POCIS-style samplers. Using Oasis HLB nylon-POCIS configurations and classical POCIS configurations, Belles et al. [19] reported similar dissipation rates for caffeine-$^{13}C_3$ (k_e = 0.047 d^{-1}) and DIA-d_5 (k_e = 0.044 d^{-1}); however, direct comparisons are still challenging because flow across samplers was not explicitly explained in previous work and data herein suggests that the flow can significantly influence elimination. Using classical POICS configurations and a flow rate of approximately 2-3 cm/s, Mazzella et al. [21] reported a similar dissipation rate for DIA-d_5 (k_e = 0.044 d^{-1}) while also reporting dissipation of atrazine (k_e = 0.020 d^{-1}). Similar dissipation rates reported for caffeine-$^{13}C_3$ and DIA-d_5 using both nylon- and classical-POCIS configurations suggest that diffusion out is not membrane limited. Therefore, for these particular PRCs, diffusion through and thickness of the aqueous boundary layer is likely the rate-limiting step of PRC elimination as opposed to membrane permeation/diffusion. Consequently, PRC elimination rates should differ based on

flow across sampler membranes due to changes within the aqueous boundary layer. Changes in analyte mass transfer based on the thickness of the aqueous boundary layer also influence sampling rates [12, 13], which is the primary benefit for using PRCs for in-situ flow calibrations.

3.3 Influence of flow

Elimination of PRCs occurred faster at higher flow conditions for both NOCIS configurations (Fig. 2, Table 2). For NOCIS configurations containing Dowex Optipore L-493, average PRC elimination occurred 2.9x and 2.1x faster under fast and slow flow conditions, respectively compared to static conditions (Table 2). For NOCIS configurations containing Oasis HLB, average PRC elimination occurred 4.1x and 3.8x faster under fast and slow flow conditions, respectively compared to static conditions (Table 2). Although elimination rates from Dowex Optipore L-493 decreased with decreasing flow conditions, the overall range and extent of elimination was minimal compared to Oasis HLB (Table 2). In contrast, elimination rates from Oasis HLB were greatest between static and flow conditions, despite the negligible differences between fast and slow flow treatments. Moreover, better regression fittings were maintained for PRC elimination from Oasis HLB under static conditions with R-square values of 0.78 or better (Table 2). Few other investigations have calibrated PRC elimination under more than one flow condition [20]. Belles et al. [20] reported DIA elimination rates from classical POCIS configurations of 0.245 d^{-1} and 0.145 d^{-1} for fast and slow stirred conditions, respectively. These elimination rates align well with results reported in Table 2 for DIA-d_5 elimination from NOCIS configurations containing Oasis HLB.

Differences in PRC elimination rates between flow treatments were best described by calculating flow dependence where the elimination rates observed for both fast and slow conditions are normalized based on elimination rates obtained from static conditions (Fig. 3). This calculation demonstrates the magnitude of difference between static elimination rates as compared to elimination rates observed under flow conditions. Regardless of sorbent, flow dependence for each PRC was greatest under fast conditions as compared to slow conditions (Fig.

3). Due to greater differences in PRC elimination between static and flow conditions (Fig. 2, Table 2), PRC flow dependence was greater for NOCIS configurations containing Oasis HLB (Fig. 3). For example, mean flow dependence for DIA-d_5 was 2.4 and 5.4 for Dowex Optipore L-493 and Oasis HLB, respectively.

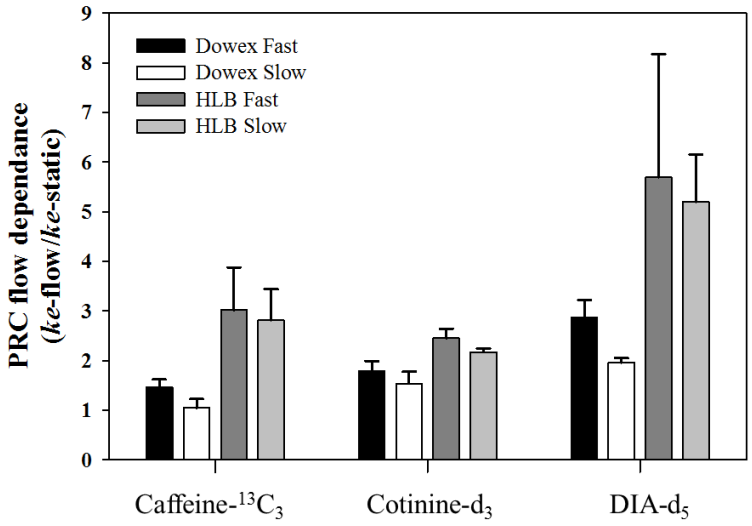


Fig. 3. Mean (standard deviation) flow dependence of performance reference compounds (PRCs) demonstrating first order elimination from samplers containing Dowex Opitpore L-493 and Oasis HLB. Flow dependence for fast and slow flow conditions were calculated by normalizing the respective elimination rates (k_e) by elimination rates obtained from static conditions.

It has been well documented that changes in hydrodynamic conditions can significantly influence contaminant uptake for various passive sampler configurations, especially when uptake is controlled by changes in the aqueous boundary layer [2, 5, 12-18] (Chapter IV). Sampling rate variations were also observed for NOCIS configurations during the validation study as calculated from the 8-d time point of the in-situ exposure using Equation 2 (Table S5). Similar to variations in PRC elimination, flow dependency can also be used to express differences in analyte sampling rates across flow treatments (Fig. 4, Table S6). Therefore, expressing this variation as flow dependency can provide a normalized metric for comparison across different sampler designs.

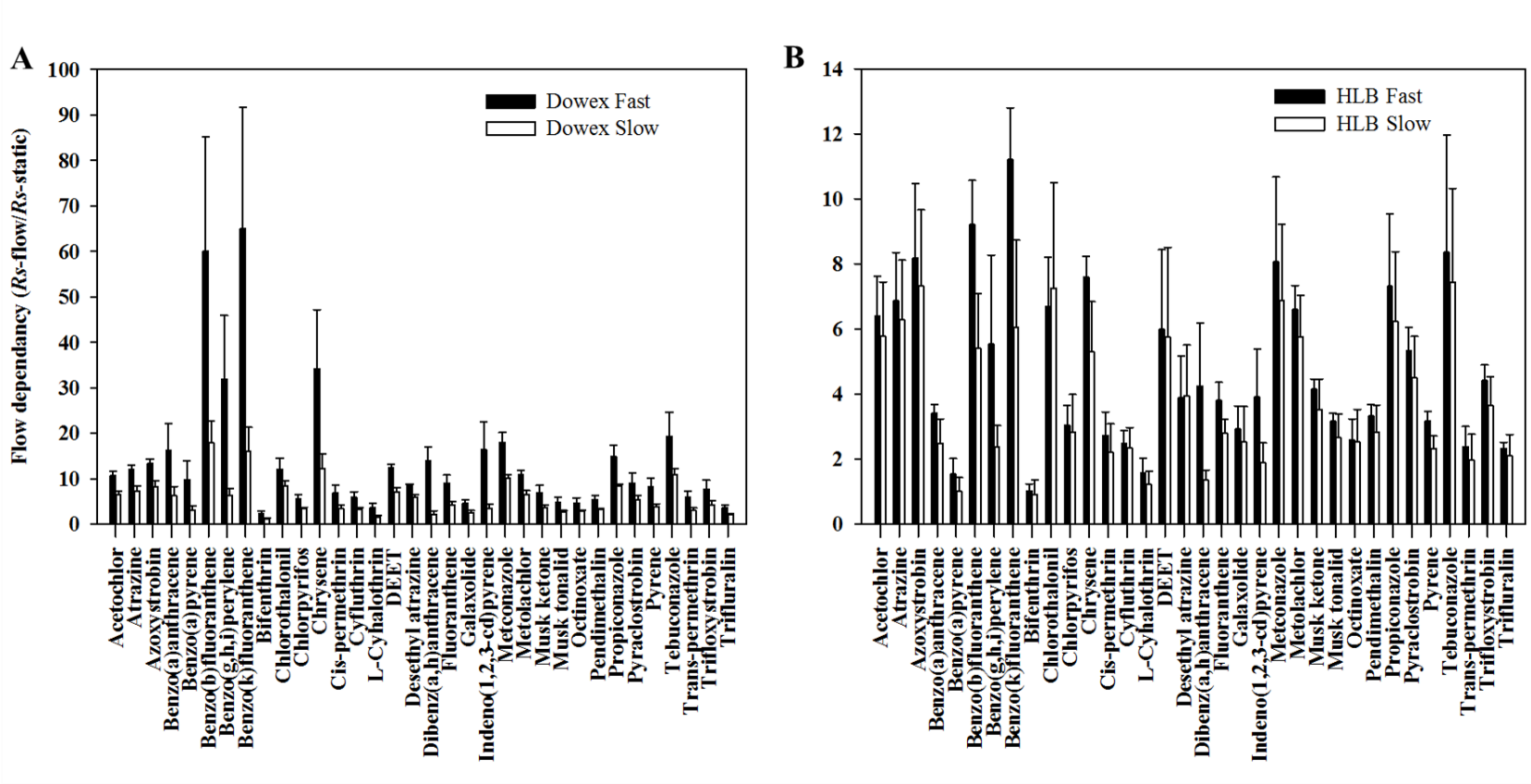


Fig. 4. Mean (standard deviation) flow dependence of analyte sampling rates (R_S) under fast (black) and slow (white) flow conditions for samplers containing Dowex Opitpore L-493 (**A**) and Oasis HLB (**B**). Flow dependence for fast and slow flow conditions were calculated by normalizing the respective R_S by rates obtained from static conditions. Sampling rates were calculated from the 8-d time point of the validation study using Equation 2.

Mean flow dependence of sampling rates for Dowex Optipore L-493 was 13.9 (range: 2.3-65.0) and 5.8 (range: 1.1-17.9) for fast and slow flow conditions, respectively (Fig. 4, Table S5). Mean flow dependence of sampling rates for Oasis HLB was 4.8 (range: 1.0-11.2) and 3.4 (range: 0.9-7.4) for fast and slow flow conditions, respectively (Fig. 4, Table S5). These values are similar to previously reported values where sampling rates under fast flow conditions were 9.5x and 4.8x greater than static conditions for NOCIS configurations containing Dowex Optipore L-493 and Oasis HLB, respectively (Chapter IV). The flow dependency of many analytes is much greater than found for PRCs (Fig. 3 and Fig. 4), especially for Dowex Optipore L-493.

3.4 PRC validation study

3.4.1 Uncorrected rates

Estimated PRC elimination rates obtained during the eight-day PRC validation study (Table S7) were similar to rates obtained from regression models from the PRC calibration study (Table 2) and had the same order of release from both sorbents. As such, similar release kinetics were obtained between both studies. Mean percentage of initial PRC concentrations in Dowex Optipore L-493 at the conclusion of the validation study were 30% (14), 40% (14), and 53% (6) for fast, slow, and static flow conditions, respectively. Mean percentage of initial PRC concentrations in Oasis HLB at the conclusion of the validation study were 7% (4), 8% (5), and 42% (19) for fast, slow, and static flow conditions, respectively.

For comparison between previous investigations (Chapter IV), analyte R_S were determined at the conclusion of the validation study using Equation 2 and average measured water concentrations (Table S4). Median R_S estimated from the eight-day time point of the validation study were 696 mL/d (range: 54-1006 mL/d), 388 mL/d (range: 8-698 mL/d), and 72 mL/d (range: 4-257 mL/d), respectively for fast, slow, and static conditions from NOCIS configurations containing Dowex Optipore L-493 (Table S4). Similarly for NOCIS configurations containing Oasis HLB, median R_S were 715 mL/d (range: 59-1043 mL/d), 620 mL/d (range: 19-992 mL/d), and 156 mL/d (range: 14-429 mL/d) for fast, slow, and static

conditions, respectively (Table S4). Estimated R_S were 30-35% lower than sampling rates obtained during previous calibration studies conducted under the same fast (9.3 cm/s) and static (0 cm/s) conditions and exposure units (Chapter IV).

3.4.2 Validation study sampling rate corrections

Using Equation 6, analyte R_S calibrated using fast flow conditions (Chapter IV) were corrected based on elimination rates determined from PRC calibration and validation studies. Performance reference compound correction factors applied to calibrated R_S are provided in Table 3 for all flow rates and PRCs. To evaluate the effectiveness of PRC corrections, corrected R_S were used in Equation 2 to estimate water concentrations within the validation study. Estimates obtained for all analytes (n=34) are presented in Figure 5 as percentage of actual measured water concentration (Table S4) from the validation study. As expected, the most dramatic improvement in water concentration predictions occurred for static conditions, due to the greatest differences between in-situ and calibration flow conditions (i.e. 9.3 cm/s). The complete data set presented in Figure 5 is reported in the supplementary information for both Dowex Optipore L-493 (Table S8) and Oasis HLB (Table S9).

Table 3. Mean (standard deviation) correction factors for each flow treatment applied to calibration sampling rates (R_{Scal}) calculated from the ratio of $k_{ePRCcal}$ to $k_{ePRClab}$ (EQ 6). To match analyte *Rs* calibration study flow rates (Chapter IV) correction factors were normalized by $k_{ePRClab}$ obtained from fast flow conditions (Table 2).

	Dowex Optipore L-493			Oasis HLB		
PRC	Fast	Slow	Static	Fast	Slow	Static
Caffeine-$^{13}C_3$	1.212 (0.086)	0.871 (0.114)	0.837 (0.090)	0.821 (0.059)	0.772 (0.035)	0.284 (0.064)
Cotinine-d_3	1.106 (0.186)	0.941 (0.057)	0.629 (0.148)	0.818 (0.051)	0.726 (0.008)	0.334 (0.007)
DIA-d_5	1.199 (0.131)	0.821 (0.105)	0.419 (0.039)	1.312 (0.204)	1.298 (0.373)	0.262 (0.109)

For Dowex Optipore L-493, predicted water concentrations were not significantly improved by PRC corrections. On average, PRC corrected estimates were 13% lower, 15% higher, and 74% higher than uncorrected estimates, respectively for fast, slow, and static flow conditions as compared to estimates calculated from calibration sampling rates (Fig. 5, Table S8). Predictions resulting from PRC corrections had the greatest improvements across all flow rates using DIA-d_5 where predictions averaged 65.7%, 47.2%, and 22.1% of measured concentrations for fast, slow, and static flows, respectively (Fig. 5, Table S8). Cotinine-d_3 provided the best correction under fast flow conditions where predictions averaged 73.7% of measured concentrations; however, no PRC improved the prediction accuracy of water concentrations under fast flow conditions as compared to estimates generated from calibration sampling rates (Fig. 5, Table S8). Regardless of PRC or flow treatment, applying PRC corrections to NOCIS configurations containing Dowex Optipore L-493 had little effect on estimated water concentrations as compared to estimates generated from calibration experiments. Since PRC elimination had minimal flow dependence (Figure 3) as compared to analytes, little improvement was possible.

Across all PRCs, average corrected water estimates for Oasis HLB were 7%, 15%, and 273% higher than water estimates calculated from calibration sampling rates (Fig. 5, Table S9). Caffeine-$^{13}C_3$ provided the most accurate prediction across all flow conditions where estimates averaged 84.3%, 71.1%, and 71.9% of measured values, respectively for fast, slow, and static flow conditions (Fig. 5, Table S9). Cotinine-d_3 provided similar prediction accuracies at fast (84.8%) and slow (75.6%) conditions; however, cotinine-d_3 corrections provided the lowest accuracy (58.0%) of the three PRCs under static conditions (Fig. 5). Average water estimates provided by DIA-d_5 were lowest under fast (53.0%) and slow (42.6%) flow conditions while providing the best predicted concentrations under static (87.0%) conditions (Fig. 5). These data demonstrate the benefits of incorporating multiple PRCs within POCIS-style samplers to provide accurate estimates of water concentrations over a range of flow conditions. For instance, as

compared to water concentrations estimates generated from calibration experiments, caffeine-$^{13}C_3$ and cotinine-d_3 correction provided improved estimates across all flow conditions; however, DIA-d_5 provided the best improvements under static conditions (Fig. 5, Table S9).

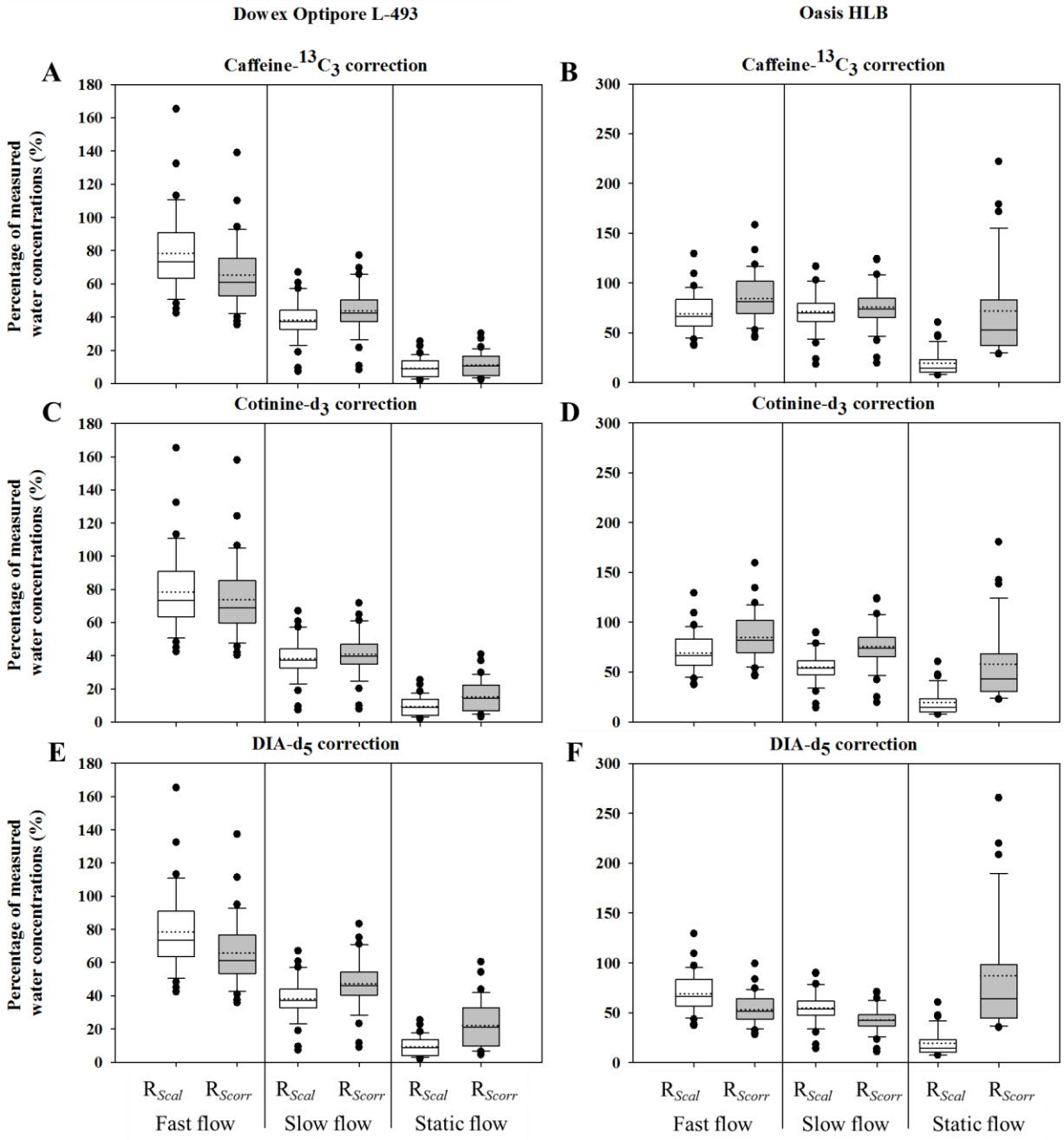


Fig. 5. Estimated water concentrations for 34 target analytes from the validation study for Dowex Optipore L-493 (Left Column) and Oasis HLB (Right Column) under fast, slow, and static conditions. White boxes represent water estimates based on sampling rates from the calibration study (Chapter IV), whereas grey boxes represent water estimates based on sample rates corrected by caffeine-$^{13}C_3$ (**A,B**), cotinine-d_3 (**C,D**), and DIA-d_5 (**E,F**). Estimates provided as box and whisker plots with median (solid horizontal line), mean (dotted horizontal line), inner quartile range (25th and 75th percentiles; box), the 10th and 90th percentiles (whiskers), and outliers (dots).

Performance reference compounds have been suggested for correcting in-situ analyte R_S with the assumption that exchange kinetics are similar between PRC elimination and analyte accumulation [21]. If the mass transfer kinetics of all targeted analytes have similar responses to flow as a PRC, a single PRC may be applicable for correcting the entire data set [20, 21]. However, several studies have suggested that multiple PRCs should be incorporated into samplers to account for differential exchange kinetics [21, 22, 24]. Our data for NOCIS samplers containing Oasis HLB support these claims because no single PRC could account for the wide variation in flow dependencies observed for the entire targeted analyte list (Fig. 5). Therefore, we matched PRCs to analytes based on relative flow dependencies to present a best-fit correction approach. To assign PRCs to target analytes, thresholds were determined by taking the geometric mean of Caffeine-$^{13}C_3$ and DIA-d_5 flow dependencies at fast flow conditions for NOCIS configurations containing Oasis HLB, however, this approach was not attempted with Dowex Optipore L-493 due to the minimal flow dependency differences in PRC elimination (Fig. 3). For Oasis HLB, parings were made based on fast flow conditions and were sorted by magnitude of flow dependence. Analytes with flow dependencies less than 1.7 were not PRC-corrected, analytes with flow dependencies between 1.7 and 4.0 were corrected by Caffeine-$^{13}C_3$ and analytes with flow dependencies > 4.0 were corrected by DIA-d_5. A summary plot of the results from this best-fit approach is provided in Figure 6 for Oasis HLB and water estimates that were included in the best-fit PRC approach are provided as bolded numbers within Table S9. As compared to single PRC adjustment strategy (Fig. 5), analyte sampling rates using the best-fit approach (R_{Sbest}) showed improvement in estimated water concentrations during static conditions (Fig. 6). Significantly, the range of high outliers was reduced so that no estimates were >2x (i.e. 200%) of the measured mean value. Similar to single PRC corrections, the frequency of estimated values below 50% of the true measured values was reduced to 41% (n=14) as compared to 97% (n=33) for estimates generated from calibration studies. As evident by outlier range, PRC correction did not work uniformly across all target analytes, especially when flow conditions

were similar to flow conditions of the calibration study (Chapter IV). The accuracy of the predictions were similar to that found for the individual PRCs (Fig. 5); however, the outlier range was slightly improved (i.e. reduced) suggesting that using multiple PRCs reduced the magnitude of variation (Fig. 6).

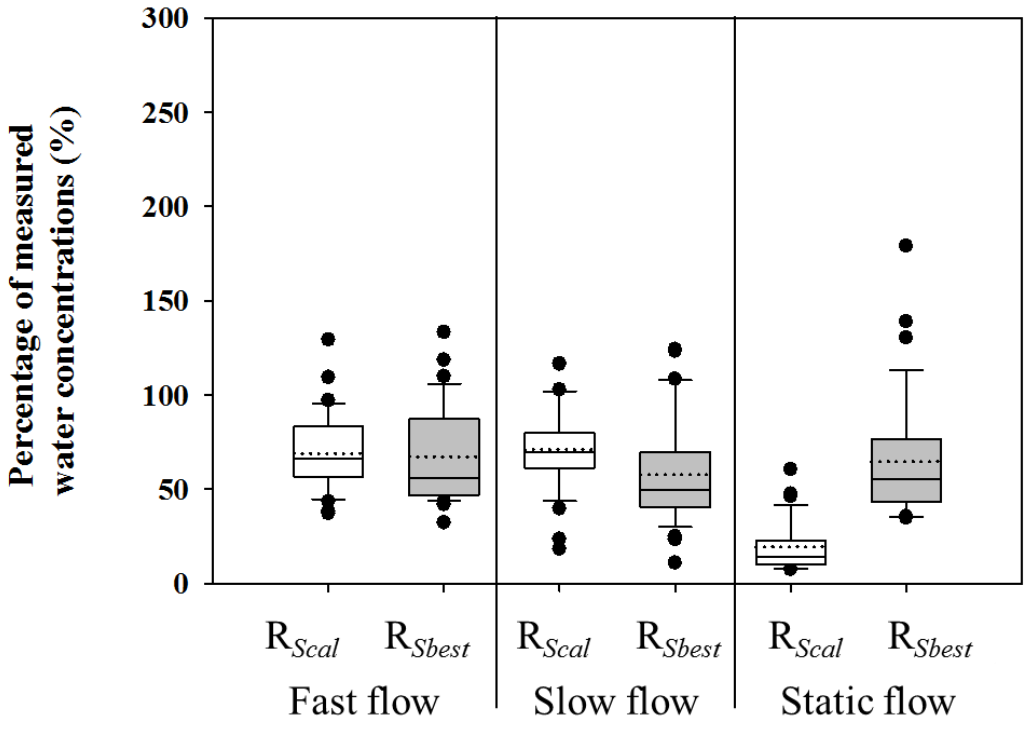


Fig. 6. Estimated water concentrations for 34 target analytes from the validation study for Oasis HLB under fast, slow, and static conditions. White boxes represent water estimates based on sampling rates from the calibration study (Chapter IV), whereas grey boxes represent water estimates based on sample rates corrected using the best-fit PRC approach. Analytes with flow dependencies < 1.7 were not corrected, analytes with flow dependencies between 1.7 and 4.0 were corrected by caffeine-$^{13}C_3$, and analytes with flow dependencies > 4.0 were corrected by DIA-d_5.

Previous investigations evaluating the effectiveness of PRC corrections have typically conducted laboratory calibrations for sampling rates and PRC elimination under one flow condition followed by in-situ exposures at field locations [20, 21]. Our approach was similar in that calibration experiments were conducted separately from the PRC validation study; however, the in-situ validation reported herein was conducted under highly controlled conditions with

known flow rates and contaminant concentrations. This was done purposefully to evaluate the effectiveness of PRC corrections under stable conditions.

4. Conclusions

Our results suggest that Oasis HLB is more amenable to PRC approach as compared to Dowex Optipore L-493. Thus, if Dowex Optipore L-493 is used, calibration study flow conditions should be similar to expected field conditions. For Oasis HLB, the PRC approach has potential to decrease the impact of flow on sampling rate accuracy when the impact of flow is substantially different than calibration conditions. However, when in-situ flow conditions are similar to calibration conditions, PRC adjustment did not decrease variability, which is somewhat inherent when additional measurements are added to the system but are not necessary. In these situations, the benefits of PRC correction may be outweighed by error in measurement and slight difference among samplers, thus reducing the overall effectiveness of PRC adjustment. The potential to reduce major errors with PRCs greatly improves the feasibility of using NOCIS configurations containing Oasis HLB for environmental monitoring, especially when in-situ flow conditions unknown or dramatically different from calibration flow conditions. Although there is greater variability in measuring water concentrations with NOCIS (or integrative samplers in general) compared to collecting and extracting discrete water samples, this variability is much less than what is likely obtained from collecting a few discrete water samples (Chapter II).

Acknowledgments

The authors claim no conflict of interest for this research.

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Appendix

Supporting Information:

CHARACTERIZATION OF PERFORMANCE REFERENCE COMPOUND KINETICS AND ANALYTE SAMPLING RATE CORRECTIONS UNDER THREE FLOW REGIMES USING POCIS-STYLE NYLON ORGANIC CHEMICAL INTEGRATIVE SAMPLERS

Summary of Contents:

Table S1. Analyte analysis method, retention time (RT), select ions monitored (SIM). The two qualitative (Qual) ions had to be within 20% of expected ratio, relative to the quantitative (Quant) ion response, to report the analyte without flagging the data as qualitatively uncertain.

Analyte	GC Method	RT (min)	SIM ions Quantitative: Qualitative (m/z)
DEET	1	6.85	119:190, 191
Desethyl atrazine	1	7.76	172:174, 187
Trifluralin	1	7.98	306:264, 290
Atrazine-d_5 (surrogate)	1	8.69	205:178, 220
Atrazine	1	8.72	200:173, 215
Chlorothalonil	1	8.99	266:264, 268
Galaxolide	1	9.56	243:258, 213
Musk tonalid	1	9.66	243:258, 187
Acetochlor	1	9.89	146:162, 223
Metolachlor	1	10.79	162:238, 240
Musk ketone	1	10.82	279:128, 294
Chlorpyrifos	1	10.88	197:258, 314
Pendimethalin	1	11.58	252:253, 281
Fluoranthene	1	11.68	202:200, 203
Pyrene	1	12.22	202:200, 203
Octinoxate	1	14.50	178:161,179
Tebuconazole	1	14.91	125:163, 250
Propiconazole	2	9.75	259:173, 191
Trifloxystrobin	2	9.94	116:131, 222
Benzo(a)anthracene-d_{16} (surrogate)	2	10.82	240
Benzo(a)anthracene	2	10.75	228:226
Chrysene	2	10.84	228:226
Bifenthrin	2	11.27	181:165, 166
Metconazole	2	11.41	125:250, 319
λ-Cyhalothrin	2	12.66	181:141, 197
Trans-permethrin	2	13.58	183:163, 164
Cis-permethrin	2	13.78	183:163, 165
Benzo(b)fluoranthene	2	13.88	252:253
Benzo(k)fluoranthene	2	13.96	252:254
Cyfluthrin	2	14.71	163:165, 226
Benzo(a)pyrene	2	14.83	252:250
Pyraclostrobin	2	16.06	132:164, 325
Azoxystrobin	2	17.14	344:388, 403
Benzo(g,h,i)perylene	2	17.14	276:277

Dibenz(a,h)anthracene	2	17.72	278:276
Indeno(1,2,3-cd)pyrene	2	18.02	276:277
Desisopropyl atrazine-d_5	3	6.98	178:160, 180
Cotinine-d_3	3	7.52	179:122, 147
Atrazine-d_5	3	7.98	205:178, 220
Lindane	3	8.02	181:219, 254
Caffeine-$^{13}C_3$	3	8.67	197:111, 196
Fluoranthene-d_{10}	3	9.93	210:212, 213

Table S2. Mean (standard deviation) analyte extraction recoveries from fortified quality control samples for the three sorbent medias and water. Expected recoveries are based on analysis of spiking solutions (n=10) conducted throughout all experiments.

Analyte	Percentage of expected (SD)		
	Dowex Optipore L-493 (n=6)	Oasis HLB (n=6)	Water (n=7)
Acetochlor	91.2 (4.2)	95.5 (5.0)	89.2 (7.1)
Atrazine	91.3 (3.5)	92.2 (4.9)	96.1 (6.6)
Azoxystrobin	110.8 (10.0)	106.5 (16.7)	167.4 (18.9)
Benzo(a)anthracene	81.1 (4.0)	85.0 (2.7)	79.5 (12.6)
Benzo(a)pyrene	73.7 (4.9)	85.3 (4.4)	33.0 (14.3)
Benzo(b)fluoranthene	79.1 (4.2)	85.5 (6.5)	68.9 (5.5)
Benzo(g,h,i)perylene	84.7 (6.7)	100.4 (9.6)	95.5 (11.8)
Benzo(k)fluoranthene	73.9 (7.0)	82.0 (5.6)	63.4 (8.6)
Bifenthrin	92.6 (5.0)	87.4 (5.4)	75.8 (3.9)
Chlorothalonil	205.3 (11.8)	143.0 (17.1)	100.3 (30.8)
Chlorpyrifos	82.1 (6.4)	83.6 (4.8)	64.5 (8.3)
Chrysene	75.6 (4.8)	81.7 (4.7)	73.5 (9.8)
Cis-permethrin	114.4 (6.4)	107.7 (9.5)	105.6 (6.4)
Cyfluthrin	111.1 (6.4)	100.6 (13.3)	125.6 (12.1)
λ-Cyhalothrin	120.1 (6.3)	103.3 (8.5)	115.4 (6.3)
DEET	85.7 (5.8)	87.9 (6.6)	87.9 (6.3)

Desethyl atrazine	96.3 (4.7)	95.3 (4.3)	98.1 (7.7)
Dibenz(a,h)anthracene	87.7 (5.6)	102.8 (8.8)	105.9 (12.8)
Fluoranthene	86.9 (5.9)	89.1 (4.3)	92.2 (6.8)
Galaxolide	86.1 (5.3)	88.5 (6.7)	57.6 (6.4)
Indeno(1,2,3-cd)pyrene	77.3 (6.1)	102.3 (6.4)	92.0 (13.0)
Metconazole	84.2 (4.2)	86.6 (5.0)	93.1 (7.0)
Metolachlor	90.9 (3.9)	92.4 (4.9)	88.8 (6.7)
Musk ketone	89.5 (4.1)	90.3 (5.2)	85.9 (7.0)
Musk tonalid	85.5 (4.9)	88.3 (5.2)	61.3 (7.1)
Octinoxate	78.3 (4.5)	77.7 (5.0)	54.0 (7.0)
Pendimethalin	91.4 (6.1)	91.4 (4.4)	69.6 (8.3)
Propiconazole	88.0 (4.8)	88.8 (4.5)	79.2 (5.2)
Pyraclostrobin	99.0 (8.5)	88.0 (19.2)	141.6 (14.9)
Pyrene	81.7 (5.4)	85.2 (3.8)	81.8 (7.2)
Tebuconazole	75.2 (7.0)	76.6 (6.1)	84.0 (6.3)
Trans-permethrin	112.7 (6.3)	105.2 (8.1)	99.5 (4.9)
Trifloxystrobin	91.3 (6.1)	95.5 (5.0)	77.6 (4.7)
Trifluralin	89.4 (5.7)	92.2 (4.9)	43.6 (7.4)

Table S3. Mean (standard deviation) PRC extraction recoveries from fortified quality control samples for the three sorbent medias and water. Expected recoveries are based on analysis of spiking solutions (n=10) conducted throughout all experiments.

Analyte	Percentage of expected (SD)		
	Dowex Optipore L-493 (n=6)	Oasis HLB (n=6)	Water (n=4)
Atrazine-d_5	97.3 (10.8)	94.8 (8.0)	103.0 (2.7)
Caffeine-$^{13}C_3$	105.4 (14.2)	113.9 (8.2)	103.9 (2.1)
Cotinine-d_3	104.5 (6.6)	104.0 (10.8)	88.1 (6.8)
Desisopropyl atrazine-d_5	100.6 (4.7)	100.1 (5.4)	115.0 (8.2)
Fluoranthene-d_{10}	102.1 (4.7)	104.1 (8.0)	71.1 (2.9)
Lindane	87.9 (5.5)	94.0 (4.2)	104.5 (5.8)

Table S4. Mean (standard deviation) measured and percentage of expected of water concentrations in experimental units during the validation study for fast, slow, and static treatments. For each flow treatment, water samples were obtained daily before water exchanges (n=4) and after 5-h after exchanges (n=4). Expected recoveries are based on analysis of spiking solutions (n=10) conducted throughout all experiments.

Analyte	Fast (9.3 cm/s)		Slow (5.0 cm/s)		Static (0 cm/s)	
	Measured (ng/mL)	Percent of expected (%)	Measured (ng/mL)	Percent of expected (%)	Measured (ng/mL)	Percent of expected (%)
Acetochlor	0.46 (0.07)	97.7 (14.5)	0.48 (0.09)	101.2 (18.0)	0.51 (0.04)	106.6 (9.2)
Atrazine	0.54 (0.07)	110.7 (14.3)	0.57 (0.11)	117.2 (22.5)	0.60 (0.05)	122.4 (10.7)
Azoxystrobin	0.64 (0.10)	129.0 (20.6)	0.65 (0.12)	131.5 (25.0)	0.76 (0.12)	154.8 (24.4)
Benzo(a)anthracene	0.03 (0.01)	32.0 (10.4)	0.03 (0.00)	29.8 (4.8)	0.02 (0.01)	25.2 (13.8)
Benzo(a)pyrene	0.01 (0.00)	7.8 (3.6)	0.01 (0.00)	6.3 (1.7)	0.01 (0.01)	10.4 (8.1)
Benzo(b)fluoranthene	0.05 (0.01)	59.3 (10.7)	0.04 (0.01)	50.3 (12.1)	0.05 (0.02)	54.5 (26.5)
Benzo(g,h,i)perylene	0.06 (0.02)	56.5 (17.7)	0.05 (0.01)	47.3 (8.7)	0.07 (0.04)	67.4 (38.4)
Benzo(k)fluoranthene	0.04 (0.01)	43.8 (10.3)	0.04 (0.01)	39.4 (11.3)	0.04 (0.02)	47.2 (23.4)
Bifenthrin	0.09 (0.03)	87.5 (34.7)	0.08 (0.03)	79.6 (29.1)	0.08 (0.03)	76.8 (32.4)
Chlorothalonil	0.48 (0.14)	143.5 (41.0)	0.42 (0.13)	125.3 (40.1)	0.40 (0.15)	119.7 (44.5)
Chlorpyrifos	0.07 (0.01)	79.6 (13.9)	0.07 (0.01)	74.2 (14.6)	0.06 (0.01)	62.6 (8.3)
Chrysene	0.05 (0.01)	55.7 (11.0)	0.05 (0.01)	53.1 (11.8)	0.05 (0.02)	52.1 (25.1)
Cis-permethrin	0.06 (0.02)	64.3 (16.5)	0.06 (0.02)	56.5 (18.3)	0.07 (0.03)	69.3 (27.2)
Cyfluthrin	0.07 (0.01)	65.9 (9.3)	0.06 (0.02)	53.2 (17.1)	0.07 (0.03)	64.2 (23.5)

λ-Cyhalothrin	0.08 (0.02)	76.0 (19.7)	0.07 (0.02)	68.7 (21.5)	0.08 (0.03)	74.2 (31.9)
DEET	0.44 (0.07)	92.7 (14.0)	0.47 (0.09)	99.2 (18.8)	0.50 (0.05)	104.9 (10.0)
Desethyl atrazine	0.56 (0.07)	114.4 (13.5)	0.58 (0.12)	118.1 (23.7)	0.59 (0.05)	121.2 (10.8)
Dibenz(a,h)anthracene	0.09 (0.04)	94.2 (38.6)	0.09 (0.03)	95.2 (29.9)	0.10 (0.07)	109.6 (71.3)
Fluoranthene	0.10 (0.02)	111.2 (20.5)	0.11 (0.02)	116.2 (19.1)	0.08 (0.02)	90.9 (16.4)
Galaxolide	0.30 (0.05)	62.1 (11.4)	0.30 (0.07)	61.8 (14.9)	0.25 (0.02)	53.1 (3.8)
Indeno(1,2,3-cd)pyrene	0.08 (0.03)	88.8 (31.6)	0.07 (0.02)	75.4 (18.9)	0.08 (0.05)	95.0 (58.2)
Metconazole	0.51 (0.08)	96.9 (15.7)	0.54 (0.12)	102.5 (22.3)	0.58 (0.10)	111.0 (18.5)
Metolachlor	0.46 (0.06)	96.3 (13.2)	0.49 (0.09)	102.1 (19.2)	0.51 (0.04)	106.7 (9.1)
Musk ketone	0.43 (0.05)	89.3 (11.3)	0.45 (0.08)	94.2 (17.7)	0.43 (0.04)	90.3 (9.0)
Musk Tonalid	0.31 (0.06)	63.0 (12.8)	0.30 (0.07)	62.7 (15.4)	0.24 (0.03)	50.2 (5.3)
Octinoxate	0.28 (0.06)	46.8 (9.6)	0.24 (0.05)	40.0 (7.9)	0.21 (0.06)	34.6 (9.9)
Pendimethalin	0.39 (0.05)	75.8 (9.9)	0.39 (0.07)	76.3 (13.6)	0.30 (0.05)	58.8 (10.7)
Propiconazole	0.48 (0.09)	87.4 (16.9)	0.51 (0.12)	93.2 (21.9)	0.55 (0.07)	100.1 (13.2)
Pyraclostrobin	0.61 (0.11)	124.5 (23.5)	0.60 (0.13)	123.3 (26.3)	0.67 (0.12)	137.2 (23.8)
Pyrene	0.07 (0.01)	78.0 (15.7)	0.07 (0.02)	81.7 (18.6)	0.05 (0.02)	56.4 (23.0)
Tebuconazole	0.54 (0.12)	96.9 (22.1)	0.54 (0.11)	97.7 (19.3)	0.59 (0.06)	107.4 (11.7)
Trans-permethrin	0.07 (0.02)	65.2 (18.4)	0.06 (0.02)	55.6 (20.7)	0.07 (0.02)	63.7 (23.3)

Trifloxystrobin	0.43 (0.11)	78.2 (20.0)	0.46 (0.13)	84.0 (23.4)	0.45 (0.07)	81.1 (13.2)
Trifluralin	0.26 (0.06)	54.0 (12.0)	0.22 (0.07)	45.8 (13.4)	0.17 (0.02)	33.8 (4.9)

Table S5. Mean (standard deviation) sampling rates (R_S) for NOCIS configurations containing Dowex Optipore L-493 and Oasis HLB as calculated from the 8-d time point of the validation study exposure using Equation 2.

	Analyte Sampling rates (mL/d)					
	Dowex Optipore L-493			Oasis HLB		
Analyte	Fast (9.3 cm/s)	Slow (5.0 cm/s)	Static (0 cm/s)	Fast (9.3 cm/s)	Slow (5.0 cm/s)	Static (0 cm/s)
Acetochlor	717 (101)	431 (74)	67 (4)	717 (60)	643 (141)	114 (14)
Atrazine	693 (109)	421 (69)	57 (4)	671 (77)	614 (146)	99 (12)
Azoxystrobin	651 (85)	399 (67)	49 (8)	646 (77)	576 (84)	82 (17)
Benzo(a)anthracene	952 (211)	374 (82)	61 (10)	957 (135)	702 (260)	280 (21)
Benzo(a)pyrene	348 (107)	109 (20)	37 (4)	377 (66)	254 (125)	254 (52)
Benzo(b)fluoranthene	707 (230)	214 (46)	12 (1)	675 (114)	412 (199)	74 (13)
Benzo(g,h,i)perylene	169 (53)	34 (9)	6 (1)	156 (73)	68 (25)	28 (3)
Benzo(k)fluoranthene	676 (235)	167 (44)	11 (1)	614 (113)	344 (197)	55 (7)
Bifenthrin	170 (23)	83 (10)	78 (13)	170 (18)	144 (51)	171 (24)
Chlorothalonil	1006 (159)	698 (104)	84 (6)	949 (70)	992 (246)	146 (34)
Chlorpyrifos	642 (87)	405 (79)	119 (18)	840 (24)	761 (178)	284 (55)

Chrysene	929 (181)	339 (63)	29 (6)	920 (156)	646 (232)	121 (11)
Cis-permethrin	695 (120)	351 (53)	103 (7)	752 (84)	603 (180)	285 (53)
Cyfluthrin	505 (67)	287 (39)	87 (10)	583 (26)	552 (108)	242 (45)
λ-Cyhalothrin	290 (62)	128 (17)	82 (10)	325 (77)	251 (57)	213 (30)
DEET	729 (119)	414 (73)	59 (5)	659 (80)	626 (164)	123 (47)
Desethyl atrazine	514 (66)	370 (40)	61 (4)	382 (29)	384 (67)	106 (34)
Dibenz(a,h)anthracene	54 (14)	8 (2)	4 (0)	59 (26)	19 (6)	14 (1)
Fluoranthene	844 (172)	406 (69)	94 (3)	917 (52)	684 (170)	245 (34)
Galaxolide	851 (143)	479 (77)	185 (2)	898 (66)	761 (205)	319 (64)
Indeno(1,2,3-cd)pyrene	73 (20)	16 (4)	5 (1)	74 (26)	36 (13)	19 (1)
Metconazole	618 (78)	347 (48)	34 (2)	728 (105)	619 (122)	95 (21)
Metolachlor	697 (98)	418 (70)	64 (4)	711 (59)	622 (134)	108 (5)
Musk ketone	831 (173)	455 (68)	122 (11)	844 (53)	715 (178)	204 (4)
Musk Tonalid	863 (145)	473 (65)	178 (11)	925 (67)	776 (218)	294 (33)
Octinoxate	826 (118)	533 (81)	181 (23)	850 (137)	817 (201)	338 (49)
Pendimethalin	826 (120)	490 (63)	153 (15)	899 (63)	762 (178)	272 (16)
Propiconazole	659 (82)	376 (59)	45 (5)	712 (104)	604 (117)	102 (21)
Pyraclostrobin	701 (104)	416 (71)	79 (9)	759 (74)	635 (130)	143 (11)
Pyrene	954 (200)	449 (74)	115 (7)	1043 (62)	776 (209)	332 (38)
Tebuconazole	641 (115)	366 (52)	34 (5)	689 (145)	621 (129)	90 (26)

Trans-permethrin	654 (102)	331 (44)	112 (8)	703 (97)	576 (163)	306 (56)
Trifloxystrobin	730 (84)	409 (67)	97 (16)	730 (99)	602 (137)	166 (10)
Trifluralin	912 (152)	541 (48)	257 (24)	995 (73)	900 (243)	430 (30)

Table S6. Mean (standard deviation) flow dependence for Dowex Optipore L-493 and Oasis HLB. Flow dependence for fast and slow flow conditions were calculated by normalizing the respective R_S by rates obtained from static conditions. Sampling rates were calculated from the 8-d time point of the validation study (Table S5) using Equation 2.

Analyte	Flow dependence ($R_{Sflow}/R_{Sstatic}$)			
	Dowex Optipore L-493		Oasis HLB	
	Fast (9.3 cm/s)	Slow (5.0 cm/s)	Fast (9.3 cm/s)	Slow (5.0 cm/s)
Acetochlor	10.7 (0.9)	6.4 (0.9)	6.4 (1.2)	5.8 (1.7)
Atrazine	12.0 (1.0)	7.3 (1.1)	6.9 (1.5)	6.3 (1.8)
Azoxystrobin	13.3 (1.0)	8.2 (1.3)	8.2 (2.3)	7.3 (2.3)
Benzo(a)anthracene	16.1 (6.0)	6.3 (2.0)	3.4 (0.3)	2.5 (0.7)
Benzo(a)pyrene	9.8 (4.1)	3.1 (0.9)	1.5 (0.5)	1.0 (0.4)
Benzo(b)fluoranthene	60.0 (25.3)	17.9 (4.8)	9.2 (1.4)	5.4 (1.7)
Benzo(g,h,i)perylene	31.9 (13.9)	6.2 (1.7)	5.5 (2.7)	2.4 (0.7)
Benzo(k)fluoranthene	65.0 (26.6)	16.0 (5.2)	11.2 (1.6)	6.0 (2.7)
Bifenthrin	2.3 (0.7)	1.1 (0.3)	1.0 (0.2)	0.9 (0.5)
Chlorothalonil	12.1 (2.4)	8.4 (1.2)	6.7 (1.5)	7.3 (3.2)
Chlorpyrifos	5.5 (1.0)	3.4 (0.3)	3.0 (0.6)	2.8 (1.2)
Chrysene	34.1 (13.0)	12.1 (3.3)	7.6 (0.6)	5.3 (1.5)
Cis-permethrin	6.8 (1.7)	3.5 (0.8)	2.7 (0.7)	2.2 (0.9)
Cyfluthrin	5.9 (1.2)	3.3 (0.4)	2.5 (0.4)	2.3 (0.6)
λ-Cyhalothrin	3.6 (1.0)	1.6 (0.4)	1.6 (0.5)	1.2 (0.4)
DEET	12.3 (0.9)	7.0 (1.0)	6.0 (2.5)	5.7 (2.7)
Desethyl atrazine	8.3 (0.5)	6.0 (0.6)	3.9 (1.3)	3.9 (1.6)
Dibenz(a,h)anthracene	14.0 (3.1)	2.2 (0.7)	4.2 (1.9)	1.4 (0.3)
Fluoranthene	9.0 (1.8)	4.3 (0.7)	3.8 (0.6)	2.8 (0.4)
Galaxolide	4.6 (0.7)	2.6 (0.4)	2.9 (0.7)	2.5 (1.1)
Indeno(1,2,3-cd)pyrene	16.3 (6.1)	3.5 (0.8)	3.9 (1.5)	1.9 (0.6)
Metconazole	18.0 (2.1)	10.1 (0.8)	8.1 (2.6)	6.9 (2.4)

Metolachlor	10.9 (0.9)	6.5 (0.8)	6.6 (0.7)	5.8 (1.3)
Musk ketone	6.9 (1.7)	3.7 (0.5)	4.1 (0.3)	3.5 (0.9)
Musk Tonalid	4.9 (1.0)	2.7 (0.4)	3.2 (0.2)	2.6 (0.7)
Octinoxate	4.6 (1.1)	2.9 (0.1)	2.6 (0.7)	2.5 (1.0)
Pendimethalin	5.4 (0.9)	3.2 (0.3)	3.3 (0.4)	2.8 (0.8)
Propiconazole	14.8 (2.5)	8.3 (0.5)	7.3 (2.2)	6.2 (2.1)
Pyraclostrobin	9.1 (2.3)	5.3 (1.1)	5.3 (0.7)	4.5 (1.3)
Pyrene	8.3 (1.9)	3.9 (0.6)	3.2 (0.3)	2.3 (0.4)
Tebuconazole	19.3 (5.3)	10.8 (1.5)	8.4 (3.6)	7.4 (2.9)
Trans-permethrin	5.9 (1.3)	3.0 (0.6)	2.4 (0.6)	2.0 (0.8)
Trifloxystrobin	7.7 (2.0)	4.3 (0.9)	4.4 (0.5)	3.6 (0.9)
Trifluralin	3.6 (0.7)	2.1 (0.1)	2.3 (0.2)	2.1 (0.6)

Table S7. Mean (standard deviation) performance reference compound (PRC) elimination rate for NOCIS configurations containing Dowex Optipore L-493 and Oasis HLB as calculated from the 8-d time point of the validation study using Equation 5.

PRC	PRC elimination rates (d^{-1})					
	Dowex Optipore L-493			Oasis HLB		
	Fast (9.3 cm/s)	Slow (5.0 cm/s)	Static (0 cm/s)	Fast (9.3 cm/s)	Slow (5.0 cm/s)	Static (0 cm/s)
Caffeine-$^{13}C_3$	0.100 (0.007)	0.072 (0.009)	0.069 (0.007)	0.288 (0.021)	0.270 (0.012)	0.100 (0.023)
Cotinine-d_3	0.150 (0.025)	0.128 (0.008)	0.085 (0.020)	0.489 (0.030)	0.434 (0.005)	0.200 (0.004)
Desisopropyl atrazine-d_5	0.247 (0.027)	0.169 (0.022)	0.085 (0.008)	0.321 (0.050)	0.318 (0.091)	0.064 (0.027)

Table S8. Mean estimated water concentrations expressed as percentage of actual measured water concentrations (Table S4) for 34 target analytes during the PRC validation study for Dowex Optipore L-493 under fast, slow, and static conditions. Estimates are provided based on sampling rates (R_S) from the calibration study (Chapter IV) and sampling rates corrected by caffeine-$^{13}C_3$, cotinine-d_3, and DIA-d_5.

Analyte	Percentage of measured water concentrations (%)											
	R_{Scal}			R_{Scorr} - caffeine-$^{13}C_3$			R_{Scorr} - cotinine-d_3			R_{Scorr} - DIA-d_5		
	Fast (9.3 cm/s)	Slow (5.0 cm/s)	Static (0 cm/s)	Fast (9.3 cm/s)	Slow (5.0 cm/s)	Static (0 cm/s)	Fast (9.3 cm/s)	Slow (5.0 cm/s)	Static (0 cm/s)	Fast (9.3 cm/s)	Slow (5.0 cm/s)	Static (0 cm/s)
Acetochlor	74.8	44.9	7.0	62.3	51.4	8.4	70.0	47.9	11.5	62.6	55.5	16.8
Atrazine	72.2	43.9	6.0	60.2	50.2	7.2	67.7	46.8	9.9	60.4	54.1	14.4
Azoxystrobin	68.6	42.1	5.2	57.0	48.1	6.3	64.2	44.9	8.6	57.7	51.9	12.5
Benzo(a)anthracene	96.4	37.9	6.2	80.6	43.1	7.4	91.0	40.5	10.0	80.5	47.0	14.8
Benzo(a)pyrene	99.3	31.1	10.4	83.3	35.5	12.5	94.4	33.2	17.1	82.5	38.4	25.0
Benzo(b)fluoranthene	108.5	32.9	1.9	91.1	37.4	2.2	103.4	35.1	3.0	90.2	40.7	4.4
Benzo(g,h,i)perylene	94.0	18.9	3.1	78.4	21.5	3.7	89.7	20.1	5.0	79.5	23.2	7.3
Benzo(k)fluoranthene	165.3	40.7	2.6	139.0	46.2	3.1	157.9	43.4	4.3	137.3	50.3	6.2
Bifenthrin	55.7	27.1	25.4	46.3	31.3	30.2	52.1	29.0	40.8	46.7	33.6	60.4
Chlorothalonil	87.5	60.7	7.3	72.9	69.6	8.7	82.0	64.8	11.8	73.2	75.1	17.4

Chlorpyrifos	53.0	33.4	9.8	44.1	38.1	11.7	49.5	35.6	15.8	44.3	41.2	23.4
Chrysene	102.9	37.5	3.2	85.9	42.9	3.8	96.9	40.0	5.1	86.1	46.2	7.5
Cis-permethrin	113.2	57.2	16.8	94.3	65.7	20.1	106.4	61.2	27.4	95.0	71.1	40.1
Cyfluthrin	89.8	51.0	15.4	74.5	58.6	18.5	84.0	54.6	25.1	75.8	63.3	36.9
λ-Cyhalothrin	65.1	28.6	18.4	53.8	32.9	21.9	60.9	30.6	29.8	55.4	35.5	43.8
DEET	74.5	42.3	6.0	62.1	48.4	7.3	69.8	45.1	9.9	62.2	52.2	14.5
Desethyl atrazine	79.5	57.2	9.5	66.2	65.8	11.5	74.3	61.0	15.7	66.7	70.5	22.9
Dibenz(a,h)anthracene	48.2	7.3	3.4	40.0	8.3	4.1	45.6	7.8	5.7	40.9	9.0	8.3
Fluoranthene	79.9	38.4	8.9	66.7	43.9	10.7	75.2	41.0	14.6	66.7	47.5	21.3
Galaxolide	65.7	37.0	14.3	54.8	42.3	17.2	61.7	39.4	23.5	55.0	45.7	34.4
Indeno(1,2,3-cd)pyrene	42.3	9.4	2.7	35.3	10.7	3.2	40.3	10.0	4.3	35.8	11.5	6.4
Metconazole	60.2	33.8	3.3	50.1	38.9	4.0	56.3	36.0	5.5	50.5	41.6	8.0
Metolachlor	70.0	42.0	6.4	58.3	48.0	7.7	65.5	44.8	10.5	58.6	51.8	15.4
Musk ketone	71.5	39.1	10.5	59.7	44.8	12.6	67.4	41.7	17.0	59.7	48.3	25.1
Musk Tonalid	65.3	35.8	13.4	54.4	41.1	16.1	61.3	38.2	21.9	54.6	44.2	32.2
Octinoxate	44.9	29.0	9.8	37.4	33.7	11.7	41.9	30.7	15.9	37.5	35.3	23.5
Pendimethalin	79.1	47.0	14.7	65.9	53.9	17.6	74.1	50.1	23.8	66.2	58.0	35.1

Propiconazole	63.1	36.1	4.3	52.5	41.3	5.2	59.0	38.4	7.0	53.0	44.4	10.3
Pyraclostrobin	80.6	47.8	9.1	67.0	54.7	10.8	75.5	51.0	14.7	67.9	59.0	21.6
Pyrene	78.1	36.7	9.4	65.2	42.1	11.3	73.6	39.3	15.4	65.2	45.5	22.6
Tebuconazole	63.6	36.3	3.4	53.0	41.6	4.0	59.9	38.7	5.4	53.4	44.7	8.0
Trans-permethrin	132.4	67.0	22.7	110.1	77.2	27.2	124.2	71.7	36.9	111.3	83.3	54.2
Trifloxystrobin	65.6	36.8	8.7	54.6	42.1	10.4	61.2	39.2	14.0	55.0	45.3	20.8
Trifluralin	53.8	31.9	15.2	44.9	36.8	18.1	50.4	34.0	24.6	44.9	39.3	36.2

Table S9. Mean estimated water concentrations expressed as percentage of actual measured water concentrations (Table S4) for 34 target analytes during the PRC validation study for Oasis HLB under fast, slow, and static conditions. Estimates are provided based on sampling rates (R_S) from the calibration study (Chapter IV) and sampling rates corrected by caffeine-$^{13}C_3$, cotinine-d_3, and DIA-d_5. Bolded values were used for the "Best-Fit" PRC correction.

Analyte	Percentage of measured water concentrations (%)											
	R_{Scal}			R_{Scorr} - caffeine-$^{13}C_3$			R_{Scorr} - cotinine-d_3			R_{Scorr} - DIA-d_5		
	Fast (9.3 cm/s)	Slow (5.0 cm/s)	Static (0 cm/s)	Fast (9.3 cm/s)	Slow (5.0 cm/s)	Static (0 cm/s)	Fast (9.3 cm/s)	Slow (5.0 cm/s)	Static (0 cm/s)	Fast (9.3 cm/s)	Slow (5.0 cm/s)	Static (0 cm/s)
Acetochlor	65.9	59.1	10.5	80.7	81.5	39.0	81.0	81.5	31.3	**50.9**	**46.2**	**47.4**
Atrazine	67.0	61.3	9.9	82.0	84.6	36.9	82.4	84.6	29.7	**51.6**	**47.8**	**44.8**
Azoxystrobin	60.0	53.5	7.6	73.4	73.7	28.8	73.8	73.7	22.9	**46.2**	**42.5**	**35.5**
Benzo(a)anthracene	83.4	61.2	24.4	**102.2**	**84.5**	**88.1**	102.8	84.5	72.9	64.3	46.7	104.2
Benzo(a)pyrene	**89.7**	**60.4**	**60.5**	110.1	83.4	221.9	110.6	83.4	180.5	69.2	44.7	265.3
Benzo(b)fluoranthene	93.7	57.3	10.2	115.0	79.1	36.7	115.6	79.1	30.5	**72.3**	**42.7**	**43.0**
Benzo(g,h,i)perylene	70.0	30.6	12.7	84.9	42.3	46.0	86.9	42.3	38.0	**52.1**	**23.3**	**54.5**
Benzo(k)fluoranthene	129.3	72.4	11.5	158.2	100.0	41.4	159.5	100.0	34.4	**99.2**	**52.8**	**48.6**
Bifenthrin	**46.0**	**39.0**	**46.2**	56.3	53.8	171.4	56.6	53.8	138.3	35.4	29.9	208.3
Chlorothalonil	70.3	73.6	10.9	86.2	101.5	41.3	86.1	101.5	32.6	**54.8**	**57.2**	**51.0**
Chlorpyrifos	55.4	50.2	18.7	**67.7**	**69.2**	**70.7**	67.9	69.2	56.2	42.8	39.0	86.9
Chrysene	87.4	61.4	11.5	107.1	84.7	41.3	107.7	84.7	34.3	**67.3**	**46.6**	**48.8**
Cis-permethrin	97.1	77.9	36.8	**118.6**	**107.4**	**138.9**	119.4	107.4	110.3	74.6	60.5	170.4
Cyfluthrin	83.3	78.8	34.5	**101.6**	**108.5**	**130.3**	102.0	108.5	103.5	64.2	64.4	160.1
λ-Cyhalothrin	**54.3**	**42.0**	**35.6**	66.0	57.9	133.1	66.9	57.9	106.6	41.2	33.7	162.5

DEET	70.4	66.9	13.2	86.2	92.2	51.1	86.7	92.2	39.5	**54.2**	**51.8**	**64.2**
Desethyl atrazine	89.7	90.1	24.9	**109.8**	**124.2**	**95.9**	110.3	124.2	74.7	69.3	70.9	119.4
Dibenz(a,h)anthracene	43.4	14.1	10.3	52.7	19.4	37.1	53.9	19.4	30.7	**32.4**	**10.9**	**43.8**
Fluoranthene	72.5	54.0	19.3	**88.6**	**74.5**	**70.1**	89.0	74.5	57.7	56.0	42.1	83.1
Galaxolide	57.8	49.0	20.5	**70.7**	**67.5**	**77.6**	71.0	67.5	61.6	44.6	38.0	95.6
Indeno(1,2,3-cd)pyrene	37.2	18.1	9.6	**45.3**	**25.0**	**34.8**	46.1	25.0	28.6	28.1	13.8	41.5
Metconazole	58.5	49.7	7.6	71.5	68.6	28.8	72.0	68.6	22.8	**44.9**	**39.0**	**35.4**
Metolachlor	62.2	54.3	9.5	76.1	74.9	34.7	76.4	74.9	28.3	**48.1**	**42.5**	**41.7**
Musk ketone	60.6	51.3	14.7	74.2	70.8	53.7	74.5	70.8	43.8	**46.9**	**39.9**	**64.3**
Musk Tonalid	57.2	48.1	18.2	**70.1**	**66.3**	**65.7**	70.3	66.3	54.4	44.2	37.2	77.8
Octinoxate	38.3	36.9	15.2	**46.9**	**50.8**	**56.7**	47.3	50.8	45.7	29.4	28.6	69.1
Pendimethalin	71.2	60.3	21.6	**87.2**	**83.2**	**79.3**	87.5	83.2	64.6	55.0	47.0	95.3
Propiconazole	57.0	48.3	8.1	69.7	66.5	30.7	70.2	66.5	24.3	**43.8**	**37.9**	**37.8**
Pyraclostrobin	74.2	62.0	14.0	90.7	85.5	51.6	91.2	85.5	41.9	**57.1**	**48.6**	**62.2**
Pyrene	70.5	52.4	22.4	**86.4**	**72.3**	**81.2**	86.6	72.3	66.9	54.6	40.7	96.3
Tebuconazole	57.5	51.9	7.5	70.3	71.5	28.7	71.0	71.5	22.5	**43.9**	**40.6**	**35.6**
Trans-permethrin	109.3	89.5	47.5	**133.3**	**123.3**	**179.0**	134.4	123.3	142.3	83.6	70.1	219.7
Trifloxystrobin	54.6	45.0	12.4	66.8	62.0	45.1	67.2	62.0	37.0	**42.0**	**35.1**	**53.9**
Trifluralin	48.6	44.0	21.0	**59.5**	**60.6**	**76.5**	59.7	60.6	62.8	37.5	34.1	91.3

VITA

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