ABSTRACT

HELTSLEY, REBECCA MARIE. Novel Methods for Monitoring Chlorinated Contaminants in Aquatic Environments. (Under the direction of Dr. Damian Shea)

Chemical exposure assessment is a fundamental component of ecological risk assessment. Without proper tools to determine exposure, final decisions regarding risk must be estimated. In this study, novel methods for improving exposure assessment in aquatic ecosystems were evaluated.

Methods for rapid extraction, detection, and quantification of two dioxin-like compounds in tissue, sediment, and water were developed and validated. Methods were notably sensitive and reproducible. The methods were applied to fish samples with induced ethoxyresorufin-O-deethylase activity. Although the compounds were not detected, this study demonstrated for the first time the analysis of tetrachloroazobenzenes with low ppb method detection limits.

A novel passive sampling device (PSD), a tool used to monitor waterborne concentrations of organic contaminants, consisting of polydimethysiloxane (PDMS) as the polymeric sorption material was developed. Uptake rates were determined for a suite of >50 polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) into PDMS disks in order to investigate the device's potential as an *in-situ* sampler for hydrophobic organic contaminants. Uptake rates ranged from 0.03 to 0.7 L/g*d. Compounds with log K_{ow} values >4.5 remained in the linear uptake phase, demonstrating the ability of PDMS to function as a time-integrated PSD. Results indicate that PDMS offers great potential to be an effective alternative to conventional sampling and other passive sampling techniques.

In the final phase of this project, PDMS samplers were evaluated as mobile PSD attached to flathead catfish. Also, the potential for using adipose fin clips from catfish as a non-lethal sampling technique to estimate concentrations of PCBs and OCPs in muscle tissues was investigated. Results indicated no practical/physical barriers for attaching mobile PSDs to aquatic organisms. Mobile samplers provided accurate estimates of location specific concentrations of waterborne contaminants that fish were exposed to during the sampling period. Adipose fin concentrations were highly correlated ($r^2 = 0.77$) with those found in the muscle, which requires mortality. This study represents the first time mobile PSDs and adipose fin clips have been used for exposure assessment of organic contaminants.

NOVEL METHODS FOR MONITORING CHLORINATED CONTAMINANTS IN AQUATIC ENVIRONMENTS

By

REBECCA MARIE HELTSLEY

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APPROVED BY:

Dr. Damian Shea Chair of Advisory Committee	Dr. Chris Hofelt
Dr. Margie Oleksiak	Dr. David Danehower

This dissertation is dedicated to,

My mom and dad, Sandra and James, who gave me their unconditional love and encouragement to complete this scientific journey I set out on so many years ago

Jeff, my wonderful husband for his unwavering support and love

My sisters and brother, my best friends, who have always been there for me

Kerouac and Branson who always remind me of the simple pleasures in life

Biography

She's not for sure where it all began, as a young aspiring chef in the kitchen with her mother measuring flour and sugar ever so precisely as a chemist would prepare a standard or floating down Skaggs Creek in a canoe with her father trying to keep his little "froggies" (his four little girls) quiet so they could appreciate all the sounds of nature. However it happened upon Rebecca Marie Heltsley, her love for science came at an early age. Born in 1975, she grew-up in a large family, three sisters and one brother, in a small town in Kentucky. It was her love of the being outdoors and interest in environment issues that led her to pursue scientific degrees at Western Kentucky University. In 1994, she followed in the foot steps of her three big sisters and started studying chemistry, biology, and environmental science. Her last year at WKU as an undergraduate she took a class named Toxicology. From environmental chemistry to vertebrate toxicity, Dr. Houston introduced this exciting subject to Rebecca and it was in that classroom that she realized there was a field that connected her degrees. Continuing on at WKU, she obtained her masters in chemistry under the supervision of Dr. Wei-Ping Pan (who joked that his American name was "Peter"), with a focus on developing analytical methods for measuring hydrocarbons in fly ash. Although, she loved working in the 24-hour combustion lab she knew a career in coal analysis just wasn't for her. So in 2000, Rebecca pursued her new found love of environmental toxicology and began at North Carolina State University with research focusing on developing novel methods to monitor organic contaminants in aquatic environments. Upon finishing at NCSU, Rebecca will begin postdoctoral work with the National Institute of Standards and Technology in Charleston, South Carolina.

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Chapter 1. Strategies for Monitoring Hydrophobic Organic Contaminants in Aquatic Environments.

Introduction

Environmental exposure to complex chemical mixtures poses a potential risk to both human and ecological health. Even ultra-trace concentrations (part per trillion or lower) can be toxicologically relevant, especially for chronic effects and their detection can be important to understanding the fate of these chemicals. Measuring chronic exposure to chemicals is a continual challenge. For aqueous exposure, water samples are typically grabbed at a single point in time, extracted, and analyzed to estimate exposure. However, these grab samples only represent a moment in time and thus provide no information on chronic exposure unless many sequential samples are collected and analyzed. Grab samples typically require a large volume of water that introduces practical problems with sample handling and the use of large volumes of solvents for extraction. Even when continuous pumping systems are employed to deliver water to a continuous liquid-liquid extraction (LLE) device or a large volume solid phase extraction (SPE) sampler, concerns exist with analyte loss or sample contamination. In addition, these sampling techniques measure the total concentration of a chemical when often only a fraction is biologically available (bioavailable) to an organism.

Due to limitations associated with traditional sampling techniques coupled with the analytical challenges of measuring residue concentrations below detection limits in grab samples, biomonitoring organisms have frequently been used to assess potential exposure of aquatic organisms to chemicals. However, inherent problems exist with organism-based approaches as well. Aquatic organisms (particularly vertebrates) can metabolize many organic contaminants and they typically have rapid response times, depurating accumulated chemicals when exposure decreases. The accumulation of

contaminants in organisms can also be influenced by environmental conditions (1) and it is not easy to model or correct for these influences in the field. These factors often lead to great uncertainty over how well chemical residues measured in an organism represent exposure in nature. Because exposure estimates are a fundamental component of human and ecological risk assessments, the development of better methods for measuring contaminant exposure in aquatic systems is needed.

Passive sampling devices (PSDs) are an alternative approach to chemical exposure assessment that utilize a non-living substrate to sequester and preconcentrate hydrophobic organic contaminants from aquatic environments in a time-integrated fashion. PSDs typically contain a polymeric membrane that is permeable to many chemicals and can accumulate chemicals from the environment in a lipid-like phase (e.g., in an internal solvent or in the polymer itself). Using laboratory-derived and field-verified uptake rates (calibration), PSDs are deployed in the field for a fixed amount of time, the accumulated chemical residue is measured, and then the average exposure during deployment is back-calculated with a simple equation based on the calibration. PSDs also preconcentrate contaminants allowing for the detection of ultra-trace residues that are normally beyond the capabilities of standard sampling techniques, providing an estimate of time-weighted-average (chronic) exposure, and may provide a good measure of the *bioavailable* concentration because the polymer membrane appears to exclude the same chemical forms of contaminants that are excluded by biological membranes. The use of PSDs has improved our understanding of the occurrence and fate of hydrophobic chemicals in the environment (2-4).

Uptake into PSDs follows first order uptake kinetics. Analytes sequestered by a PSD will be in one of the following three uptake phases: linear (integrative), curvilinear, or equilibrium (figure 1). Ambient water concentrations can be estimated from PSDs using integrative sampling rates (time-weighted average, TWA, model), equilibrium partitioning coefficients and/or exchange rate constants. Selection of the appropriate model, linear or equilibrium is dependent on the physico-chemical properties of the investigated target analytes, exposure duration, and exposure conditions (temperature, biofouling, hydrodynamics). When using PSDs to estimate concentrations of target analtyes in water, it is extremely important to know what region of the curve best describes specific compounds at a given point in time. Without this information, PSD data may be misinterpreted (5).

Integrative samplers are characterized by a low to moderate PSD surface area to volume ratio. Thus, the PSD will serve as a near infinite sink for target contaminants and uptake will remain in the linear phase throughout the exposure period, commonly greater than two weeks. Residues of hydrophobic organic contaminants (HOCs) from episodic events will be retained in the sampler due to the small loss rates and large amount of time required to reach equilibrium. This approach provides a time-weighted average (TWA) concentration of contaminants. Semipermeable membrane devices (SPMDs) (5), low density polyethylene (LDPE) strips (6), and polydimethylsiloxane (PDMS) disks (7) are classified as cumulative dose (integrative) samplers.

The equilibrium-based method relies on the sampler approaching or attaining steady state with the surrounding water within the exposure period. This method is commonly used because it simplifies the modeling required to estimate environmental

concentrations and has been shown to correlate well with body burdens of biota (8). By using an equilibrium device, the ambiguity associated with not knowing what phase specific compounds are in at a given time is diminished, however, establishing that the target analytes have attained equilibrium is required. Unlike integrative samplers, equilibrium samplers are characterized by having a low capacity and high loss rate. Episodic events may not therefore be accurately represented. Examples of steady state samplers include solid phase microextraction (SPME) fibers (9) and thin films of LDPE or PDMS (data not published).

The following equation is a mathematical expression for the overall uptake curve (Figure 1),

$$C_{PDMS} = C_W * K_{PSD}(1-\exp[-k_e t]) \quad (1)$$

where C_{PDMS} is the concentration in the PSD (ngL^{-1}), C_W is the concentration of chemical in water (ngL^{-1}), K_{PSD} is the PSD-water partition coefficient (Lng^{-1}), k_e is the elimination rate constant or exchange rate constant (day^{-1}) for both overall uptake and elimination, and t is the deployment period (days). If k_e and K_{PDMS} are known (from laboratory experiments), C_W can be estimated by measuring C_{PSD} and rearranging eq 1 to solve for C_W (5). For contaminants with a high octanol-water partition coefficient ($\log K_{OW} > 4.5$) or when $k_e t$ is small ($<\ln 2$), the uptake of the chemical will generally remain in the linear or integrative phase during typical deployments (4, 5) and equation 1 can be reduced to

$$C_W = C_{PSD}/k_u * t \quad (2)$$

where k_u is the uptake rate constant (L/g*d). A more convenient way of expressing uptake is defined as the effective sampling rate (R_S), the volume of water from which the analyte is quantitatively extracted per unit time (L/d) (5). The R_S can be determined in

controlled laboratory conditions (calibration) with a fixed C_W and then used to estimate C_W by using equation 3

$$R_S = N_{PSD} / C_W * t \quad (3)$$

where N_{PSD} is the amount (ng) of chemical sorbed by the PSD (10). In order for a PSD to be considered an integrative sampler, R_S values must be independent of concentration which is a key aspect in determining TWA concentrations of analytes in water. Effective sampling rates also should remain relatively constant over an extended period (5).

The following equation can be used to estimate water concentrations using equilibrium partitioning approaches,

$$C_W = C_{PSD\text{-}E}/K_{PSD} \quad (4)$$

where $C_{PSD\text{-}E}$ is the concentration of target analytes in the PSD at equilibrium (ng/g). Log K_{OW} can be used to estimate K_{PSD}, however this relationship should be used with caution. For many PSDs, this relationship begins to fail around log K_{OW} of approximately 6.0 (5, 7, 11). The relationship between K_{PSD} and the rate constants (uptake rate, k_u and elimination rate, k_e) is given by,

$$K_{PSD} = k_u/k_e = (R_s/m_{PSD})/k_e \quad (5)$$

where m_{PSD} is the weight of the PSD sampler.

Release kinetics from PSDs can be modeled using equation 6,

$$C_{PSD} = C_{PSD\text{-}0} \exp(-k_e t) \quad (6)$$

where $C_{PSD\text{-}0}$ is the concentration in the PSD at time zero. This parameter is usually known (5). Elimination rates (k_e) can be used to estimate concentrations of target analytes in water using equation 1. Elimination from the PSDs is one of the easiest kinetic

parameters to measure because it avoids confounding factors such as keeping a fixed concentration in water.

The following equations can be used as general guidelines to compute appropriate exposure times and/or to select the modeling approach to employ for calculating ambient water concentrations from PSDs

$$t_{50} = t_{1/2} = -\ln 0.5\ K_{PSD}\ V_{PSD} / R_s \quad (7)$$

$$t_{90} = -\ln 0.1\ K_{PSD}\ V_{PSD} / R_s \quad (8)$$

where t_{50} is the time required to accumulate 50% of the equilibrium concentration in the sampler and t_{90} is the time required to accumulate 90% of the equilibrium concentration in the sampler. Integrative sampling only occurs in the linear phase (t_{50}) of analyte uptake. Once the sampler has exceeded the linear phase the equilibrium or steady state approach is used to estimate ambient water concentrations and therefore the device would no longer provide a TWA concentration of contaminants (5).

Regardless of which sampling approach is selected, transfer of analytes through membranes can potentially be limited by several barriers including, aqueous boundary layers, biofouling, and the polymer matrix itself. (Figure 2a). When using equilibrium-based approaches, these barriers may not be significant and can largely be ignored. However, it is important to remember that environmental variables, such as temperature, biofouling, and flow across the membrane, can affect the time to reach equilibrium. Therefore, the investigator must demonstrate that the PSD has reached equilibrium. Uptake rates used for estimating TWA concentrations in water are affected by environmental conditions such as, temperature, biofouling, and flow as well as PSD-based barriers (5). For example, as seen in Figure 2a, the aqueous boundary layer

thickness is essentially controlled by the shear flow across the membrane. Under stagnant conditions, the thickness is around 1000 μm whereas in turbulent conditions, the thickness is reduced to < 100 μm. This range in thicknesses can significantly affect sampling rates, decreased boundaries result in a faster uptake rate for compounds with log K_{OW}s > 4.5. Because it is impossible to determine uptake rates for large number of environmental conditions that can exist, therefore performance reference compounds (PRCs) are used to account for these influences and thus more accurately estimate water concentrations. PRCs are analytically, non-interferring compounds added to PSDs prior to deployment that share similar physico-chemical characteristics as the target analytes. The loss of the PRC is proportional to the uptake of target analtyes (isotropic exchange kinetics) (Figure 2b). Therefore, site-specific PRC loss can be used to adjust the uptake rate accordingly. In the case of equilibrium sampling, PRC loss should be > 80% which would indicate compounds with log K_{OW}s similar to the PRC will have attained equilibrium (3, 5).

The SPMD is the best characterized PSD to date. SPMDs were developed for monitoring and assessing trace concentrations of hydrophobic organic contaminants into low-density polyethylene (LDPE) filled with the lipid triolein (2−5). Organic contaminants with a molecular mass less than about 600 Daltons selectively partition through the pores of the LDPE and concentrate into the SPMD lipid. However, SPMDs have some disadvantages including the need to isolate analytes from the triolein, possible anisotropic behavior of sorption/desorption due to the partial crystallinity of the polyethylene membrane, and higher cost of materials relative to some alternatives (2–5).

Use of PSD technology continues to expand and advance. Today, there are a number of passive sampling techniques that can preconcentrate numerous contaminants including, heavy metals (12, 13), nonpolar organics (2, 3, 5, 6−11) and polar organic chemicals (14). This growth can be attributed to the long-standing desire of scientists to simplify sampling techniques by not only reducing the cost but also decreasing the amount of labor needed for sample handling and processing while estimating chemical exposure in water over time and providing a surrogate of bioavailability in aqueous environments.

Passive sampling devices have become an effective tool for environmental research and analysis. The work presented in this dissertation is a significant advance in the use of passive sampling for hydrophobic environmental contaminants and demonstrates new applications with important environmental implications. The overall goal was to investigate new polymers that retained the advantages of current passive sampling approaches, but simplified the construction and use by utilizing easily-obtained materials that did not require any cleanup steps and required little or no solvent.
The applicability of the PSD developed here is demonstrated by several studies which examine a suite of chlorinated compounds that may exert adverse effects on ecosystems and human health through a variety of pathways. Because of their widespread, historic use, polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) were investigated, along with two other chlorinated chemicals that have dioxin-like toxicities, tetrachloroazobenzene and tetrachloroazoxybenzene. These last two chemicals are found in the environment due to the application of contaminated chloraniline herbicides and/or the break-down of these same herbicides. The commonality of these groups of

contaminants is that they are relatively persistent and lipophilic, thereby having high potential to bioaccumulate in aquatic organisms, and are of significant environmental concern.

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Figure Legends.

Figure 1. Plot of three phases of passive sampling device uptake. Integrative sampling is characterized by the linear phase and provides an estimate of the cumulative dose (time-weighted average) concentration of contaminants over a specified exposure period (5).

Figure 2. Potential barriers to analyte mass transfer into a passive sampling device (a) and an illustration of why the permeable reference compound (PRC) approach can be used to adjust passive sampling device uptake rates (b). This example demonstrates how biofouling proportionally decreases the elimination of the PRC while at the same time decreasing the uptake of target analytes (5)

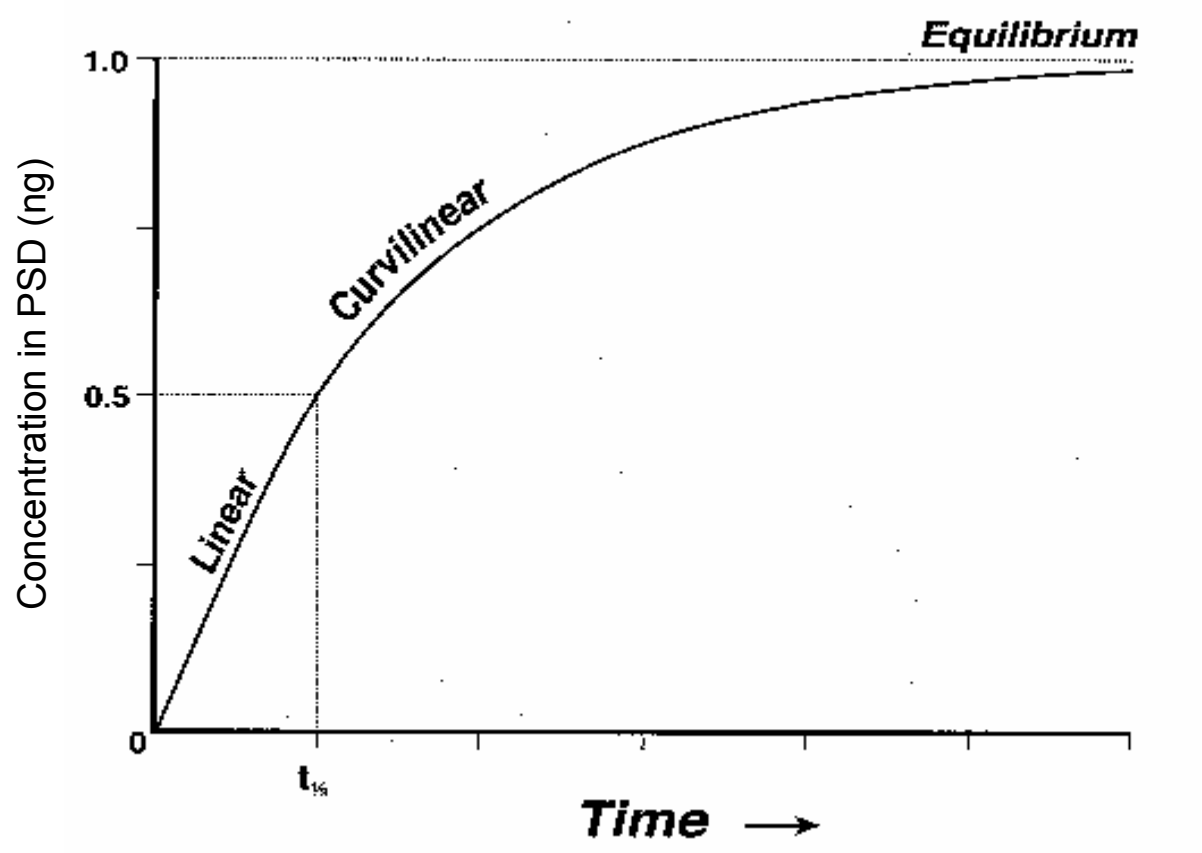

Figure 1.

a. b.

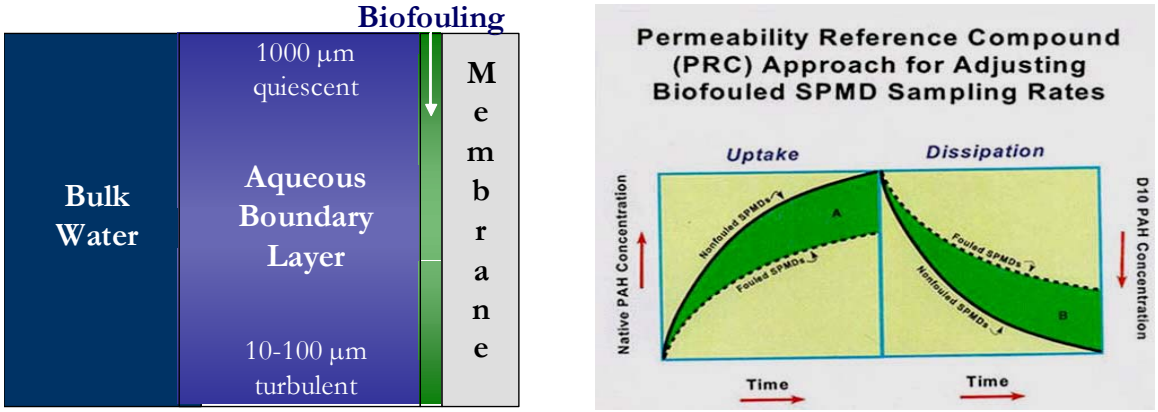

Figure 2.

Chapter 2. Development and Use of a Novel Passive Sampling Device for Detecting and Measuring Chlorinated Contaminants.

REBECCA M. HELTSLEY,*

KATRINA WHITE,

DAMIAN SHEA,

North Carolina State University, Department of Environmental and Molecular Toxicology, Box 7633, Raleigh, North Carolina 27695-7633

* Corresponding author telephone: 919-515-1960; fax: 919-515-7169; e-mail: rmheltsl@unity.ncsu.edu.

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Abstract. Environmental exposure to complex mixtures of persistent organic pollutants poses a potential risk to both human and ecological health. Even ultra-trace concentrations can be toxicologically relevant, can result in food-chain transfer, and can be important to understanding the fate of these chemicals. Measuring chronic exposure to chemicals at these trace levels is a continual challenge due to inadequate sensitivity of existing analytical methods and fluctuations in environmental concentrations that are not captured with traditional grab sampling. We have developed a novel passive sampling device that provides *in-situ* analyte enrichment of non-polar chemicals from water and serves as an infinite sink over several weeks of exposure. This allows simple linear uptake kinetics to be used to estimate chronic exposure at ultra-trace concentrations. The device consists of polydimethysiloxane (PDMS), a polymeric sorption material that is compatible with both solvent extraction and thermal desorption. Laboratory calibration experiments were conducted to determine the uptake and elimination rates and partition coefficients for a broad suite of chlorinated compounds into PDMS disks in order to investigate the device's potential as an environmental *in-situ* sampler for hydrophobic organic contaminants. Uptake rates ranged from 0.002 to 0.027 $L/g*cm^2$. Compounds with log K_{ow} values >4.5 remained in the linear uptake phase for the duration of the experiment (25 days) demonstrating the ability of PDMS to function as a time-integrated sampling device. Our results indicate that PDMS offers great potential to be an effective alternative to conventional sampling and other passive sampling techniques.

Introduction

Concerns regarding exposure to finite concentrations of aqueous contaminants have led to the development of improved techniques for assessing the presence and potential toxicological impacts of environmental contaminants in aquatic ecosystems. Passive sampling is one of the most promising strategies currently employed to monitor ultra-trace and/or bioavailable concentrations of waterborne contaminants. Passive sampling technology has provided a means for a more comprehensive understanding of the behavior of persistent and bioaccumulative pollutants well beyond the scope of what conventional methods of estimating aquatic exposure to these chemicals has provided in the past. The use of passive samplers has rapidly expanded over the past decade. We now have the capabilities to passively monitor a broad array of contaminants including historic compounds of concern and emerging contaminants like pharmaceuticals, in multiple environmental matrices (1−10).

Two of the most recognized passive sampling configurations, the semipermeable membrane device (SPMD) and the solid phase microextraction (SPME) fiber, function based on the same premise, preconcentration of contaminants into a lipid-like phase, but accomplish this with two very distinct configurations. The SPMD is the most extensively characterized passive sampling device (PSD) to date. SPMDs have been used in many ways including: monitoring nonpolar organic contaminants in aquatic environments, development of the concept of using performance monitoring compounds to quantify and correct for environmental effects on uptake; as well as providing a matrix to assess potential toxicity. Characterized by a low surface area to volume ratio, SPMDs are designed to function as infinite sink samplers thereby providing a means to estimate time

weighted average (TWA) concentrations during a specific exposure period (1−5). In contrast, SPME fibers are low capacity samplers configured specifically to reach equilibrium by increasing the surface area to volume ratio, resulting in rapid extraction and concentration of contaminants from aqueous samples. SPME fibers are a convenient extraction method for higher concentration solutions in the laboratory. However, they cannot serve as an integrative field measuring device due to their limited sorptive capacity, though research is currently underway to broaden the application of SPME fibers to become an *in situ* environmental sampler (5−10). Both passive sampling designs have proven to be powerful investigative tools that offer a broad range of applicability with respect to different chemical classes.

Polydimethylsiloxane (PDMS) is a hydrophobic polymeric sorption material that has been extensively employed as a nonpolar coating for gas chromatographic columns and SPME fibers. The use of PDMS as a receiving phase has many advantages. PDMS, the simplest silicone rubber, has a unique flexibility resulting in one of the lowest glass transition temperatures (Tg = -128) of any polymer (11). This unique flexibility coupled to its amorphous structure allows the diffusion of small molecules into PDMS (12). It is a thermally inert material which allows PDMS to be thermally desorbed directly into a GC inlet, as is commonly done with SPME fibers. However, if thermal desorption is not available, PDMS can be extracted with minimal amounts of solvent with no additional clean-up or fractionation steps necessary. In addition to all the advantages listed above, PDMS can be purchased in a variety of thicknesses and configurations making it easy to adjust the surface area to volume ratios to create either a low or high capacity sampler. In accordance with the need to improve chemical exposure assessment, this paper introduces

an alternate approach to passive sampling technology in which PDMS film is utilized as the polymeric sorption phase.

General models of chemical uptake kinetics have been applied to PSDs to illustrate their basic function as an integrative sampler. Aqueous contaminant concentrations can be estimated from PDMS using laboratory-derived effective sampling rates (TWA model) with the following equation,

$$C_W = N_{PDMS} / R_s * t \quad (1)$$

where C_W is the concentration of chemical in water (ng/L), N_{PDMS} is the amount (ng) of chemical sorbed by the PDMS, Rs is the effective sampling rate (L/d) and t is the deployment period (days) (13). Because PSDs sample the bioavailable fraction from the water column, it has been demonstrated that low concentrations of total organic carbon (TOC) (14) or dissolved organic carbon (DOC) (13) can affect the uptake of contaminants into PSDs in a similar fashion as it affects uptake into biota (14). This is especially true for compounds with low water solubilities that readily sorb to organic carbon. Therefore, when determining uptake rates it is important to account for the fraction of chemical truly dissolved in the water column ($C_{W\text{-}td}$). In order to account for DOC, equation 1 can be modified to correct for measured amounts of DOC in the water,

$$C_{W\text{-}td} = (1 + K_{DOC}*C_{DOC}) N_{PDMS}/ R_s *t \quad (2)$$

where $C_{W,td}$ is the total dissolved concentration of contaminant in the water (ngL^{-1}), K_{DOC} is the dissolved organic carbon-water partition coefficient (L/kg) and is estimated using Burkhard's relationship that $\log K_{DOC} = 0.11 \log K_{OW}$ (15), and C_{DOC} is the concentration of DOC measured in the water (kg/L) (13).

The primary aim of this work was to provide comprehensive calibration data to illustrate the potential of PDMS as an integrative PSD for a suite of chlorinated contaminants including polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), and tetrachloroazobenzenes. This work will allow researchers to use PDMS to estimate waterborne concentrations of these chlorinated compounds in a time-integrated or equilibrium fashion.

Methods

Chemicals and Materials. Acetone, dichloromethane (DCM), hexane (>95%), toluene, and anhydrous sodium sulfate used for standard preparation, silanizing glassware, residue recovery, and enrichment were Ultra resi-analyzed grade purchased from J.T. Baker Inc. (Phillipsburg, NJ). Mixtures of polychlorinated biphenyls (PCBs) and organochlorine pestides (OCPs) were purchased from AccuStandard Inc. (New Haven, CT). 3,3',4,4'−tetrachloroazobenzene and 3,3',4,4'−tetrachloroazoxybenzene were purchased from ChemService Inc. (West Chester, PA). Stock solutions and calibration standards were prepared in hexane. PDMS disks were submerged in copper (II) sulfate (Sigma Aldrich Co, St. Louis, MO) solution every three days throughout each of the calibration experiments (excluding the K_{PDMS} determination) to prevent biofouling and no biofouling was observed using this treatment. Virgin PDMS (0.16 cm) manufactured by Diversified Silicone Products Inc. (Santa Fe Springs, CA) was purchased from McMaster Carr (Atlanta, GA). To avoid contamination of samples all glassware, aluminum foil, stainless steel wiring, and sodium sulfate were baked out (300 °C) overnight or rinsed three times each with acetone, DCM, and hexane.

Film Preparation. PDMS disks were prepared with the following configurations: 0.16 cm thickness sheeting, 0.40 cm in diameter with a surface area of approximately 0.44 cm^2, and the average weight of an individual disk was = 0.02 g. Laboratory calibration data from this study are applicable to PDMS films with similar configurations. However, if modifications of the PDMS design are made, the surface area can be used to adjust calibration data accordingly. Prior to use, PDMS disks were pre-extracted with acetone on a shaker table (350 rpm) for ~30 minutes (repeated five times). The disks were dried in a 60 °C oven for ~10 minutes.

Calibration Experiments.

Static Exposure. PDMS disks were exposed to test chemicals at nominal concentrations (500−1,000 ng/L) in a static renewal system. The system consisted of a 1 L silanized beaker (wrapped in foil to prevent photodegradation) that contained deionized water that had been spiked with test chemicals dissolved in hexane and allowed to equilibrate for 12 hours. Analyte-fortified equilibrated water was renewed every 12 hours to ensure that water concentrations remained constant. Triplicate disks were removed on days 0, 0.5, 1, 3, 6, 11, 15, 19, and 23. After collection, individual disks were stored in the freezer (−20 °C) until extraction. Water samples were extracted every 12 hours throughout the experiment to monitor water concentrations. After day 16, daily water extracts were combined for analysis. The temperature of the system remained at 24 ± 1 °C. PRC compounds were not included in the uptake experiment.

PRC Loading Techniques. Two methods were utilized to load PRCs (Cl1 (02) and Cl2 (11)) and target analytes (PCBs, OCPs, and chlorobenzenes) into the disks for elimination studies, the methods will be referred to as the hexane and methanol (MeOH) loading methods. The MeOH loading method was adapted from Booij *et al*. (16) with minor modifications. Briefly, PDMS disks were placed in a 50:50 (v/v) methanol−water solution that had been spiked with the compounds of interest. The mixture was placed on a shaker table (300 rpm), disks were removed in triplicate at 1, 2, 5, and 22 hours, and at 24 hours the disks were removed and the solution was extracted (described below) in order to determine the PDMS−solution partition coefficient (K_{MS}). The following equation was used to predict the total amount of PRC to be added to the system

$$N_t = N_M [(V_S + nm_mK_{MS})/m_mK_{MS}] \quad (3)$$

where N_t is the total amount (ng) of PRC to be added to the system, N_M is the target amount (ng) per sampler, V_S is the solvent volume (mL), n is the total number of samplers in the system, m_m is the mass of the sampler (g), and K_{MS} is the PDMS-solution partition coefficient (mL/g) (16). The hexane loading method was adapted from Brown *et al*. (1999). Briefly, PDMS disks were layered singly in a petri dish, hexane (3.5 mL) spiked with compounds of interest was added to the dish and allowed to evaporate for ~20 minutes in a hood. Ten of the loaded disks from each method were removed and extracted to determine the average amount of compound loaded per disk (time zero), loading efficiency, and variation between disks. The remaining disks were stored in the freezer (−20 °C) for the elimination experiment and for a stability study to determine how storage over time affected the final loading amount. Loaded disks were also stored at

ambient temperatures to determine if there is any loss of the PRCs. Each loading experiment was conducted at ambient temperatures (22−25 °C).

Elimination Experiment. Loaded PMDS disks (18 disks per loading method) mounted on stainless steel wires were placed into a 19 L aquarium that contained ~17 L of deionized water. Renewal rates were calculated in order to maintain a constant water concentration of approximately zero in the static renewal system. Triplicate disks (three from each loading method) and a 500 mL water sample were collected on days 0, 1, 5, 12, 30, 45, and 56. The elimination experiment was conducted at ambient temperatures (24−27 °C).

K_{PDMS} Determination. Individual PDMS disks were placed into 200 mL of DI water spiked with the appropriate amount of chemical. Previous experiments (data not shown) revealed a swelling in the PDMS membrane following spiking DI water with the hexane solution. The source of the swelling was presumed to be due to the hexane. Swelling of PMDS in the presence of hexane is consistent with observations made by Brown *et al*. (17). In an attempt to decrease the swelling of the membrane, the spiked aqueous solution was allowed to vent for 30 minutes prior to shaking. After venting, the system was closed with Teflon coated lids and allowed to shake at 200 rpm for the duration of the experiment at 25 ± 1 °C. For the first 72 hours, the jars were vented daily. Triplicate jars were removed on days 11, 20, 32, 42, 55, and 61. Water was extracted immediately and disks were stored in the freezer (−20 °C) until extraction.

Sample Processing. PDMS disks were shaker-table extracted with 0.5 mL of acetone for 3 hours at 300 rpm. Extracts were quantitatively transferred into a 2 mL vial for analysis. Water samples were extracted immediately by liquid-liquid extraction with DCM, solvent exchanged, and concentrated to 1.0 mL for analysis. The methanol−water solution collected from the PRC loading experiment was brought up to > 80% water and extracted as described above. The fraction of dissolved organic carbon (DOC) in water was determined by carbon, hydrogen, and nitrogen (CHN) analysis with an elemental analyzer. Mean DOC concentration was 2.6 mg/L.

Instrumental Analysis and Quality Control. PCBs and OCPs were analyzed using an HP6890 GC equipped with electronic pressure control connected to an HP 5973 MSD or an electron capture detector (GC-ECD). For GC-ECD analysis, extracts were injected in the pulsed splitless mode and separated on a 30 m x 0.32 mm ZB-50 (0.25 μm film thickness) fused silica capillary (Phenomenex, Inc. Torrance, CA). Column temperature was programmed as follows: Initial temperature 60 °C for 1.4 min, 20 °C/min to 210 °C, 1 °C/min to 250 °C, 10 °C/min to 300 °C, hold for 7 min. The injector and detector were set at 300 °C. For GC-MS analysis, extracts were injected in pulsed splitless mode and separated on a Restek 30 m x 0.25 mm Rtx-5 (0.25 μm film thickness) MS with Integra-Guard column. The pressure was ramped to 30 psi before injections with a 1 min hold time. The pressure was then dropped to give a constant flow of 1 mL/min for the duration of the run. The temperature program was as follows: Initial temperature 50 °C for 1.0 min, 25 °C/min to 100 °C, 15 °C/min to 245 °C, 0.5 °C/min to 247 °C, 10 °C/min to 300

°C hold for 5 min. The injector and detector were set at 300 °C. Selected ion monitoring (SIM) was used for the analysis.

Response factors were generated using a four-point calibration curve, and response was monitored using a mid-level calibration standard. The relative percent difference between the mid-level check was always less than 15% for all analytes. Sample concentrations were calculated using the generated response factors and were based on the known amount of TCMX injected. Data quality was assessed using procedural blanks, replicate analyses, matrix spikes and surrogate internal standards. Surrogate and matrix spike recoveries were 70 – 116%, results were not corrected for these recoveries. Method blanks were either not detected or <10% of the measured value. Method detection limits for PDMS ranged from, 0.004 – 0.08 ng/g disk (GC-ECD).

Data Analysis. SAS (v8.02, Cary, NC) was used to determine if intercepts of the uptake curves were significant ($p<0.001$). Sigma Plot v7.101 was used to determine the exchange rate constants. Standard errors were calculated from both linear and nonlinear regressions.

Results and Discussion

Uptake Curves. The performance of PDMS as a TWA sampler was assessed in a 23 day static renewal system. In order for PDMS to be considered an integrative sampler, the slope of the uptake curve should remain relatively constant over an extended deployment period, generally greater than two weeks. If deviations in the slope occur soon after deployment the advantages of the passive sampling technique over traditional low

volume methods (liquid-liquid extraction, LLE) starts to diminish. The uptake of PCBs, OCPs, and tetrachloroazobenzenes with log K_{OW}s ranging from 2.9–8.2 were investigated (Table 1). Characteristic uptake curves for four representative compounds are shown in Figure 1. The coefficients of determination (R^2) for linear uptake curves were close to one ($R^2 > 0.90$) indicating a good relationship between the two variables.

Test chemicals with a log $K_{OW} \geq 4.5$ demonstrated linear kinetic control throughout the 23 day exposure (Figure 1, PCB 8, heptachlor epoxide, PCB 209). This allows the use of equation 1 for estimating waterborne concentrations of target analytes and thus provides a true cumulative dose concentration. Out of 50 compounds investigated in this experiment, only eight have a log $K_{OW} < 4.5$ (table 1). Isomers of BHC, ranging in log K_{OW} from 3.7 − 4.1, began approaching equilibrium for this PDMS configuration at a point between days 1 and 3. On the other hand, endosulfan I and II (log K_{OW} of 3.6) stayed in the linear kinetic phase throughout the duration of the experiment. However, endosulfan sulfate, the persistent breakdown product of endosulfans, began approaching equilibrium between days 6 and 8. Similarly, chlorothalonil which has the lowest log K_{OW} of all compounds examined (log $K_{OW} = 2.9$) remained in the linear uptake phase. The failure of log K_{OW} to accurately predict linearity appears to be a function of structural properties for the individual compounds. For example, the isomers of BHC, which possess a cyclohexane ring, may exhibit a faster uptake because of decreased steric hindrance to partitioning into the membrane and therefore would reach equilibrium with the surrounding water more quickly.

The different kinetic phases demonstrated for OCPs with similar log K_{OW}s illustrates how significant it is to determine what region of the curve the specific

compound is in at a given time prior to using PSDs for estimating aqueous exposure conditions. For example, the use of equation 1 for estimating C_W would not be valid for any of the BHC isomers. For compounds that approach or attain steady state during a deployment, an equilibrium approach should be used to estimate C_W,

$$C_W = C_{PDMS\text{-}E}/K_{PDMS} \quad (4)$$

where C_W is the estimated analyte concentration in water (ng/L), $C_{PDMS\text{-}E}$ is the concentration of analyte in the PDMS at equilibrium (ng/g), and K_{PDMS} is the equilibrium PDMS-water partition coefficient (L/g) (5). Equilibrium-based water estimations no longer provide a TWA concentration, and it is important to note that for these particular compounds, PDMS with this configuration may not represent episodic contaminant releases. However, equilibrium-based approaches are commonly used to estimate chemical concentrations largely because of the simplicity.

Linear regression was used to model the uptake data and determine if intercepts could be neglected. Generally, intercepts were low (<0.8 ng) if significant. However, even low intercepts must be considered when deploying PDMS in the field. Field deployments should be long enough to diminish any effects from this initial spike in uptake (18). Because the intercepts generated from this experiment were typically low, a deployment of two or more days should be ample time to negate nonzero intercepts. By deploying the disks for at least two days, the intercept would be 10% of the total amount in the disk, which is the criterion for acceptance established by Booij *et al*. (18). This conclusion is in concordance with the observation of Luellen and Shea who also observed minor nonzero intercepts (13).

Effective Sampling Rates. The effective sampling rates (R_s) for target analytes were calculated using equation 1 for each of the sampled time points after day one while the disk remained in the linear phase. Average values (based on three disks) are reported in Table 1 with corresponding standard errors. PDMS sampling rates were determined in controlled laboratory conditions in which aqueous concentrations of target analytes were held relatively constant (a stipulation for using equation 1). This was confirmed by collecting and immediately analyzing exposure water twice daily; the average coefficient of variation (% CV) for the water was <25%, and the average water concentrations ranged from 350−1100 ng/L (70−110% of the nominal concentration). Rates remained relatively constant throughout the exposure, the R_s values obtained ranged from 0.0010−0.0115 L/d (12 – fold difference). This range of sampling rates is relatively small compared to the 200,000 – fold difference in K_{OW} values (log K_{OW} 2.9−8.2) for the compounds tested. Because the isomers of BHC attained steady state rather quickly, the sampling rates listed in Table 1 for these compounds should be considered estimates.

The apparent volume of water quantitatively extracted from PDMS disks is lower than other commonly used PSDs, such as SPMDs or low density polyethylene (LDPE) strips. However, when the PCB sampling rates of PDMS are expressed on a surface area normalized basis to the standard SPMD (460 cm^2, PDMS R_s were multiplied by a factor of 460 cm^2/0.440 cm^2) and plotted against log K_{OW} (figure 2a and b), there is reasonably good agreement between both samplers. Uncorrected (a) and corrected (b) PCB effective sampling rates for either DOC or TOC levels by PDMS disks and SPMDs, respectively, are compared in figures 2a and b. The SPMD data, determined by Meadows *et al*., includes uptake rates for ~75 PCB congeners which provided a robust data set for

comparing the two PSDs (14). The uncorrected data, presented in Table 1 and Figure 1a, illustrated a decrease in the effective sampling rate as hydrophobicity increases for both SPMDs and PDMS disks (average uncorrected ratio of SPMD R_s/PDMS R_s for PCBs common to both studies is 0.9). This observation is consistent for other chemical classes with log K_{OW} > 5, such as polycyclic aromatic hydrocarbons (PAHs) and OCPs (observed in this study) (13, 19). However, the factors contributing to the general decrease in R_s are complex and may not be the same amongst chemical classes or PSDs (polymeric membranes).

When considering the decrease of R_s for high log K_{OW} PAHs reported for SPMDs by Huckins *et al*. and Luellen and Shea, the pore size of the LDPE membrane may have resulted in the observed decreased uptake rate. The maximal pore diameter for mass transfer through LDPE membranes is about 10 Å, and the molecular dimensions of hydrophobic PAHs actually extends beyond this limit (5, 13). Conversely, high log K_{OW} PCBs are < 9 Å in breadth (20) and should have less impedance through the LDPE membrane. Meadows *et al*. suggested that the lower sampling rates for extremely hydrophobic PCBs reported in their study could be due to the extremely low water solubility of PCBs in concert with undetectable levels of organic molecules which may be acting as an additional sorptive phase and resulting in a lower uptake. Therefore, once corrections were made with an implicit TOC concentration (estimated at about 0.5 mg/L, half the detection limit of the method used to analyze for TOC) this trend was no longer observed. In accordance with Meadows *et al*., we observed the same trend for corrected DOC PDMS sampling rates (Figure 2b) (14). After corrections were made, the sampling rates for both PSDs increase with increasing hydrophobicity (average corrected ratio of

SPMD R_s/PDMS R_s for compounds shared amongst the two studies was 1.0). As seen in Figure 2a and b, DOC corrections do not affect lower hydrophobicity analtytes. However, the difference in uncorrected and corrected data for lipophilic compounds is much more pronounced demonstrating the importance of measuring DOC in the exposure water. Because organic carbon levels can vary drastically amongst exposure sites, the uncorrected sampling rates for PDMS are presented in Table 1 this would allow other investigators to apply equation 2 to correct for site-specific measured levels of TOC or DOC.

Loading of PRCs. Site conditions have been shown to affect the uptake of contaminants into PSDs. Performance reference compounds (PRCs) are a relatively new approach for determining site-specific sampling rates in order to improve the accuracy of estimated water concentrations. Two methods for spiking PRCs into PDMS disks were assessed in this study, the MeOH and Hexane loading methods. Each method was modified from previous studies that investigated loading compounds into PDMS films or silicone tubing (16, 17). Both methods proved to be a simple approach for loading compounds into PDMS, a solvent-free PSD. Results obtained from the loading indicate that each method is robust and the data obtained provides encouraging evidence of the potential use of PRCs in PDMS to estimate how site-specific conditions may influence the uptake of target analytes. The within-batch variation was comparable for both methods. The MeOH loading method had a lower average % CV of 5%, values ranged from 1−18%, with the larger CV values representing the superhydrophobic PCBs; the hexane loading method had an average of 8% variation, but the range did not extend as high as the MeOH loading method (ranging from 2−9%). It should be noted that the between batch variation

(inter-batch) was 19%. Therefore, to reduce variability, when deploying loaded disks the same batch of PDMS should be used if possible. This higher inter-batch variability is in agreement with results obtained by Booij *et al*. (16).

The efficiency of loading for both methods was excellent: hexane loading efficiency was approximately 88% and the MeOH loading efficiency was 85%. One advantage of the MeOH loading method is that the methanol-solution partition coefficient (K_{ms}) can be calculated (values reported in Table 1). The K_{ms} can then be used to determine the total amount of PRC to be added to the system to achieve the desired target amount of PRC per sampler (equation 3) (16). However, when regressing log K_{MS} verses log K_{OW}, there is a strong relationship ($y = 0.57x - 0.12$, $r^2 = 0.80$) between the two variables indicating that log K_{OW} could be used to estimate K_{MS} or vice versa. Regardless of which loading method is selected, the overall percent loss (~15%) should be accounted for in order to attain optimal loading into PDMS.

In order to determine if loaded disks stored at ambient temperatures for extended periods of time would eliminate PRCs into the atmosphere, loaded disks wrapped in clean aluminum foil were stored at ambient temperatures or at -20 °C for six weeks. Disks stored either at ambient temperatures or at -20 °C over a six week period showed no loss of PRCs (data not shown). By deploying PRC loaded PDMS disks, other investigators can apply the sampling rates derived from a static system in this study to various exposure conditions with a series of simple corrections outlined by Huckins *et al*. (4).

Exchange Rate Constants. Release kinetics were modeled by the following equation,

$$C_{PDMS} = C_{PDMS\text{-}0} \exp(-k_e t) \quad (5)$$

where $C_{PDMD\text{-}0}$ is the concentration in the PDMS at time zero and this parameter is usually known (5), k_e is the elimination rate constant or exchange rate constant (day^{-1}) for both overall uptake and elimination, and t is deployment period (days). Only minor differences were observed with the elimination rates calculated for both hexane and MeOH loaded disks (Figure 3). Therefore, MeOH loaded values are reported in table 1. Measured values of k_e range from 0.001−2.45 d^{-1}. As expected, the isomers of BHC as well as endosulfan sulfate, all compounds that quickly reached steady-state in the uptake experiment, had the fastest elimination rates. Predicted k_e values from the uptake study are similar to those measured in the elimination experiment. On average, elimination derived k_e values were 1.5 x higher than the uptake predicted k_e values. This observation is consistent with data of Booij *et al*. and Huckins *et al*. (4, 18). Elimination rates obtained from the hexane and MeOH loading methods are similar (Figure 3, average ratio of k_e MeOH/k_e hexane is 0.92). For compounds with a log K_{OW} value < 6.2, k_e values decrease with increasing hydrophobicity. However, for the superhydrophobic compounds, k_e values actually begin to show a slight increase. By excluding compounds with a log K_{OW} > 6.2, there is a stronger correlation between log k_e and log K_{OW} (r^2 = 0.7).

When examining the relationship between log k_e and log K_{PDMS}, there is a steady decrease in k_e values with increasing K_{PDMS} (Table 1). This relationship indicates that diffusion through the aqueous boundary layer is the rate-limiting step for all the compounds studied. However, because there were only three compounds with a log K_{OW}

< 3.6 in this experiment, it is difficult to predict if these less hydrophobic compounds are under membrane or aqueous control. According to Vrana *et al*. and Verbruggen *et al*. compounds with a log K_{OW} < 3.6 will be accumulated under membrane control (21, 22).

K_{PDMS}. The membrane-water partition coefficient, K_{PDMS}, can be expressed as the ratio of concentration of analyte in the membrane (C_M) over concentration of analyte in water (C_W). It can be viewed as a measure of a compound's affinity for the membrane, i.e., a higher K_{PDMS} value indicates a stronger affinity for the membrane. K_{PDMS} values were measured for all 52 test chemicals (Table 1). Although the K_{PDMS} experiment ran for 56 days, in this turbulent system, steady state was achieved on or before the earliest sampling point which was 10 days. K_{PDMS} values correspond to the uptake and elimination data. The isomers of BHC which came into equilibrium quickly and eliminated rapidly in the calibration experiments exhibit the lowest K_{PDMS} values while most compounds that remained in the linear uptake phase have log K_{PDMS} values > 3.5.

K_{PDMS} from Table 1 and SPME fiber values are plotted against hydrophobicity in Figure 4 (23, 24). Despite the scattering in the data, it is obvious that log K_{PDMS} values, regardless of configuration, i.e. fiber or disk, increase with increasing log K_{OW} until approximately log K_{OW} 6.2 at which point log K_{PDMS} values remain constant or slightly decrease with increasing log K_{OW} (23). This relationship not only exists for PDMS but has also been reported for polyacrylate (PA) fibers (K_{PA}) as well. However, based on the mixture of compounds investigated by Verbruggen *et al*. and Vaes *et al*. (data not shown), this trend occurs at a lower Log K_{OW} for PA (approximately log K_{OW} 5.7 and 4.2, respectively) (22, 24).

It is interesting to note, that k_e values generated in this study decrease with increasing log K_{OW} up to a $K_{OW} \cong 6.2$, beyond which they actually remain constant or increase slightly (Figure 3). K_{PDMS} can also be defined as the ratio of the uptake rate constant (k_u) versus the elimination rate constant (k_e). Because uptake rates remain relatively constant (12–fold difference) over the wide range of log K_{OW} compounds studied in this experiment, a decrease in K_{PDMS} values should be expected because k_e values slightly increase for the superhydrophobic compounds. It can be concluded from the results of this study and others, that this phenomenon is related to interactions between chemical classes and the polymeric membrane investigated. For example, PA is a more rigid polymer, therefore diffusion into the PA fiber may exhibit greater resistance than that of the PDMS, resulting in the decreased K_{PA} values observed at a lower K_{OW} for both Vaes *et al*. and Verbruggen *et al*. (22, 24). Despite the different sources of PDMS for the SPME fiber and the disk compared in Figure 4, the average ratio of $K_{PDMS\text{-}disk}$ /$K_{PDMS\text{-}fiber}$ for the 13 compounds investigated in both studies is approximately 1.0. (ratios ranging from 0.75 to 1.2 for compounds with log K_{OW}s between 3.9 and 7.4). Because the measured K_{PDMS} values are consistent in these studies, we feel that these values could be used to estimate K_{PDMS} for a variety of sources of PDMS.

K_{PSD} (equilibrium PSD-water partition coefficient) values are often needed to estimate water concentrations of chemicals of concern from PSDs (eq 4). However, due to the limited K_{PSD} published values this is often not possible unless this partition coefficient is extrapolated from log K_{OW}, and this relationship usually begins to fail around log K_{OW} of approximately 6 as seen in this work and others (19, 22). The extensive K_{PDMS} and R_s values presented in this work and in White *et al*. (2003) will

allow for the estimation of water concentrations for the most commonly identified PCBs, OCPs, and PAHs in environmental water systems using either equilibrium-based or integrative sampling (19).

Potential PDMS Interferences. The entire sample handling and processing procedures associated with PDMS are far less difficult when compared to carrying out a tissue or sediment extraction. However, interferences can be problematic when analyzing samples using GC-MS. Co-extractable siloxanes from the PDMS respond very well in electron ionization MS. Preliminary results indicate that siloxanes interfere with quantitation of PCBs and OCPs more readily than PAHs when analysis is performed by GC-MS. However, siloxanes do not respond on GC-ECD which was the instrument most commonly used in this study. Use of size exclusion chromatography (SEC) as a pretreatment for samples prior to GC was found to reduce the amount of siloxanes by as much as 80%. Current studies are underway to determine if the grade of PDMS and/or the pre-extraction method utilized influence the amount of co-extractable material.

PDMS Overview. This paper presents a low-cost alternative to current passive sampling technology. PDMS disks are robust in design and can be deployed in any environment with no maintenance required during exposure. The design of this time-integrated sampler also offers ease of deployment for any person and a simplistic extraction or thermal desorption upon retrieval. This work and other investigations (19) have demonstrated the ability of PDMS disks to act as an integrative sampler over time for hydrophobic persistent organic pollutants. However, if an equilibrium-based approach is

desired, PDMS can easily be configured to satisfy this need, simply by decreasing the thickness of the sheeting. As passive sampling technology continues to expand based on the need to reduce cost and labor for environmental sampling, we believe our device as well as other passive sampling approaches will play an integral role in assessing the presence and potential impacts of aquatic contamination.

Figure 5 is a representation of the many passive sampling approaches available today. It clearly illustrates the relationship between uptake of contaminants and the surface area to volume ratio (SA/V) of different samplers. A steady-state sampler, characterized by a high SA/V ratio employs a design which is customized for attaining steady-state within a brief exposure period. The polyacrylate SPME fiber (PA fiber) seen in Figure 5 is an example of a steady-state sampler (22). This design offers simplicity when determining instantaneous water contaminant concentrations because the only calibration data needed is the K_{PSD} value which can be estimated with caution from the log K_{OW} up to a log K_{OW} value of 6. Other factors must be considered when using an equilibrium sampler including, site conditions that may affect time to reach equilibrium and it must be demonstrated that equilibrium has been achieved. The advantage of the steady-state sampler is its straightforward use although, it does not provide a TWA concentration as do the other four samplers in Figure 5 which include the PDMS, SPMD, and LDPE (14, 19, 25). An integrative sampler is characterized by a low to moderate SA/V ratio. As seen in this paper and other manuscripts that utilize passive sampling approaches in an integrative fashion, more calibration data is needed to estimate water concentrations. However, a TWA concentration derived from a PSD can be considered basically effortless when compared to traditional techniques. The ease of estimating a

cumulative dose with a PSD, positions scientists one step closer to bridging the many unknowns in environmental risk assessment.

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Table 1. PDMS Calibration Data for Chlorinated Compounds.

Target Analyte	MW	Log K_{OW}[a]	PDMS Sampling Rate R_S (Ld^{-1}) ± SE[b]			PDMS k_e (d^{-1})[c] ± SE			Log K_{ms}	Log K_{PDMS} ± SE		
Hexachlorobenzene	285	5.5	0.0115	±	0.0004	0.0086	±	0.0005	3.39	4.20	±	0.08
Alpha - BHC	291	3.9	0.0013	±	0.0042	0.6029	±	0.0303	2.40	3.27	±	0.01
Lindane	291	4.1	0.0010	±	0.0075	0.4502	±	0.1579	2.28	3.05	±	0.01
Beta-BHC	291	3.7	0.0012	±	0.0035	0.6421	±	0.1789	1.36	1.94	±	0.01
Heptachlor	373	5.4	0.0093	±	0.0003	0.0041	±	0.0007	3.22	5.57	±	0.03
Delta - BHC	291	3.7	0.0012	±	0.0315	2.4529	±	0.1920	1.90	2.44	±	0.03
Aldrin	365	5.7	0.0086	±	0.0004	0.0037	±	0.0006	3.57	5.49	±	0.06
Chlorothalonil	266	2.9	0.0011	±	0.0005	0.0060	±	0.0008	1.48	4.73	±	0.03
Chlorpyrifos	351	5.7	0.0120	±	0.0002	0.0137	±	0.0010	3.67	5.31	±	0.06
Heptachlor Epoxide	389	4.6	0.0077	±	0.0003	0.0165	±	0.0008	2.65	4.29	±	0.01
Trans-Chlordane	410	6.0	0.0087	±	0.0003	0.0038	±	0.0006	3.18	5.09	±	0.03
trans-nonachlor	444	5.8	0.0074	±	0.0004	0.0037	±	0.0007	2.67	4.87	±	0.05
2,4'-DDE	318	5.8	0.0075	±	0.0006	0.0035	±	0.0007	2.67	5.07	±	0.02
Cis-chlordane	410	5.9	0.0075	±	0.0003	0.0036	±	0.0007	2.77	4.42	±	0.13
Endosulfan I	407	3.6	0.0078	±	0.0003	0.0158	±	0.0009	2.77	4.42	±	0.13
4,4'-DDE	318	5.9	0.0052	±	0.0003	0.0025	±	0.0006	3.65	5.09	±	0.06
Dieldrin	381	5.2	0.0072	±	0.0003	0.0106	±	0.0007	2.76	4.54	±	0.01
2,4'-DDD	320	6.0	0.0063	±	0.0003	0.0051	±	0.0008	2.93	3.64	±	0.04
Endrin	381	5.2	0.0044	±	0.0007	0.0143	±	0.0013	2.70	4.43	±	0.01
2,4'-DDT	354	5.8	0.0049	±	0.0006	0.0023	±	0.0009	3.45	5.08	±	0.03
4,4'-DDD	320	6.0	0.0064	±	0.0004	0.0061	±	0.0007	2.89	4.84	±	0.02
Endosulfan II	407	3.6	0.0058	±	0.0004	0.0586	±	0.0038	2.11	3.86	±	0.02
4,4'-DDT	354	6.2	0.0034	±	0.0006	0.0024	±	0.0007	3.50	5.09	±	0.06
Endrin Aldehyde	383	4.9	0.0012	±	0.0006	0.0124	±	0.0020	2.57	3.08	±	0.03
Endosulfan Sulfate	423	3.7	0.0027	±	0.0017	0.2645	±	0.0187	1.65	3.19	±	0.01
Mirex	546	6.9	0.0041	±	0.0011	0.0055	±	0.0015	3.69	5.10	±	0.02
Endrin Ketone		5.0	0.0017	±	0.0004	0.0511	±	0.0077	2.08	3.58	±	0.01
Methoxychlor	346	5.1	0.0031	±	0.0004	0.0511	±	0.0077	2.08	3.58	±	0.01
PRC, Cl (02)	189	4.6		nd		0.0234	±	0.0039	2.89	4.31	±	0.03
Cl2 (08)	223	4.5	0.0117	±	0.0003	0.0130	±	0.0008	3.02	4.67	±	0.01
PRC, Cl2 (11)	223	5.3		nd		0.0111	±	0.0007	3.04	4.65	±	0.01
Cl3 (18)	258	5.2	0.0129	±	0.0003	0.0075	±	0.0006	2.94	4.56	±	0.07
Cl3 (28)	258	5.7	0.0093	±	0.0003	0.0054	±	0.0006	3.26	5.13	±	0.02
Cl4 (44)	292	5.8	0.0099	±	0.0005	0.0061	±	0.0008	3.57	5.32	±	0.03
Cl4 (52)	292	5.8	0.0088	±	0.0003	0.0037	±	0.0007	3.19	5.28	±	0.03
Cl4 (66)	292	6.2	0.0087	±	0.0003	0.0029	±	0.0007	3.52	5.13	±	0.05
Cl5 (101)	326	6.4	0.0088	±	0.0004	0.0051	±	0.0016	3.27	4.69	±	0.06
Cl4 (77)	292	6.4	0.0072	±	0.0003	0.0020	±	0.0007	3.35	5.00	±	0.04
Cl5 (118)	326	6.7	0.0048	±	0.0006	0.0024	±	0.0007	3.88	5.02	±	0.06
Cl6 (153)	361	6.9	0.0067	±	0.0007	0.0027	±	0.0008	4.26	5.16	±	0.05
Cl5 (105)	326	6.7	0.0035	±	0.0005	0.0031	±	0.0006	3.30	5.23	±	0.05
Cl6 (138)	361	6.8	0.0043	±	0.0008	0.0022	±	0.0007	3.55	4.90	±	0.05
Cl7 (187)	395	7.2	0.0056	±	0.0010	0.0024	±	0.0009	4.32	4.90	±	0.14
Cl5 (126)	326	6.9	0.0070	±	0.0006	0.0122	±	0.0019	3.66	5.04	±	0.03
Cl6 (128)	361	6.7	0.0031	±	0.0008	0.0024	±	0.0007	3.78	4.93	±	0.05
Cl7 (180)	395	7.4	0.0031	±	0.0011	0.0036	±	0.0009	4.41	5.01	±	0.05
Cl7 (170)	395	7.3	0.0069	±	0.0011	0.0031	±	0.0008	4.03	4.97	±	0.05
Cl8 (195)	430	7.2	0.0026	±	0.0012	0.0043	±	0.0010	4.45	5.11	±	0.07
Cl9 (206)	464	7.6	0.0029	±	0.0012	0.0052	±	0.0014	4.53	5.15	±	0.08
C10 (209)	499	8.2	0.0028	±	0.0011	0.0037	±	0.0014	4.42	5.16	±	0.11
TCAB	320	6.7	0.0048	±	0.0013	0.0020	±	0.0008	3.74	5.19	±	0.05
TCAOB	336	6.9	0.0036	±	0.0012	0.0012	±	0.0006	3.65	5.00	±	0.05

[a]Standard Error of n, where n = number of samples, different for each experiment

[b]Log K_{ow} (26)

[c]ke (d^{-1}) values obtained from the MeOH loaded disks in the elimination experiment

FIGURE LEGENDS

Figure 1. Uptake curves for four representative chlorinated compounds: Cl2 (08) (●), Heptachlor epoxide (○), Cl10 (209) (▲), and Delta-BHC (□).Vertical lines represent standard deviation of three individual disks.

Figure 2. Relationship between PCB Sampling rates and log K_{OW} determined for PDMS disks (○) and SPMDs (●). PDMS corrected data for 2.6 mgL^{-1} measured dissolved organic carbon and assumed log $K_{DOC} \approx 0.41$ log K_{OW}. SPMD corrected data for 0.5 mgL^{-1} total organic carbon and assumed log $K_{TOC} \approx$ log K_{OW} (14).

Figure 3. Relationship between log k_e and log K_{OW}: MeOH loading (●), hexane loading (o), and MeOH loading compounds with a log KOW < 6.2 (Δ).

Figure 4. Relationship between log K_{PDMS} or log K_{PA} and log K_{OW} for PDMS disks (●), PDMS SPME fiber (▽) (23), and PA SPME fiber (■) (22).

Figure 5. Relationship between log uptake rates (k_1, m^3/m^3*d) and surface area to volume ratios (SA/V, m^2/m^3) for a variety of PSDs (14, 19, 25).

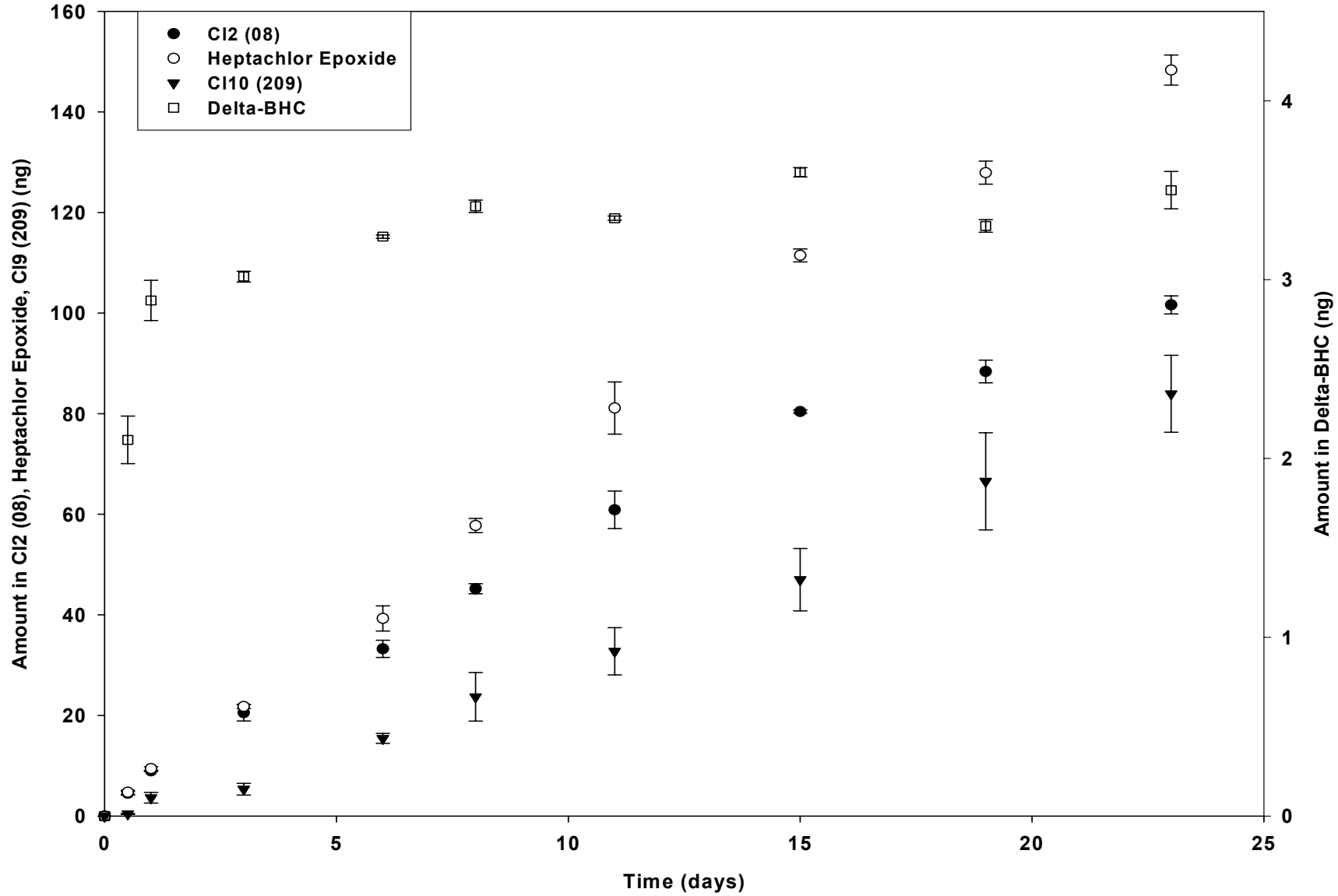
Cl2 (08)
Heptachlor Epoxide
Cl10 (209)
Delta-BHC
Amount in Cl2 (08), Heptachlor Epoxide, Cl9 (209) (ng)
Amount in Delta-BHC (ng)
Time (days)
0
20
40
60
80
100
120
140
160
0
1
2
3
4
0
5
10
15
20
25

Figure 1

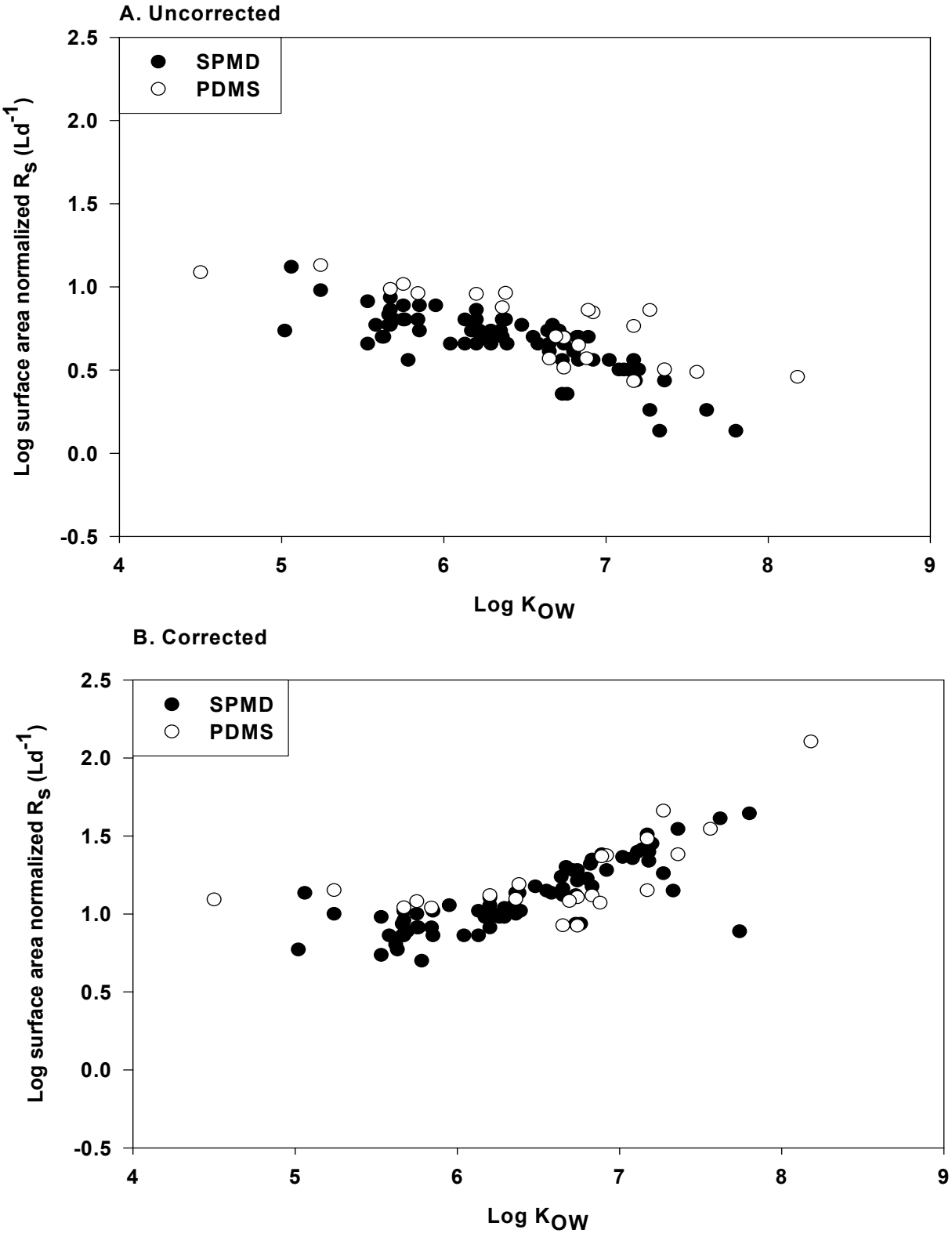
A. Uncorrected
SPMD
PDMS
Log surface area normalized R_S (Ld^{-1})
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
4
5
6
7
8
9
Log K_{OW}
B. Corrected
SPMD
PDMS
Log surface area normalized R_S (Ld^{-1})
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
4
5
6
7
8
9
Log K_{OW}

Figure 2

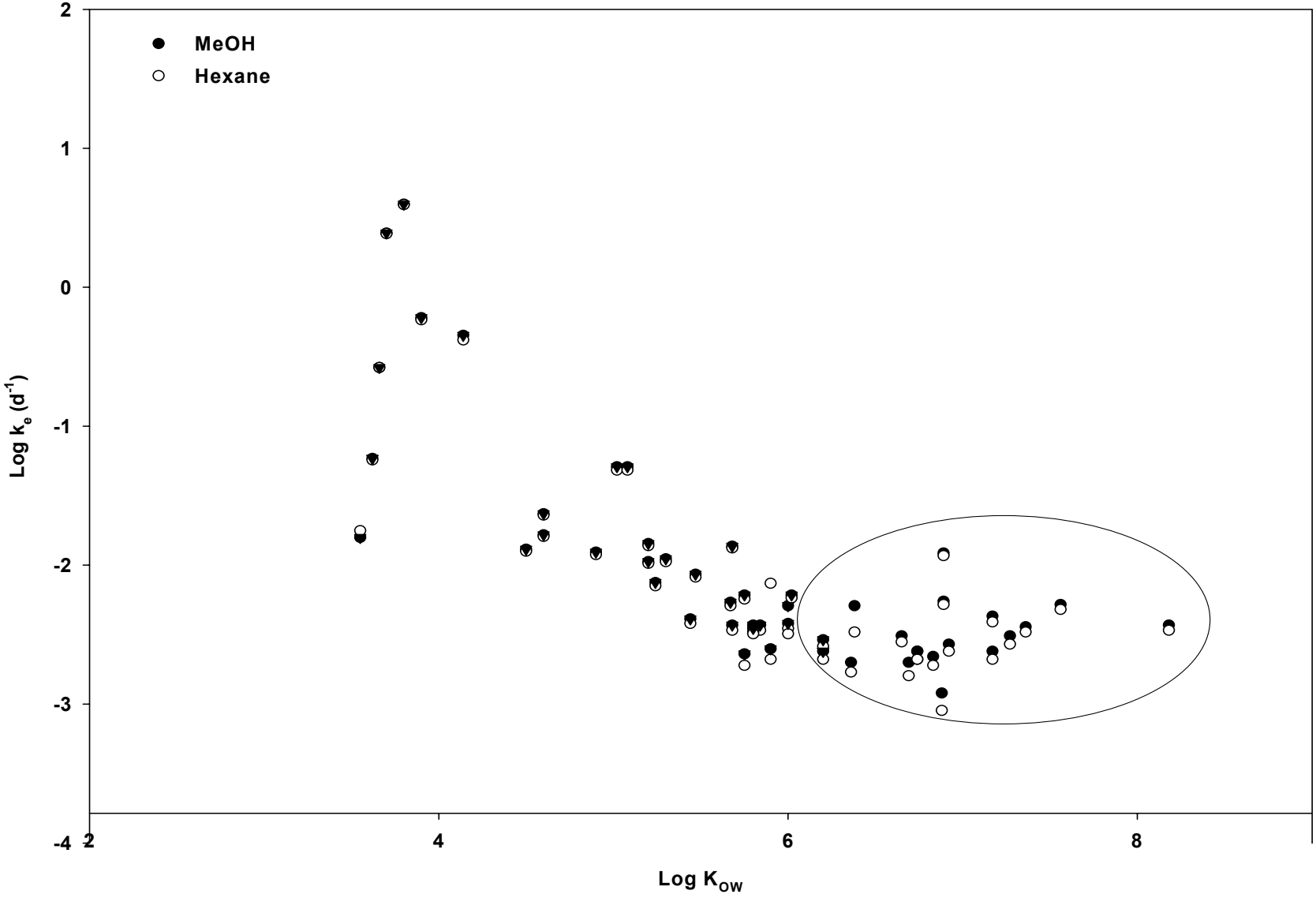

Figure 3

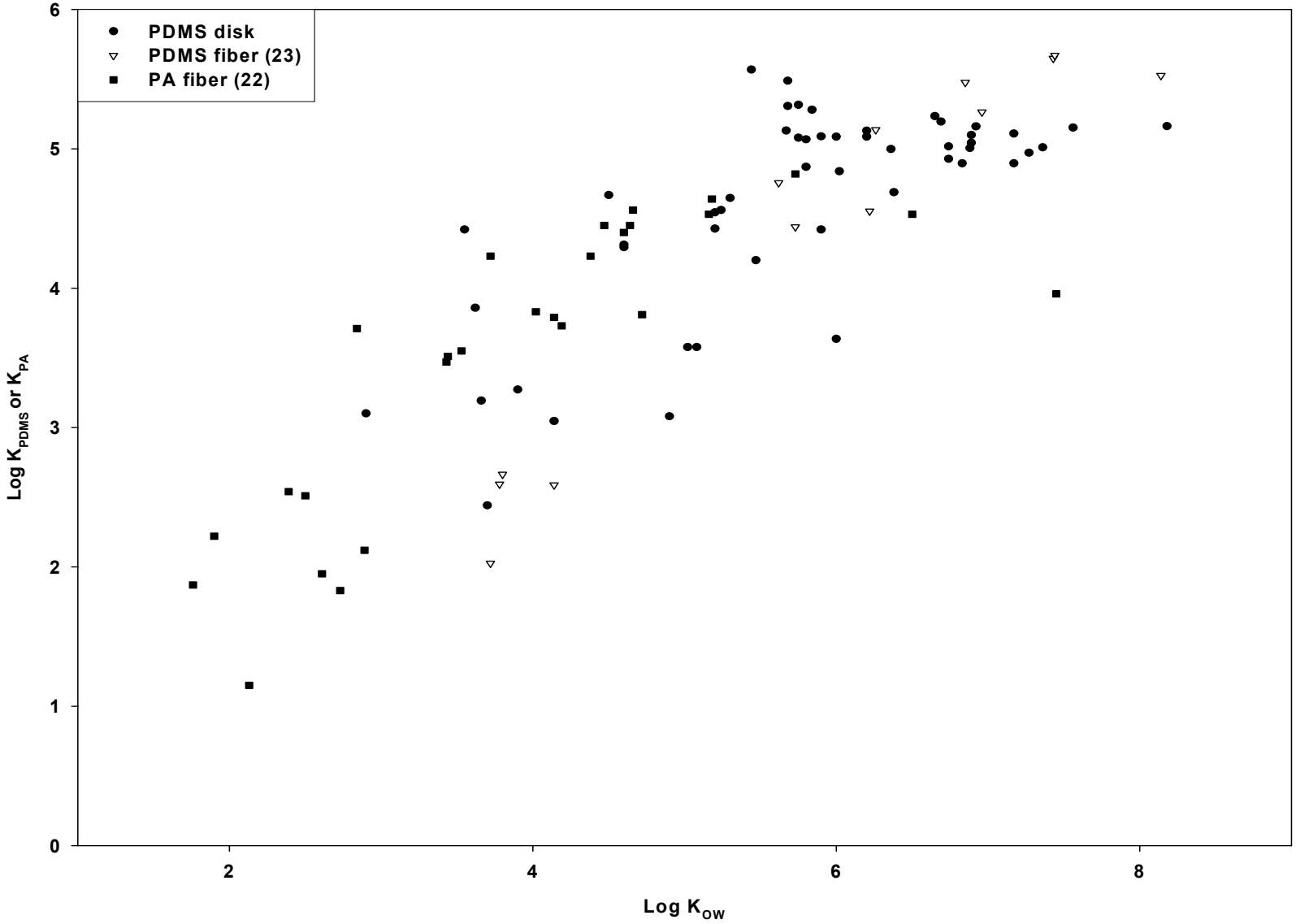
PDMS disk
PDMS fiber (23)
PA fiber (22)
Log K_{PDMS} or K_{PA}
Log K_{ow}
0
1
2
3
4
5
6
2
4
6
8

Figure 4

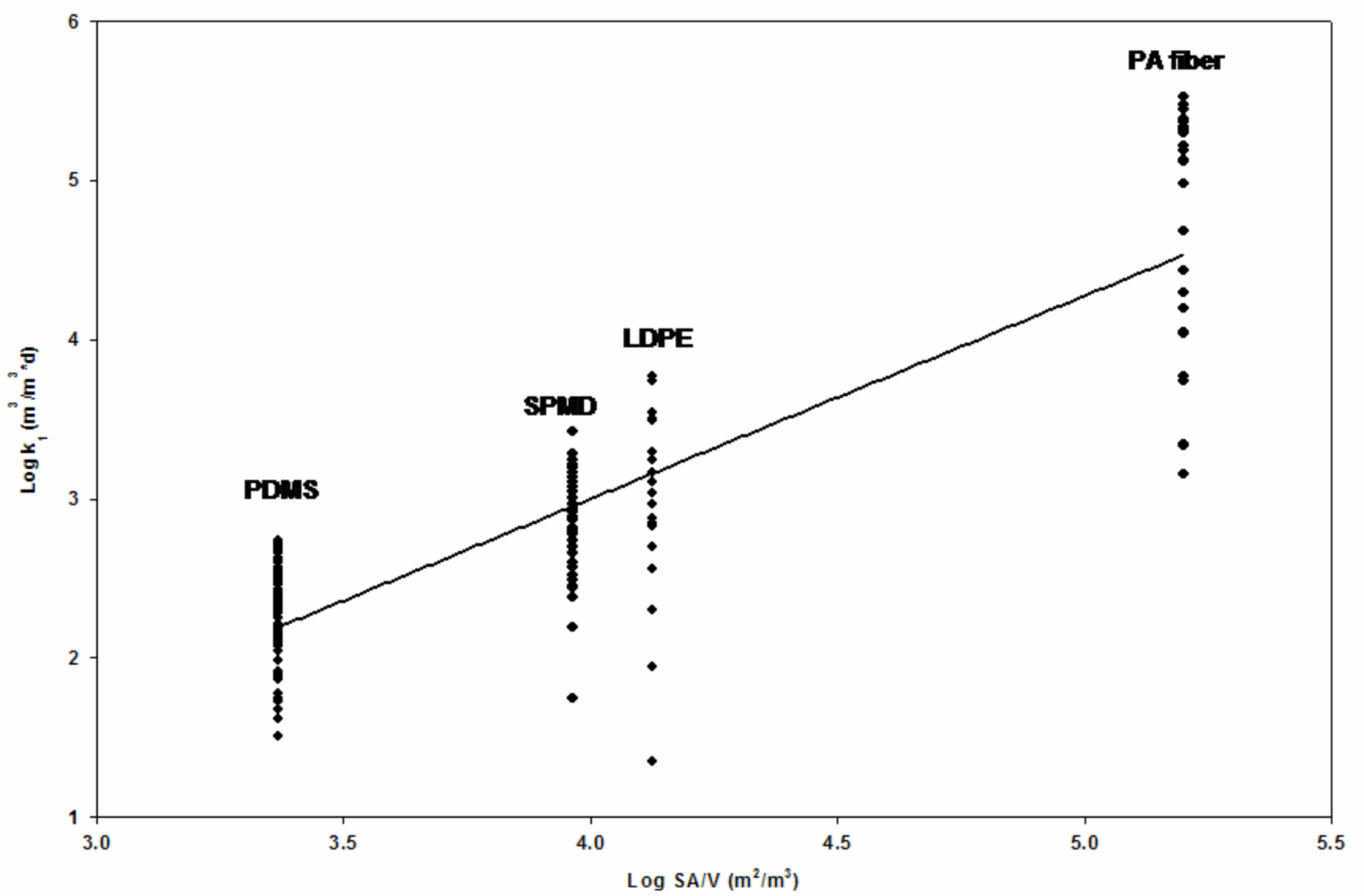
PA fiber
LDPE
SPMD
PDMS
Log k1 (m3/m3*d)
Log SA/V (m2/m3)
6
5
4
3
2
1
3.0
3.5
4.0
4.5
5.0
5.5

Figure 5

Chapter 3. Tetrachloroazobenezenes, a Review of the Published Literature.

Introduction

One of the major advantages of historic-use pesticides, such as dichlorodiphenyltrichloroethane (DDT), an insecticide used in the control of vector borne diseases like malaria and typhus, was their ability to persist in the environment. Persistence is what afforded pesticides like DDT, the nickname "miracle pesticide", due to their well-recognized effectiveness at a time when alternative control methods were unavailable. However, that same persistence is responsible for devastating effects on wildlife, including declining populations of bald eagles due egg shell thinning (1). After 30 years of heavy usage, DDT was banned in the United States in 1972. Today, 30 years after DDT was banned, its residues and potential deleterious effects still linger (2−5).

Concerns regarding the persistence of organic contaminants in the environment have resulted in the production of more water-soluble, less persistent, and environmentally friendly chemicals. Current-use pesticides undergo rigorous screening prior to their approval and application in the environment. These tests are in place to identify potential risks associated with the usage of the pesticide. Therefore, the end product is a pesticide that is capable of persisting long enough to provide the needed benefits but will degrade rapidly avoiding potential risks associated with a lengthy half-life. An important, and often unanswered question is whether or not this rapid degradation guarantees that these pesticides and their breakdown products are benign.

Complex reactions occur in the environment after the release of chemicals. These reactions can result in residue problems that are more problematic than that posed by the initial chemical residue. Certain chloraniline herbicides provide an excellent example of this problem. Upon their release, these herbicides rapidly degrade. However, this initial

step of degradation may be followed by series of reactions that can result in products that are more toxic and persistent. 3,3',4,4'-Tetrachloroazobenzene (TCAB) and 3,3',4,4'-Tetrachloroazoxybenzene (TCAOB) are products of such reactions (6−16).

Environmental contamination with TCAB and TCAOB can arise from microbial transformation of herbicides that contain the 3,4-dichloroaniline (3,4-DCA) moiety, i.e., propanil, diuron, and linuron (7−16). However, this is not the only source of TCAB and TCAOB contamination. In addition to contamination occurring via microbial transformation in the soil, TCAB and TCAOB are also formed as unwanted byproducts in the synthesis of these parent herbicides. Consequently, significant exposure to TCAB and TCAOB can result from the use of DCA-based pesticides containing the contaminants (16−22).

In 1976, Poland and co-workers were the first to identify that when TCAB and TCAOB are in E configuration, they assume a planar conformation that is isoteric to the structures of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 2,3,7,8-tetrachlorodibenzofuran (TCDBF). Upon further investigation they found that TCAB and TCAOB induce aryl hydrocarbon receptor (AhR) activity (23). Binding to the AhR is the common mechanism of dioxin-like compounds such as, polychlorinated dibenzo-p-dioxins, dibenzofurans, biphenyls, and naphthalenes. TCAB and TCAOB bind to the Ah receptor with a specific binding affinity one fifth and one-third, respectively, to that of TCDD (2−25). Typical dioxin-like effects have been reported in rodents after exposure to TCAB and TCAOB, include body weight loss, thymic atrophy, hepatotoxicity, anemia, developmental toxicity, chloracne-like lesions, and an induction of cytochrome P4501A1 (19, 24−32).

Although TCAB and TCAOB are considered dioxin-like compounds, there is limited research concerning how exposure to these compounds may impact the environment and humans. The purpose of this review is to present current information on TCAB and TCAOB regarding their chemical properties, source, fate, and observed residues in the environment, pharmacokinetics, and toxicity. This review is not meant to be exhaustive or critical, but rather to provide a summary of the existing data in on TCAB and TCAOB as environmental contaminants.

Chemical Properties

The chemical structures and physico-chemical properties of propanil, 3,4-DCA, TCAB, TCAOB, and TCDD are shown in Figure 1 and Table 1. One can see the structural similarity of TCAB, and to a lesser extent TCAOB, to TCDD. The US EPA considers compounds to have dioxin-like structures when substituent chlorines occupy: a) usually no more than one of the ortho positions; b) both para positions; and c) at least two meta positions; and d) the structure is not hindered from assuming the preferred planar configuration (33). Therefore, both TCAB and TCAOB meet the EPA criteria for dioxin-like structures.

In order to understand the fate of a chemical in the environment, exposure potential and its ability to elicit toxicity, it is essential to have a base knowledge of its physical chemical properties. Limited research has been conducted to determine physical and chemical properties of TCAB and TCAOB. Therefore, several of the properties listed in Table 1 were estimated using Molecular Modeling Pro (Molecular Modeling Pro ^{TM}, Fairfield, CA), a software program designed to calculate physico-chemical properties

based on a given molecular structure, properties that were calculated using this approach are identified with an asterisk. Although there haven't been any studies examining the half-lives of TCAB and TCAOB at the time of this review, these compounds have been detected in soil samples from rice fields that received applications of propanil two years prior to the sampling (34). This detection and other available data from lab studies and field experiments indicate that TCAB and TCAOB are likely to be persistent compounds under normal environmental conditions. Given the high lipophillicity and the low water solubility of TCAB and TCAOB, one would predict that these compounds would have an increased potential to accumulate in soil, sediment, or living organisms. Taking into consideration the proposed stability of these compounds in addition to their propensity to bioaccumulate, these compounds might be considered a threat to ecosystem and human health.

Sources of TCAB and TCAOB in the Environment

The fate of certain chloraniline herbicides is virtually unique in the fact that a chain of enzymatic and chemical reactions that are synthetic rather than degradative follow the initial step of biodegradation in soil. These reactions result in products that are more complex than the initial herbicide. TCAB and TCAOB are such products. Many theories have been proposed concerning the formation of these azobenzenes, however this subject is not fully understood. What is known is that there is a pattern of conversion of these herbicides with time to 3,4-DCA, TCAB and TCAOB, Figure 2 is a depiction of this transformation. This schematic is modified from Bartha and Pramer's 1967 research, one of the first studies conducted to investigate this phenomenon (7−16).

Pathways of formation. The transformation of chlorinated anilines in the soil into azobenzenes and polyaromatic compounds of higher complexity has been the subject of much interest in the past because of the extensive use of herbicides that contain the 3,4-DCA moiety. In soil, it has been proposed that 3,4-DCA transforms into TCAB, TCAOB (7−16), however, the origins of these occupational and environmental toxicants have not been finalized. In order to predict the amount of these compounds entering into the environment, it is necessary to have an understanding of the mechanism of formation.

In 1967, Bartha and co-workers initiated a series of investigations concerning the transformation process of certain pesticides to aniline and azo compounds in soil. Once this topic emerged, researchers began to try to resolve how azo compounds were formed in soil, and their efforts towards this subject continued on for many years. Initially they observed that propanil, a highly used pre-emergent herbicide, was cleaved by an acylamidase to 3,4-DCA and propionic acid. Propionic acid was then utilized by microorganisms as a carbon source and then transformed to carbon dioxide, water and cell substance. Two molecules of 3,4-DCA condensed to form TCAB by direct oxidation or 3,4-DCA may be biologically oxidized to 3,4-dichloronitrosobenzene (3,4-DCNB), which spontaneously condenses through chemical reactions with another aniline compound to form TCAB. This experiment implied that 3,4-DCA is an intermediate in the transformation of TCAB (6). Bartha continued this research in 1968 again suggesting that 3,4-DCA was an intermediate in the formation of TCAB from propanil, dicryl and karsil (7, 8).

In 1969 Belasco and Pease contradicted Bartha *et al*., suggesting that 3,4-DCA was not an intermediate in the formation of TCAB. These results were based on a laboratory experiment in which both 3,4-DCA and propanil were applied to the soil at the same rates and allowed to incubate for 42 days. After 42 days, TCAB concentrations were measured. The results indicated that the soils treated with 3,4-DCA yielded concentrations of TCAB that were ten to twenty times lower than that of the soils treated with propanil even though the 3,4-DCA concentrations after 42 days in each of the treated soils were similar. Therefore, if 3,4-DCA were the primary precursor to TCAB, it would have produced concentrations of TCAB that were proportional to those that propanil produced (9).

In the following year, Sprott and Corke confirmed the results of Belasco and Pease. In their experiments, they found that low percentages of TCAB were formed after applications of 3,4-DCA to four different soil types. They also suggested, based on their results, that condensation of two molecules of 3,4-DCA to TCAB could be limited to certain soil types. This study was also the only study reviewed that recognized azobenzenes as a nonpersistent residue in laboratory soils. They reported that the optimal temperature for conversion of 3,4-DCA to TCAB under their experimental conditions was 25 °C and when the temperature is increased to 30°C the formation of TCAB lowered (10).

In 1972 Bordeleau *et al*. examined the transformation of 3,4-DCA in a reaction chamber that contained peroxidase, hydrogen peroxide, 3,4-DCA, and a reaction terminator. The products of the reaction were collected in a vessel and analyzed. They found that the peroxidatic oxidation of 3,4-DCA produced 3,4-dichloroanilide and 3,4-

dichlorophenylhydroxylamine. These compounds then either reacted together or with themselves to produce 3,3',4,4'-tetrachlorohydrazobenzene (TCHB). TCHB then underwent rapid autooxidation to produce TCAB. Therefore, Bordeuleau *et al*. determined that peroxidase was responsible for catalyzing the formation of TCAB from 3,4-DCA (11).

In light of the previous work, Burge reexamined how peroxidase-mediated activity was related to the formation of TCAB. Burge found peroxidase activity in one out of the five soils tested. He indicated that not all soil peroxidases have the ability to form TCAB in soil from propanil or directly from 3,4-DCA. He also backed up the results of Belasco and Pease in 1969, concluding that 3,4-DCA was not involved in the main reaction for TCAB formation (13).

Following Burge, Lay and Ilnicki examined the effect of soil sample preparation on peroxidase activity and formation of TCAB in samples that were treated with 3,4-DCA. They found a linear relationship between the formation of TCAB and peroxidase activity in soil that was pretreated with carbon and nitrogen sources. Therefore, this work supported previous work that suggested that a peroxidatic mechanism is responsible for the formation of azo compounds from propanil-derived DCA (14).

Kaufman *et al*. (12) investigated the formation TCAOB from the treatment of ^{14}C-ring-labeled 3,4-DCA in *Fusarium oxysporum* Schlecht cultures. The probable pathway discovered in culture involved the condensation of 3,4-dichloronitrosobenzene and 3,4-dichlorophenylhydroxylamine to form TCAOB. However, the formation of TCAOB in this experiment was believed to be an artifact dependent upon the experimental culture

conditions. Variations in the conditions employed led to the formation of TCAB alone or TCAB and TCAOB (12).

In 1979 Corke *et al*. proposed a pathway for the formation of TCAB from *Escherichia coli* that was somewhat contradictory to Bordeuleau *et al*.'s peroxidase system. The pathway involved the conversion of 3,4-DCA and other anilines into biphenyls, azocompounds, and bistriazenes through the intermediate diazonium cation. The diazonium cation proved to be an essential intermediate in the formation of the metabolites because when it was removed from the system there was a 90% reduction in their production. The bacteria that were used in this system were pure cultures that can often times be isolated from soils, sediments, and sewage treatment plants. The requirements for this conversion to proceed were aniline, nitrate nitrogen or nitrite nitrogen that was available, and an environment with adequate reducing conditions which allowed the microorganisms to reduce nitrate to nitrite. It is important to note that the bacteria used in this experiment had no in peroxidase activity, which Bordeuleau *et al*. (11) reported necessary for the conversion of 3,4-DCA to TCAB (15). Bunce *et al*. (16) agreed with Corke *et al*. reporting that in the presence of nitrate ions and 3,4-DCA, TCAB, tetrachlorobiphenyls, and dihydroxytetrachlorobiphenyls were formed through the diazonium cation.

The transformation of chloraniline herbicides to TCAB has now been well documented. Although a definite mechanism of formation has not been established there are several proposed pathways of formation. What is known is that these chemicals are undergoing extensive transformations after application to soil and the persistent residue resulting from these complex reactions is one of great concern.

Contamination of Commercial Herbicides. In the late 1970s researchers began to analyze chloraniline herbicides for the presence of TCAB and TCAOB. Interest in these two contaminants was a result of the written reports that identified TCAB and TCAOB as the cause of several chloracne outbreaks where workers had been exposed to TCAB- and TCAOB-contaminated propanil and methazole (35−40). The interest of researchers was further sparked when TCAB and TCAOB were found to be isosteric to TCDD (23).

The conditions of heat and mild oxidation, employed during the synthesis of 3,4-DCA or further conversion to herbicides (propanil, linuron, and diuron), promote the condensation of two molecules of 3,4-DCA to form TCAB and TCAOB (23). Table 2 outlines TCAB and TCAOB contamination in commercial herbicides and clearly illustrates that the chloraniline herbicides that were analyzed are all contaminated with detectable concentrations of these compounds. 3,4-DCA is used as an intermediate in the production of propanil, anilide herbicide, and two-substituted urea pesticides, linuron and diuron. In addition to 3,4-DCA being an intermediate in the pesticide manufacturing process, it is used by Dow chemical as an intermediate in the production of polyethers and as a cross-linkage agent in epoxy tar products (41). The percent active ingredient for the pesticides analyzed was not reported in a number of the studies; therefore, the percents found in the table are based on review of the past and current literature pertaining to amounts of active ingredients in these pesticides.

Residues in the Environment

In 1957 a Monsanto scientist, Dr. Clarence Huffman, recognized the herbicidal properties of propanil; since that time propanil has become one of the major post-emergence herbicides used in rice-growing areas of the United States and other countries (42). Propanil has a short half-life in the field and in water (Table 1). In fact, several studies have analyzed for propanil in areas where it is a heavily used herbicide, and they have found that there are rarely any detectable residues (41−46). However, as stated before, pesticides that degrade rapidly after providing a benefit does not guarantee that they are always safe. Propanil is one of the sources of TCAB and TCAOB in the environment due to contamination and degradation (6−21). Therefore, because propanil is widely used in the rice industry, monitoring of propanil and TCAB and TCAOB residues is very important to assess the safety of the environment and food, given the known toxicity associated with these compounds.

Soil Residues. There have been numerous laboratory studies examining the transformation processes of propanil in soil. However, field studies that analyze for the presence of TCAB and TCAOB in propanil-treated soils are scarce (Table 3). Given what is known about the toxicity of TCAB and TCAOB it is imperative that more information is gathered to expand upon what has already been discovered. For example there is not a clear understanding of the environmental persistence and fate, which are both essential in order to further establish the potential toxicity associated with these dioxin-like compounds. Also, there are no studies that analyze for TCAOB, the more potent of the two compound of interest, in the field or the laboratory.

Table 3 clearly illustrates the residues that have been detected in laboratory soils and field soils. The longest running experiment in the laboratory was 280 days done by Burge in 1972. In this study, 100 цg/g of propanil was applied to five different soil types. The results indicated that TCAB accumulation continued to increase throughout the experiment. Results from other laboratory studies are in agreement with Burge in that TCAB does indeed continue to accumulate throughout the length of the experiment (7−9, 12, 46). There have only been five field studies conducted to examine if TCAB is produced in the environment. In 1970, Kearney *et al*. did the most lengthy field study that investigated the potential of formation of TCAB and degradation of TCAB over a two-year period in rice-producing soils in Arkansas. The results indicated that TCAB concentrations were the greatest in the top 10 cm of the soil and that TCAB has been detected two-years after propanil applications (42).

Pharmacokinetics and Toxicity of TCAB and TCAOB.

Research on the toxicity of TCAB and TCAOB began in 1976 when Poland and co-workers identified TCAB and TCAOB as being isosteric to the most potent dioxin and one of the most toxic chemicals known to laboratory animals, TCDD (23). Following this discovery, research continued in this area addressing how potentially toxic these compounds may be to humans. This research continued for many years and is still ongoing. The information found in the literature focuses primarily on vertebrate toxicity. No studies with regard to TCAB or TCAOB accumulation in aquatic invertebrates could be found by this reviewer. The toxicity data for these organisms is lacking, and the

current knowledge of TCAB and TCAOB suggests that there is a great need to characterize toxicity associated with these ecological receptors.

Pharmacokinetics. Pharmacokinetic studies of TCAB and TCAOB in rodent models began in 1984 to examine the reported differences in biological potency and binding affinity to the AhR between these compounds and TCDD (23, 31, 47). Burant and Hsia (48) were the first to investigate the absorption, distribution, metabolism and excretion of TCAB and TCAOB in male Sprague-Dawley rats. The rats were given a single dose of 10 mg of ^{14}C-labeled TCAB or TCAOB via stomach intubations. The urine, feces, and various organs were collected and analyzed for radioactivity using a scintillation counter. The results of the experiment indicated that 66% of the TCAB was excreted via the urine and feces within the first 24 hours of the experiment, whereas, only 37% of the TCAOB was eliminated via urine and feces. The half-lives were estimated to be 18 hours and 34 hours for TCAB and TCAOB, respectively. The majority of the remaining radioactivity left in the rats was found in adipose tissue. Metabolism of TCAB was predicted to be through ring hydroxylation, which renders a product that is more polar and less lipophillic than the parent molecule therefore explaining the reduced toxicity previously reported as compared to TCDD which is not metabolized as readily (49).

Pillai *et al*. (50) and Zeigler *et al*. (51) re-examined the pharmacokinetics of radiolabeled TCAB and TCAOB in male Fischer 344 rats that received treatments intravenously or orally. The reported half-lives and clearance rates for TCAB and TCAOB were 4 hours with a clearance rate of 12.3 ml/min·kg and 7 hours with a clearance rate of 11.0 ml/min·kg, respectively. They also found that the adipose tissue

had the greatest amount of radioactivity. Based on their results, they concluded that both TCAB and TCAOB undergo extensive metabolism to chloraniline derivatives via azo reduction. Therefore, these compounds when compared to TCDD have decreased absorption and an enhanced elimination, which renders them less toxic. However, they did report that other toxicities associated the chloraniline derivatives produced after metabolism may be a concern.

Chloracne. Once TCAB and TCAOB were found to be isosteric to TCDD, a well-known acnegen, several incidences of chloracne that had occurred before this discovery were confirmed and written up formally with TCAB or TCAOB being the etiological agents (23, 35, 36). Chloracne is defined as an acneiform eruption due to poisoning by halogenated aromatic compounds having a specific molecular shape. It can be distinguished from acne vulgaris by observing the distribution of the acne, unusual age onset, and clustering of similar cases in a factory or town (35).

In the early 70s, there were at least eight outbreaks of chloracne in chemical plants that resulted from exposure to TCAB- and TCAOB-contaminated herbicides. Most of these cases (75%) were not reported (35−38). Factory employees who manufactured herbicides such as methazole and propanil were reporting symptoms occurring in both exposed areas and covered areas. These symptoms included epidermal cysts, comedones, and papules, which resulted in scarring on exposed areas of the body. The symptoms would usually manifest one to two months after exposure to the contaminated pesticides. TCAOB has been reported as having extreme acnegenic potential, which was physically

observed when four family members developed chloracne solely from exposure to contaminated clothing or tools from a pesticide manufacturing facility (35).

In 1980, Taylor and Lloyd did a follow up study on one of the incidences of chloracne where 15 workers in a methazole plant in 1972 had reported symptoms. Follow-up studies of patients with chloracne are needed to evaluate the skin and look for evidence of systemic disease. Only one-third of the patients responded to the questionnaire and agreed to be re-examined and have their children re-examined as well. Three out of the five responders still had evidence of chloracne and the other two were free of any symptoms. The two children had no evidence of active chloracne but did have mild scarring (40).

Vertebrate Toxicity. The toxicity of TCAB and TCAOB in the rodent model has been well documented. From the late 70's to present time scientists have been exploring this issue to determine if these compounds should be considered occupational health hazards. The origin of this research is rooted in Poland and co-workers discovery that revealed TCAOB and TCAB as potent inducers of hepatic aryl hydrocarbon hydroxylase (AHH). They determined this by injecting chick embryos with TCDD and TCAOB and then comparing the response. From this experiment they calculated that TCAOB expressed 96% of the activity that was seen with TCDD (TCDD producing 100% activity).

In the same study they also found that TCAB and TCAOB compete with radiolabelled TCDD for specific hepatic cytosol binding sites. The biological potency (ED_{50}) was estimated from the log dose response curves obtained in the AHH induction experiment. The ED_{50} *in vivo* was then compared with the binding affinity observed *in*

vitro and they found that they were in good agreement. TCAOB was slightly more potent than TCAB. From these results, it was concluded that the capacity of TCDD, TCAB, and TCAOB to induce AHH activity corresponded with their ability to elicit acne (23).

Since the findings of Poland *et al*. (1976), many other scientists continued to investigate the potential toxicity associated with these compounds. Over two decades later, research was still thriving with the main objective being to establish if there were any other parallels connecting one of the most potent dioxins with TCAB and TCAOB. From these continued research efforts, a great deal of information has been revealed regarding the vertebrate toxicity of these environmental and occupational toxicants, information that does indeed establish a relationship between the toxicological effects produced by TCDD and TCAB/TCAOB (25−30).

It wasn't quite a year after Poland *et al*. (1976) established the initial relationship between TCDD and TCAB/TCAOB, when TCAB was evaluated to determine the potential health hazard *in vitro* systems. They treated mouse embryo fibroblasts (C3H/10 T ½) with varying concentrations of TCAB (ranging from 0.47 цg/mL to 15.10 цg/mL) and then examined the cells to see if there was any cytotoxicity associated with the treated versus control cells. The results indicated that TCAB was toxic to mammalian cells. The cells treated with 1 цg/mL produced type III foci, which are known to produce tumors in C3H mice (52). TCAB was also found to be slightly mutagenic when examined by the salmonella microsome assay (53).

The first investigation concerning the toxicity of TCAB *in vivo* was conducted shortly thereafter when Hsia and co-workers demonstrated that TCAB had carcinogenic potential. There were three separate experiments in this study. Each utilized male

Sprague-Dawley rats that were intraparenchymal (ip) injected with TCAB or TCAOB at a range of doses. The following is a description of the dose regimens; 1 mg/kg/day, 10 mg/kg/day, 25 mg/kg/day, or 50 mg/kg/day for a total of five days and then sacrificed, a single dose of 10 mg/kg/day and sacrificed 48 hours later, or a single dose of 10 mg/kg or 100 mg/kg and sacrificed in pairs on days 7 and 14. Microsomes from the treated animals were prepared to evaluate the induction of cytochrome P450. Both TCAB and TCAOB demonstrated a 2.7 fold increase in cytochrome P450 as compared to controls. TCAOB was the most potent inducer of P450; it had the ability to sustain an induction for 14 days after a single ip injection (54).

The following year a chronic study conducted by Hsia *et al*. (1980) examined the general body health status of prolonged exposure to TCAB and TCAOB in rats. Male Sprague-Dawley rats were fed either a control rat chow or rat chow that contained 100 ppm of TCAB or TCAOB for 120 days. Each animal was weighed twice a week,and at the end of the experiment the rats were anesthetized. Blood was collected to evaluate the hematocrit, red blood cell count, white blood cell count, and hemoglobin. Various organs were collected and liver microsomes were prepared to measure the AHH activity using benzo [a] pyrene as a substrate.

The results indicated that the overall health of the rats was affected when they consumed approximately 25 mg of TCAB or TCAOB over 120-day period. In general, TCAOB had a greater impact on the rats than the TCAB. Decreases in body weight were reported along with a reduction of red blood cells, which could be indicative of the early stages of aplastic anemia. The low amount of dietary exposure to TCAOB (approximately 0.2 mg/animal/day) induced cytochrome P450 enzymes. The liver and

spleen increased in weight while the testis decreased in weight. The increase in liver weight can probably be explained by an increased proliferation of the smooth endoplasmic reticulum of the hepatocytes (26).

The P450 that is induced by TCAB and TCAOB is cytochrome P4501A1 (i.e., CYP1A1 enzyme activity) (23). The binding of these compounds to the AhR can lead to chromatin disruption, increased promoter accessibility and increased rates of transcription initiation of the CYP1A1 gene. There are reported differences in toxic potency of dioxin-like compounds in different species, which is associated with the binding affinities of these compounds to the AhR. TCDD is the most potent (55).

In the same year Schrankel *et al*. (30) examined how TCAB and TCAOB affected the pathology of the liver in an acute study utilizing rats and mice. They ran two experiments on male Sprague-Dawley rats, which received ip injections of TCAOB at 25 mg/kg on days one and five (sacrificed on day ten) or on days one through four (sacrificed on day five). The final experiment involved female ICR outbred Swiss albino mice that were given ip injections of 20 mg/kg/day TCAOB for five days and sacrificed the following day. The organs from each of the rodents were removed, weighed, and processed for light and electron microscopic examination.

The results of this experiment were in agreement with earlier studies performed by Hsia and Kreamer (25) and Hsia *et al*. (42). TCAOB caused pathological changes in hepatic tissues. The histopathological alterations that were observed in the rats and mice included enlargement of hepatocytes, numerous cytoplasmic vacuoles, proliferation of smooth endoplasmic reticulum, and the presence of numerous membranous arrays. There was no lipid accumulation observed in the cells and tissues of treated rodents, which

contradicts the production of fatty changes in hepatocytes and Kupffer cells of animals fed acute doses of other chlorinated aromatic hydrocarbons. Results of this study suggested that exposure to these chemicals in an occupational environment may cause histopathological alterations and therefore, these chemicals must be considered a health hazard (56).

More recently (57, 58), the toxicity of TCAB and TCAOB have been re-examined in Fischer F344 rats and B6C3F mice to establish a dose-response relationship for the histopathological findings and changes in organ weights, clinical chemistry, and selected reproductive parameters. These studies provide the most recent evidence that TCAB and TCAOB are dioxin-like compounds that upon exposure can result in adverse effects in rats and mice similar to those of TCDD. Groups of rats and mice were administered 0, 0.1, 1, 3, 10, or 30 mg TCAB or TCAOB by gavage five days a week for 21 days or 13 weeks. Organs were prepared for microscopic evaluation, blood was collected for hematology determination, and the length of the estrous cycle was analyzed for a reproductive parameter.

The major adverse effect seen in the 30 mg/kg treated rats was a 10% decrease in body weight in the TCAB-treated rats and death in the TCAOB-treated rats. Typical dioxin-like effects were reported after exposure to TCAB and TCAOB, including thymic atrophy (a hallmark of dioxin-like chemical toxicity), an increase in liver weight, increase in spleen weights accompanied by hematopoietic cell proliferation, responsive anemia, chloracne (first time reported in mice other than hairless mice), hyperplasia of the forestomach, increase in the estrous cycle, decreased epididymal sperm concentrations, decreased circulating thyroid hormone concentrations, thrombocytopenia, and a decrease

in body weight. No no-observed adverse effect level was reached in the TCAOB studies; however, it was reached in the TCAB studies (10 mg/kg/day). TCAB was reported to be about two to six orders of magnitude less potent than TCDD depending on the endpoint. For example, using circulating thyroxine concentrations as an endpoint, TCAB is two orders of magnitude less potent than TCDD. However when using thymic atrophy as the endpoint, it is six orders of magnitude less potent (57, 58).

The relative toxicities of dioxin-like compounds in relation to TCDD (toxicity equivalency factors, TEFs) are determined from *in vitro* and *in vivo* studies. The TEF for the compound of interest is a measure of potency for a specific endpoint, binding affinity to the AhR, of that compound divided by the potency of TCDD for the same endpoint. TCDD is assigned a TEF of one. Then the toxicity data from a given compound can be converted to provide a TCDD toxic equivalency (TEQ), which is calculated by multiplying the concentration of the dioxin-like compound by its TEF. The following criteria must be met in order for a compound to be included in the TEF system, it should show structural relationship to the PCDDs and PCDFs, it should bind to the AhR, it should elicit dioxin-specific biochemical toxic responses, and it should be persistent and accumulate in the food chain (59−64). Both TCAB and TCAOB meet the defined criteria except they do not accumulate in rodent models. Information regarding accumulation in other models is lacking.

The relative potencies, TEF, for TCAB have been calculated based on specific effects observed in literature. The TEFs for binding to the AhR, the EC_{50} binding to the mouse hepatic AhR, and the EC_{50} for induction of aryl hydrocarbon hydroxylase in chick embryo are all 0.2 (23−25, 60, 61). The TEFs of TCAB and other dioxin-like compounds,

PCDDs, PCDFs, and PCBs, can be found in Table 4. TCAB has one of the highest TEFs reported signifying that there is definitely a need to pursue further study in this field.

Immunotoxicity. The immune system has a critical role in maintaining health. When a toxicant suppresses the immunological function of an organism results can include increases in the incidence of infectious diseases and some types of cancer. Hsia *et al*. (26) Hsia and Kreamer (31) and Bleavins *et al*, (65) all performed studies to examine how exposure to TCAB and TCAOB affected the immune system in male Sprague-Dawley rats and Swiss-Webster mice. The treated animals suffered from severe thymic atrophy, which could compromise immune system development. From previous work, it has been demonstrated that immunotoxicity of polychlorinated biphenyls (PCBs) and TCDD is intimately associated with the Ah gene in mice (45, 46). Therefore, since TCDD and TCAB/TCAOB appear to produce similar toxic responses, the immunotoxicity associated with the compounds of interest may also be related to binding to the Ah receptor. Each of the studies also reported a decrease in body weight that was reminiscent to 'wasting syndrome', which is also seen in TCDD treated animals (27, 31).

Teratogenicity. The teratogenic potential of these compounds was also a subject that received much attention, with the goal of the research being to compare the teratogenicity of TCAB/TCAOB to TCDD, which is a known teratogen (47). In 1982 Schrankel *et al*. (30) were the first to investigate the teratogenicity of these potent environmental and occupational toxicants. Chick embryos were injected on days 4 or 11-13 with varying doses of TCAB and TCAOB ranging from 0.1 ng to 100 ug via a small hole in the shell.

The embryos were examined for viability during a 14-day period. The hatchability of the eggs injected on day 4 decreased when compared to the controls with the majority of death occurring on days 7 or 8.

When chick embryos were injected with 17 ppb of TCAB or 1.7 ppb TCAOB on days 11-13, 100% mortality occurred revealing that these toxicants are the second and third most toxic chemicals that have been examined in the chick embryo at the time of this study. The major malformation associated with chick embryos injected with TCAB and TCAOB was rump edema. The LD50 values estimated using probit analysis for the chick embryos were 44 ng TCAB and 12 ng TCAOB. The results of this study indicated that both TCAB and TCAOB are extremely toxic and potentially teratogenic in chick embryos, which warranted further study in this area given that there is a good correlation between teratogenicity in avian and rodent embryos (29, 48)

In 1984 Hassoun *et al*. (66) continued to examine the teratogenicity of TCAOB by studying how TCAOB affected Ah-receptor in responsive (C57BL and NMRI) and non-responsive (DVA/2J and AKR/Nbom) strains of mice. The mice were mated and pregnant females were given ip injections of TCAOB at various doses (6-16 mg/kg). Mice were sacrificed on day 17 and the uteri were examined for the number of alive, malformed, resorbed, and dead fetuses. In 50-90% of the responsive strain mice, TCAOB produced cleft palate and hydronephrosis at doses of 6-8 mg/kg body weight (bw) without any apparent maternal toxicity. 60% of the responsive and 40% of the non-responsive strains died when treated with 16 mg/kg of TCAOB. 95% of the remaining responsive mice had cleft palate. When the fetuses were examined by light and scanning electron microscopy, they found that the apical epithelial cells of the secondary palates

did not successfully follow the normal pattern of apoptosis. Treatment of rats with TCDD causes cleft palate by similar mechanisms to that observed in this experiment, therefore, suggesting a similar mechanism of pathogenesis for the two compounds (47, 49, 50).

A study was conducted in 1984 to assess how chronic dietary exposure to TCAOB effects reproductive efficiency of female Swiss-Webster mice. The dietary concentrations (0.1, 1.0, and 10 ppm) fed to the females were low enough so that no toxicity was expected during the 14-days they were fed prior to being mated. Following mating, the females delivered their pups. The pups were evaluated for survival and immune function. The results indicated that dietary exposure of 10 ppm of TCAOB reduced the reproductive capability of the mothers and caused moderate immuno-suppression in the offspring during *in utero* and early postnatal development. Another group of mice were fed TCAOB (total concentration of 40 ppm) for 28 days. At the conclusion of 28 days, the treated animals were sacrificed and their spleen cells were examined for chromosome damage. The results indicated that TCAOB caused an increased amount of sister chromatid breakage signifying that TCAOB may be a mutagen (67).

In summary, given the documented toxicity in vertebrates, the formation of TCAB and TCAOB during pesticide synthesis and in the environment, the TEF and TEQ values, it is surprising that we were unable to find any toxicity data for these two dioxin-like compounds in invertebrate systems. The chemical-physical properties of TCAB and TCAOB alone, warrant further investigation in order to definitively determine if these occupational and environmental contaminants pose a threat to ecological health. The consequences of TCAB and TCAOB exposure to a variety of organisms in the

environment are currently unknown. Based on the potentially high exposures and toxicity of TCAB and TCAOB there is a need for further quantify these contaminants in this field.

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Table 1. Physicochemical properties of Propanil, 3,4-DCA, TCAB, TCAOB, and TCDD.

Compound	Propanil	3,4-DCA	TCAB	TCAOB	TCDD
Formula	$C_9H_9Cl_2NO$	$C_6H_5Cl_2N$	$C_{12}H_6C1_4N_2$	$C_{12}H_6C1_4N_2O$	$C_{12}H_4Cl_4O_2$
Molecular Weight (g/mol)	218	162	320	336	322
Description	white crystals	light brown crystals	orange crystals	yellowish-orange crystals	
CAS Number	709-98-8	95-76-1	14047-09-7	21232-47-3	1746016
Melting point (^{0}C)	92-93	72	158	142	305
Boiling point (^{0}C)	>100	272	384.7	*387.6	900
Vapor Pressure (Pa @ 25 ^{0}C)	2.60×10^{-5}	0.84	$*7.37 \times 10^{-9}$	$*5.59 \times 10^{-8}$	2.00×10^{-7}
Water solubility (mg/L @ 25 ^{0}C)	225	none	1	*1	0.483
Log Kow	3.1	2.7	6.7	*6.8	6.8
field half-live	1-3 days		no data found	no data found	12 years
water half-live	2 days		no data found	no data found	1.5 years

Table 2. Contamination of commercial herbicides with TCAB (mg/g) and TCAOB (mg/g).

Type of Herbicide	% ai*	TCAB (mg/g)	TCAOB (mg/g)	Comments	References
3,4-DCA	100	13-463	< 0.1	GC/ECD, LOD 0.1-0.05 mg/g	17
Diuron	80	5.6-28.0	0.3-1.9	GC/ECD, LOD 0.1-0.05 mg/g	17
Linuron	50	5.6-28.0	< 0.05	GC/ECD, LOD 0.1-0.05 mg/g	17

Type of Herbicide	% ai	TCAB (mg/g)	TCAOB (mg/g)	Comments	References
Propanil, granular	36-44	2900		Spectrophotometry, LOD not reported	18
Propanil, emulsifiable liquied	36-44	2000-2600		Spectrophotometry, LOD not reported	18
3,4-DCA	100	60-8500		Spectrophotometry, LOD not reported	18

[a] Quantitated with Spectrophotometry, confirmed with GLC

Type of Herbicide	% ai	TCAB (mg/g)	TCAOB (mg/g)	Comments	References
3,4-DCA	100	9.0-51.0	nd-8	HPLC and GC/MS, LOD not reported	19
Propanil	36-44	1000-1400	nd	HPLC and GC/MS, LOD not reported	19
Diuron	80	28	nd	HPLC and GC/MS, LOD not reported	19
Linuron	50	9	nd	HPLC and GC/MS, LOD not reported	19
Neburon	60	nd	nc	HPLC and GC/MS, LOD not reported	19

Table 2. Contamination of commercial herbicides with TCAB (mg/g) and TCAOB (mg/g) (continued)

Type of Herbicide	% ai	TCAB (mg/g)	TCAOB (mg/g)	Comments	References
Propanil	36-44	0.1-9.85	< 0.05	GLC/ECD, LOD 0.05 ppm	20
Diuron	80	5.7-12.35	< 0.05	GLC/ECD, LOD 0.05 ppm	20
Linuron	50	6.7-28.4	< 0.05	GLC/ECD, LOD 0.05 ppm	20
Neburon	60	1.9-22.5	< 0.05	GLC/ECD, LOD 0.05 ppm	20

Type of Herbicide	% ai	TCAB (mg/g)	TCAOB (mg/g)	Comments	References
Diuron	100	0.15-3.38		GC/ECD, LOD 0.03 mg/g	21
Linuron	100	0.91-10.28		GC/ECD, LOD 0.03 mg/g	21

Type of Herbicide	% ai	TCAB (mg/g)	TCAOB (mg/g)	Comments	References
Propanil	37.5-41.0	1.1-30.0		GC/ECD, LOD ppb range	22

* % actuve ingredient

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Table 3. TCAB residues (ng/g) detected in soil and water.

Soil Laboratory experiments

Herbicide	Conc. applied	Type of soil	TCAB (mg/g)	Comments	References
Propanil	500 mg/g	sandy loam	230	30 days incubation, 28, ^{o}C LOD not reported, GC/FID	7
Propanil	500 mg/g	sandy loam	110	21 days incubation, 27 ^{o}C, LOD not reported, GC/FID	8
Dicryl	500 mg/g	sandy loam	85	21 days incubation, 27 ^{o}C, LOD not reported, GC/FID	8
Karsil	500 mg/g	sandy loam	145	21 days incubation, 27 ^{o}C, LOD not reported, GC/FID	8
Solan and Propanil	500 mg/g	sandy loam	4860	21 days incubation, 27 ^{o}C, LOD not reported, GC/MS	9
Diuron	500 mg/g	silt loam	<0.1	31 days incubation, 27 ^{o}C, LOD 0.1 ppm, GC/Microcoulometer	9
Linuron	500 mg/g	silt loam	<0.1	31 days incubation, 27 ^{o}C, LOD 0.1 ppm, GC/Microcoulometer	9
Propanil	250-500 mg/g	silt loam	35.4-143	31 days incubation, 27 ^{o}C, LOD 0.1 ppm, GC/Microcoulometer	9
3,4-DCA	100-500 mg/g	silt loam	1-4.5	42 days incubation, 27 ^{o}C, LOD 0.1 ppm, GC/Microcoulometer	9
3,4-DCA	100 mg/g	2 clays and two loams	0.0-0.6	19 days incubation, 25 ^{o}C, LOD not reported, GC	10
Propanil	85 mg/g	5 soil types	0.85-15.81	105 days incubation, 30 ^{o}C, LOD 0.3 ppm, GC/FID	62
Propanil	850 mg/g	5 soil types	11.5-222.7	105 days incubation, 30 ^{o}C, LOD 0.3 ppm, GC/FID	62
Propanil	100 mg/g	silty clay loam	11.8	30 days incubation, LOD not reported, GC/MS	12
Propanil	100 mg/g	5 soil types	1.1-16.0	280 days incubation, 25 ^{o}C, LOD 0.1 ppm, GLC/MS	13

Table 3. TCAB residues detected in soil (mg/g) and water (ng/L) (continued).

Soil Field Experiments

Herbicide	Conc. applied	Type of soil	TCAB (mg/g)	Comments	References
Diuron	2 to 4 lbs/A every 12 yrs	silt loam	<0.1	Last application 6/66, sampled 6/67, LOD 0.1 ppm, GC	9
Linuron	2 lbs/A	silt loam	<0.1	Last application 6/66, sampled 8/67, LOD 0.1 ppm, GC	9
Propanil	3.4 to 6.7 kg/ha	rice fields	<0.01-0.18	Applied 5/68, Sampled 7/68, LOD 0.01 ppm, GC/ECD	34
Propanil	13.5 kg/ha	0.0-30 cm rice fields	0.02-0.16	Applied 6/67, 6/68, Sampled 7/68, LOD 0.01 ppm, GC/ECD	34
Propanil	9 kg/ha	0.0-30 cm rice fields	0.01-0.05	Applied 5/67, Sampled 7/68, LOD 0.01 ppm, GC/ECD	34
Propanil	4.5 - 10 kg/ha	0.0-30 cm rice fields	<0.01 to 0.01	Applied in 5-7/66, Sampled 7/68, LOD 0.01 ppm, GC/ECD	34
Propanil	3.4-5.4 kg/ha	silt loam	0.01-0.02	Applied 6/68, Sampled 7 - 10/68, LOD 0.01 ppm, GC/ECD	62
Propanil	3.4-5.4 kg/ha	silt loam	0.01	Applied 6/69 , Sampled 6 - 10/69, LOD 0.01 ppm, GC/ECD	62
Propanil	3.4-5.4 kg/ha	silt loam	0.06	Applied 6/70, Sampled 6 - 10/70, LOD 0.01 ppm, GC/ECD	62
Propanil	1.2 kg/ha or 11.2 kg/ha	rice fields	0.01-0.05	6 months to 4 years, LOD 0.2 ppb, GC/ECD	64
Propanil	3.4-6.4 kg/ha	0.0 -20.0 cm Clay	nd	collected 24 hours after flooding, LOD 0.004 ppm, GC/ECD	63

[b] Condensation of two 3,4-DCA molecules was lessened due to dilution and dispersal of soil

Water

Herbicide	Conc. applied	Type of water	TCAB (mg/g)	Comments	References
Propanil	3.4-6.4 kg/ha	flooded rice fields	trace quantites	Sampled 0,24, 48, 96 hours after flood, LOD 0.002 ppm, GC/ECD	63

Table 4. Toxicity equivalency factors (TEFs) for TCDD and dioxin-like compounds.

Compound	TEFs
2,3,7,8-TCDD	1[a]
3,3',4,4'-TCAB	0.2[b]
2,3,4,7,8-PCDF	0.5[a]
1,2,3,4,7,8-HxCDD	0.1[a]
2,3,7,8-TCDF	0.1[a]
3,3',4,4'-TCB, PCB # 77	0.0001[a]

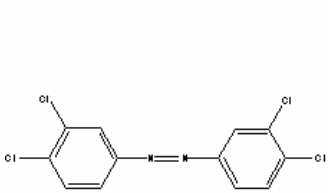
[a] Ref 66
[b] Ref 22

FIGURE LEGENDS

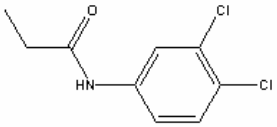
Figure 1. Structures of propanil, 3,4-DCA, TCAB, TCAOB, and TCDD. Propanil is a chloraniline herbicide applied to rice for the control of broadleaf weeds. In the environment propanil can break down to for 3,4-DCA. 3,4-DCA can condense to form TCAB and TCAOB which are structural analogs to TCDD.

Figure 2.Conversion of propanil (− −) to 3,4-DCA (—) and then further conversion to TCAB (--) (6).

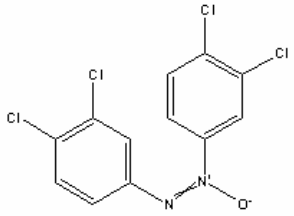
3,4-DCA



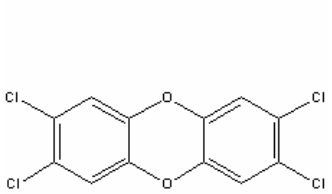
TCAB



Propanil



TCAOB



TCDD

Figure 1

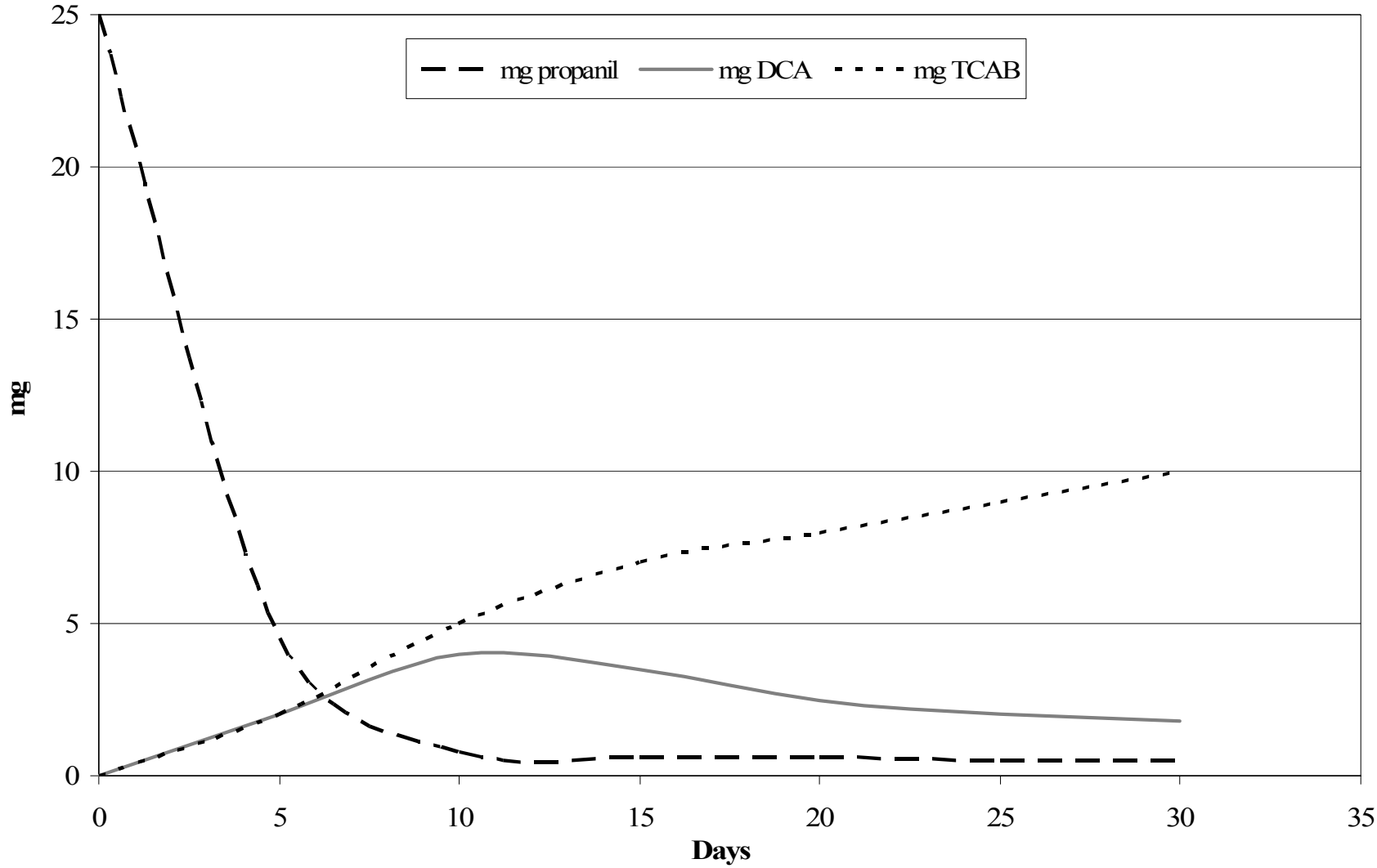

mg propanil
mg DCA
mg TCAB
25
20
15
10
5
0
mg
0
5
10
15
20
25
30
35
Days

Figure 2

Chapter 4. Sources, Detection, and Exposure of Tetrachloroazobenzenes in the Environment.

Abstract. 3,3',4,4'-Tetrachloroazobenzene (TCAB) and 3,3'4,4'-tetrachloroazoxybenzene (TCAOB) are contaminants produced during the synthesis of 3,4-dichloroaniline and dichloroanline-derived pesticides such as propanil. In addition to synthetic contamination, they are also produced as a result of the degradation of these same herbicides in soil. These compounds are structurally similar to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and act at the aryl hydrocarbon receptor to produce dioxin-like effects. Despite the widespread use of these herbicides and the known toxicity of TCAB and TCAOB, there has been only one report of quantitative determination of TCAB in animal tissue. We developed methods for rapid extraction, detection, and quantification of TCAB and TCAOB in environmental matrices using gas chromatography-electron capture detection (GC-ECD) and gas chromatography-mass spectrometer (GC-MS) operating in both electron impact and negative chemical ionization modes. The methods developed were reproducible, robust, and allowed high sample throughput. This methodology was applied to the analysis of passive sampling devices, mussels, fish, and sediment samples collected from areas adjacent to rice fields where propanil is applied as a primary herbicide. TCAB and TCAOB were not detected in tissues of fish collected at sites near high propanil use even though these fish had a highly induced ethoxyresorufin O-deethylase assay. The metabolism of these compounds in fish has never been investigated, so the lack of detection may not necessarily indicate that the fish were not exposed to TCAB and TCAOB.

Introduction

Dioxin-like compounds are an environmental concern due to their potential deleterious effects to humans and wildlife, causing weakened immune systems, developmental and reproductive problems, neurotoxicity, and cancer (1). They also are lipophilic and persistent, so these compounds tend to bioaccumulate and biomagnify in fatty tissues of biota and humans (1).

Dioxin-like compounds are a subset of chemicals that possess features common to 2,3,7,8-tetrachlorodibenzo-p-dioxin, TCDD, one of the most toxic chemicals known to man. They share similar physico-chemical properties and ultimately the exposure to this suite of compounds results in a battery of responses analogous to those observed following TCDD exposure (2). Many of the biological and toxicologic effects of dioxin-like compounds such as polychlorinated dibenzo-p-dioxins, dibenzofurans, biphenyls, and naphthalenes are mediated through the aryl hydrocarbon receptor (AhR) a ligand-activiated transcription factor (3−7). When AhR knockout mice were treated with TCDD and showed no signs of toxicity, this toxicological pathway was confirmed (6, 8). Because dioxin-like compounds bind to the AhR, exposure to these chemicals can be assayed by CYP1A-catalyzed O-deethylation of ethoxyresorufin (EROD), commonly known as EROD activity. The levels of cytochrome P450s will increase in the presence of dioxin-like chemicals. Due to this induction, the EROD assay is an extensively used, well-established *in vivo* biomarker that provides a rapid indication of exposure to toxic planar compounds. In addition to providing exposure information, EROD activity can be used to estimate the relative biological potency of chemicals when compared to TCDD (9, 10). However, it is important to note that an induced activity with a lack of analytical

detection does not always indicate lack of exposure. For example, some AhR agonists, such as certain polycyclic aromatic hydrocarbons (PAHs), are readily biotransformed and may cause enzyme induction while not being detected in the tissue (11).

In a recent study, fish samples were collected from the Mississippi River Basin adjacent to rice fields where propanil is applied as the primary herbicide (Figure 1). These fish had highly induced EROD activity in their livers. The fish were extracted and analyzed for the best characterized high affinity ligands for the AhR, but measured concentrations were insufficient to account for the observed dioxin-like activity. Previous reports have identified that the degradation of certain chloraniline herbicides can result in a series of synthetic reactions in the environment that can lead to products that cause induced EROD, 3,3',4,4'-Tetrachloroazobenzene (TCAB) and 3,3'4,4'-tetrachloroazoxybenzene (TCAOB) are products of these reactions (12, 13).

Environmental contamination with TCAB and TCAOB occurs by soil microbial transformation of herbicides containing the 3,4-dichloroaniline (3,4-DCA) moiety. However, TCAB and TCAOB are also formed as unwanted byproducts in the manufacture of 3,4- DCA and its herbicidal derivatives propanil, linuron, and diuron due to the conditions employed during their synthesis, which promote the condensation of two molecules of 3, 4-DCA to form TCAB and TCAOB (13). Propanil is widely used in the rice-growing regions of the United States (14) and TCAB has been detected in propanil at concentrations ranging from 0.1 to 2,900 μg/g (15−20).

The chemical structures and physico-chemical properties of propanil, 3,4-DCA, TCAB, TCAOB, and TCDD are shown in Figure 2 and Table 1. Certain properties listed in Table 1 were estimated using Molecular Modeling Pro (Molecular Modeling Pro ^{TM},

Fairfield, CA), a software program designed to calculate physical-chemical properties based on the given molecular structure (properties that were calculated with this approach are identified with an asterisk). At the time of this study no specific information was found regarding the environmental transport and fate of TCAB and TCAOB. However given the high lipophillicity and the low water solubility of these compounds, it indicates that TCAB and TCAOB may have an increased potential to accumulate in soil, sediment, or living organisms.

TCAB and TCAOB are confirmed structural analogs to TCDD (Figure 2), they bind to the AhR with a specific binding affinity one fifth and one-third to one-tenth (respectively) that of TCDD (21). Typical dioxin-like effects have been reported in rodents after exposure to TCAB and TCAOB, which include body weight loss, thymic atrophy, hepatotoxicity, anemia, developmental toxicity, chloracne-like lesions, and an induction of cytochrome P4501A1 (22−28).

Although TCAB and TCAOB are considered to be dioxin-like compounds, there is limited research concerning how exposure to these dioxin-like compounds may impact the environment and humans. To our knowledge, there is only one report of the quantitative determination of TCAB in biological tissues and there have been no reports of the quantitative determination of TCAOB in biological samples. This lack of knowledge is what led to the present studies. Fish exhibiting high EROD activity were collected in areas where chloraniline herbicides were used, so we began an investigation into whether TCAB and TCAOB might be causing this biomarker response. The objective of this study was to develop analytical methodology to analyze TCAB and TCAOB in environmental and biological matrices and determine if TCAB and TCAOB

were causing this increased EROD activity. This paper demonstrates analytical procedures for the detection of both TCAB and TCAOB that offer the sensitivity and reproducibility needed for the detection of these potentially harmful environmental contaminants.

Experimental

Chemicals and Reagents. 3,3',4,4'-Tetrachloroazobenzene (TCAB) and 3,3'4,4'-tetrachloroazoxybenzene (TCAOB) were purchased from ChemService Inc. (West Chester, PA). Surrogate internal standards were purchased from AccuStandard Inc. (New Haven, CT). A standard mix of TCAB and TCAOB prepared in hexane was used as the matrix spiking solution for all methods development. 2,4,5,6-tetrachloro-*m*-xylene (TCMX) used as an internal standard was purchased from ChemService Inc. (West Chester, PA). All solvents were Ultra Resi-Analyzed grade (J.T. Baker Inc., Phillipsburg, NJ). To avoid contamination of samples all glassware, aluminum foil, sample collection tools and sodium sulfate were baked at 300°C overnight prior to use or solvent rinsed three times each with acetone, methylene chloride (DCM), and hexane (>95%).

Sample Preparation. For methods development, uncontaminated tissue (fish and mussel), sediment, and DI water were used. Samples were fortified with TCAB and TCAOB. During preparation of the samples, surrogate internal standards (SIS), 4,4'-dibromooctafluorobiphenyl (DBOFB), Cl5(112), Cl8(197), acenapthene-d10, and chyrsene-d12 were spiked into the each of the samples to follow analyte recovery during extractions.

Sample Extraction. Tissue and sediment extraction. Tissue samples were extracted with 60 mL of acetone-dichloromethane (DCM) (1:1) mixture. The ratio of sample weight to solvent was 1:40. Samples were extracted for 10-12 hours (overnight) on a shaker table, centrifuged, and the extract was collected. The shaker-extraction was repeated twice with the exception of shaking for only 3-4 hours. Extracts were concentrated to a volume of 4 mL for the fish tissue and 1 mL for the mussel tissue. The ideal volume for the operating column on the lipid removal system is 1 mL however due to increased lipid saturation of the contaminant-free fish tissue we were only able to reach 4 mL. Extracts were passed through a gel permeation column (GPC) to separate lipids. Lipid fraction was determined by collecting the lipid, weighing, and evaporating. The 25 mL extract containing the analytes of interest was then concentrated to 1.0 mL and then solvent exchanged 3 times to 95% hexane. The final volume was 1.5 mL. After conditioning a 3-gram silica column with 40 mL of 95% hexane, the extract was loaded onto the column. Elution was carried out with 12 mL of hexane (F1) and 15 mL of hexane-DCM (1:1) mixture (F2). The sample was reduced to 1 mL using nitrogen and then solvent exchanged three times to 95% hexane to yield a final volume of 1 mL. Sediment was extracted by the same method used for fish except the lipid separation step was omitted.

Water extraction. Water samples were extracted using a DCM liquid-liquid extraction technique. Extracts were collected and concentrated to a volume of approximately 1 mL and then solvent exchanged three times to 95% hexane under a gently stream of nitrogen to yield a final volume of approximately 1 mL.

Instrumental Analysis and Quality Control. TCAB and TCAOB were analyzed using a HP6890 GC equipped with electronic pressure control and either an HP 5973 MSD or an electron capture detector (GC-ECD). For GC-ECD analysis, extracts were injected in the pulsed splitless mode and separated on a 30 m x 0.32 mm ZB-50 (0.25 μm film thickness) fused silica capillary (Phenomenex, Inc. Torrance, CA). The temperature was programmed as follows: Initial temperature 80 °C for 1.0 min, 20 °C/min to 250 °C, 6 °C/min to 290 °C, held for 7 min. The injector and detector were set at 250 and 300 °C, respectively. The total analysis was 21.17 minutes. Helium was used as a carrier gas at 6.06 psi. TCAB and TCAOB were analyzed using MS operating in both negative chemical ionization (GC-NCI-MS) and electron ionization (GC-EI-MS) modes. The GC was equipped with an RTX-5MS (Restek, 30 mm x 0.25mm x 0.25μm film thickness) with a 5 m Integra-Guard column. Helium was the carrier gas and high purity methane was used as the reagent gas in NCI mode. Oven temperature was as follows: 80 °C for 1 minute (EI) and 50 °C for 1 minute (NCI), 20.00 °C/min to 250°C, 6.00 °C/min to 287 °C, held for 1 minute. The total analysis time was 16.67 minutes. The injector and detector were set at 250 and 300 °C, respectively. The ion source temperature was optimized at 140 °C. Selected ion monitoring (SIM) was used for the analysis.

Response factors were generated using a four or five-point calibration curve, and response was monitored using the mid-level calibration standard. The relative percent difference between the mid-level check was less than 15% for all analytes. Sample concentrations were calculated using the generated response factors and were based on the known amount of TCMX injected. Data quality was assessed using procedural blanks, replicate analyses, matrix spikes and SIS. SIS recoveries were > 60%. No corrections for recovery were made. Method blanks were not detected. Quality assurance of the methods developed was assessed by measuring parameters such as linearity, sensitivity, repeatability, reproducibility, recovery (including surrogates and target analytes), and analysis time and labor.

Field sample collection and processing. The field sampling for this experiment was performed by the USGS, Columbia Environmental Research Center located in Columbia, MO. Samples were collected for the Biomonitoring of Environmental Status and Trends (BEST) Program: Environmental Contaminants and Their Effects on Fish in the Mississippi River Basin (29).

Site description. Draining all or parts of 32 states and 2,350 square miles before it finally reaches the Gulf of Mexico, the Mississippi River Basin (MRB) plays an integral role in this nation's crop production and provides habitat for many fish, birds, mussels, and amphibian species. It is by far the largest and most extensively farmed region in the nation. It contains approximately 65% of the total harvested cropland in the US, producing about 80 percent of the corn and soybeans, and much of the cotton, rice,

sorghum, and wheat (30−32). In order to increase crop yields, an estimated two-thirds of all pesticides used for agriculture in US, are applied to the cropland throughout this area (33). The intense use of pesticides in this area is a concern to the aquatic life and the 18 million people that rely on the basin for drinking water. For this study, sites were selected within the MRB that corresponded to high propanil use (Figure 1, area is circled) and areas where fish were collected that had an induced EROD.

Passive Sampling Devices. Low density polyethylene (LDPE) strips were deployed along the MRB and surrounding areas adjacent to areas where propanil, linuron and diuron are applied as the primary herbicides on adjacent agriculture fields (Figure 1). The passive sampling devices (PSDs) consisted of 75 µm thick 'virgin' LDPE tubing (Brentwood Plastics, Inc., St. Louis, MO), cut into 10.5 cm X 25 cm strips. The LDPE tubing was extracted with hexane for 48 h prior to use. PSDs were collected on days 0, 0.042, 0.166, 0.458, 1, 2, 3, 5, 10, and 20. Water samples were collected on the same days as the LDPE and extracted immediately as described above.

The PSDs were stored at -20 °C until extraction. Field deployed LDPE strips were extracted following methods detailed by Leullen and Shea (2002) omitting the silica column clean-up (34). Briefly, LDPE strips were cut into 2 cm^2 pieces and serially extracted three times in DCM on a shaker table. Extracts were reduced in volume, GPC was used to remove polyethylene waxes, extracts were solvent exchanged and then analyzed using the appropriate instrumentation.

Mussel, Fish, Water, Sediment, and Soil Sampling. Caged mussel and fish samples were collected on day 16 of the field sampling, at the same time water, sediment and soil were collected as described in Heltsley and Shea (in prep). Samples were extracted as described above.

Results and Discussion

Methods Development and Validation. GC-ECD is one of the most commonly used techniques for analyzing halogenated compounds in environmental samples, yielding method detection limits (MDLs) in the low or sub nanogram-per-gram range. The target compounds for this study were resolved in less than 22 minutes by employing the GC conditions described above. The extraction methods demonstrated excellent selectivity and sensitivity. There were no visible interferences indicating the clean-up procedures employed in each extraction method were effective.

Analysis of tetrachloroazobenzenes using GC-MS operating in both electron ionization and negative chemical ionization modes offers two viable alternatives to using ECD. The obvious advantage of using MS over ECD is the additional structural information provided in the mass spectrum. Electron ionization (EI) is a well-understood and commonly used ionization technique with a wealth of information provided in the mass spectra libraries for various compounds. However, in the case of tetrachloroazobenzenes, EI proved to result in over-fragmentation of the analytes. The percent relative standard deviations (% RSD) acquired for the calibration standards was greater than 25%. Therefore, based on the high RSD this method was used solely for confirmation purposes. In contrast, negative chemical ionization (NCI), a softer

ionization technique, provided less fragmentation than EI and had lower % RSDs with detection limits similar to that of the ECD. Negative chemical ionization has a greater sensitivity for halogenated, environmentally relevant compounds, however fragmentation is less documented than EI and is not represented in mass spectra libraries. For tetrachloroazobenzenes, both the ECD and GC-MS operating in NCI mode provided the sensitivity needed to detect these compounds at toxicologically significant concentrations.

The methods were checked for linearity using five different concentrations of TCAB and TCAOB in the range of 0.02 – 1 ng/μL for ECD and 0.02 – 10 ng/μL for NCI-MS. The accuracy of the measurements is expressed as the relative standard deviation (RSD), the % RSD for each of the target analytes was less than 4% for each method Linear regression analysis was performed using no weighting factors, and the correlation coefficient was > 0.99 for both methods.

Tissue Extraction. Both contaminant-free wet fish and mussels were extracted in this experiment. The MDL, average recoveries, and standard deviations are shown in Table 2 and Figure 3 (respectively). The method used in this paper has lower MDLs for TCAB than an extraction method found in the literature for biological tissues, which was 1 ppb (35) for both ECD and NCI. The average recoveries for GC-ECD in tissue ranged from 65 – 70%. It should be noted that recoveries for this method when using DCM alone as the extracting solvent ranged from 30 – 45% (data not published). The addition of acetone greatly improved the target analytes recovery in tissue. The standard deviations

of triplicate analysis for both the fish and the mussel were less than 6% for the ECD, indicating good accuracy and robustness. The coefficients of variation were less than 8%.

The recoveries calculated for tissue using GC-NCI-MS ranged from 35 – 58%. The reasons for these lower recoveries are currently being investigated. Potential problems could be associated with the tissue matrix itself and the ionization method. This conjecture is supported by the fact that surrogate and target analyte recovery in the replicate blank spikes (solvent plus surrogates and target analytes) were greater than 60% (average 72%). Negative chemical ionization also had increased standard deviations associated with the fish tissue recoveries. The mussel tissue standard deviations were less than 3%. This work demonstrates for the first time the efficient extraction of TCAOB from biological samples as well as the first attempt at using GC-NCI-MS to analyze these dioxin-like compounds.

Sediment Extraction. The recoveries of TCAB and TCAOB in the sediment ranged from 71 – 76 % for both the ECD and MS. These results were more on par with expectations because the same extracts are being analyzed by both of the detectors. The relative standard deviations of triplicate analysis were less than 15% for both instruments. The coefficients of variation ranged from 3 – 20%. The extraction procedure employed proved to be proficient in extracting TCAB and TCAOB from the wet sediment. The MDL values for sediment are the highest of all four matrices (Table 2). However, this is not uncommon because sediment in general has a higher background than other matrices.

Water Extraction. DI water was extracted using a liquid-liquid partitioning technique in this experiment. The average recovery of TCAB for the ECD was 85% with a standard deviation of 6% and a coefficient of variation of 7%. TCAOB had lower recoveries in the water than observed in the other matrices. The average recovery was 50% with a standard deviation of 9% and a coefficient of variation of 18%. Negative chemical ionization recoveries for both TCAB and TCAOB were above 100% with high standard deviations. However, the target analytes were not alone in this high recovery, the surrogates experienced similar patterns with recoveries > 120% with standard deviations above 30%. This indicates that there was interference potentially occurring with all the target compounds resulting in an additive effect. Because the additive effect occurred in all the samples extracted, this indicates that the source of this problem was coming from something that was commonly used in each extraction, but the source has yet to be identified.

Field Sampling. The developed methods, ECD as a primary method and NCI-MS as confirmation, were applied to the analysis of fish, soil, mussel, sediment, PSDs, and water that were collected in areas where propanil is applied in adjacent rice fields. Previous analysis done by the USGS indicated that fish collected in this same area had an induced EROD activity but the AhR active ligand was unknown. The fish extracts were analyzed for TCAB and TCAOB. Neither of the analytical methods developed in this paper indicated the presence of the targeted dioxin-like compounds in the fish tissue. However, it should be noted that fish do possess a low activity of monooxygenase enzymes that allow them to have a limited capacity to metabolize lower chlorinated such

di-tetra PCBs and potentially TCAB and TCAOB (36). Therefore, the lack of detection may not necessary mean these fish were not exposed because the metabolism of these compounds in fish has not been investigated. Upon determining that the fish collected had no detectable residues of TCAB, soil in the surrounding areas where the fish were collected was extracted and analyzed for TCAB. Figure 4 is a graphical representation of the positive identification of TCAB in the soil. Therefore, the soil surrounding the area where the fish had an induced EROD activity, had trace concentrations of TCAB.

In an attempt to obtain preliminary information concerning the potential contamination of TCAB at sites where EROD-induced fish were collected, mussels (*Unionacea*) were deployed/ collected, extracted and analyzed for TCAB. Bivalves have been used for years as biomonitoring organisms to establish status and trends of exposure, dose and response contaminants. Due to their limited ability to metabolize HOCs, mussels make good candidates to evaluate the contamination of aquatic ecosystems with environmental pollutants. The mussels collected from this area had detectable amounts of TCAB in their tissue. Although the concentrations were low, they correlated well with the concentrations of TCAB detected in sediment and water that was collected in the same area (Figure 5). This study represents the first to evaluate TCAB across matrices in an attempt to establish a trend of potential exposure.

Information concerning cumulative dose concentrations in water is a fundamental aspect of an ecological risk assessment. The application of PSDs stems from the limitations associated with traditional sampling approaches commonly used for monitoring contaminants in aquatic ecosystems such as, grab sampling. The data gathered with conventional techniques only represents the moment the water sample was

collected therefore, detection of episodic events may not be represented and average concentrations over time involve extensive amounts of time and labor. The advent of passive sampling techniques have offered a new approach to contaminant assessment in that they passively concentrate waterborne contaminants over time providing a time-weighted average concentration of exposure.

In order to better estimate a time-integrated measurement of TCAB in water, passive sampling devices (PSDs) were deployed in the same area where the fish were collected. In 1967 Bartha and Pramer were the first scientists to examine the conversion of propanil to 3,4-DCA and TCAB in soil (12). This phenomenon to our knowledge has not been examined in water as it is depicted in Figure 6. This figure illustrates the same trend that Bartha and Pramer saw over 40 years ago, propanil is rapidly degraded to nondetectable concentrations in the sampled water within two days. However, as the propanil begins to degrade, detectable concentrations of TCAB are present in the LDPE strips as early half a day. The time-integrated mean concentration for TCAB was approximately 0.9 ng/L. Future use of PSDs at sites potentially contaminated with TCAB may provide more accurate information regarding an estimate of the time-weighted average concentration with much less effort than required for traditional techniques.

Conclusions. The efficiency of simple extraction techniques for the determination of TCAB and TCAOB in tissue, sediment, and water was investigated. For each matrix the recoveries of TCAB ranged from 65-84% and 50-71% for TCAOB utilizing GC-ECD. This work demonstrates the first experiment to achieve this low of a MDL as well as the first presentation of TCAOB extraction in biological and water samples. The excellent

method performance was made evident via the recovery efficiency, MDL, and the reproducibility, all of which indicate that these methods would be useful in routine monitoring of TCAB and TCAOB in field samples. Method development for GC-NCI-MS and GC-EI-MS needs to be further investigated however, both of these methods show promising results with similar detection limits to that of the ECD, while providing structural information about the target analytes.

We were unable to demonstrate detectable levels of TCAB or TCAOB in fish samples from the field. However, sites with high EROD activity in collected fish had detectable concentrations of TCAB in the soil, sediment and water. Mussels deployed in areas surrounding high propanil usage had detectable concentration of TCAB that correlated well with sediment and water. Future work on this project will be to examine the induction of EROD activity in fish upon exposure to these potentially toxic compounds and their ability to metabolize them. Additional TCAB and TCAOB measurements in the field where these herbicides are applied would provide additional information needed to model these compounds in the environment and accurately predict their potential exposure to aquatic ecosystems.

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Table 1. Physicochemical properties of Propanil, 3,4-DCA, TCAB, TCAOB, and TCDD.

Compound	Propanil	3,4-DCA	TCAB	TCAOB	TCDD
Formula	$C_9H_9Cl_2NO$	$C_6H_5Cl_2N$	$C_{12}H_6C1_4N_2$	$C_{12}H_6C1_4N_2O$	$C_{12}H_4Cl_4O_2$
Molecular Weight (g/mol)	218	162	320	336	322
Description	white crystals	light brown crystals	orange crystals	yellowish-orange crystals	
CAS Number	709-98-8	95-76-1	14047-09-7	21232-47-3	1746016
Melting point (^{0}C)	92-93	72	158	142	305
Boiling point (^{0}C)	>100	272	384.7	*387.6	900
Vapor Pressure (Pa @ 25 ^{0}C)	2.60×10^{-5}	0.84	$*7.37 \times 10^{-9}$	$*5.59 \times 10^{-8}$	2.00×10^{-7}
Water solubility (mg/L @ 25 ^{0}C)	225	none	1	*1	0.483
Log Kow	3.1	2.7	6.7	*6.8	6.8
field half-live	1-3 days		no data found	no data found	12 years
water half-live	2 days		no data found	no data found	1.5 years

Table 2. Method detection limits for TCAB and TCAOB using GC-ECD, GC-NCI-MS (SIM), and GC-EI-MS (SIM) for fish (ng/g), mussel (ng/g), sediment (ng/g), and water (ng/L).

Method	Target Compound	Fish (ng/g)	Mussel (ng/g)	Sediment (ng/g)	Water (ng/L)
GC-ECD	TCAB	0.31	0.04	2.98	0.01
	TCAOB	0.10	0.07	1.61	0.01
GC-NCI-MS (SIM)	TCAB	0.03	0.08	0.78	0.01
	TCAOB	0.03	0.09	0.65	0.07
GC-EI-MS (SIM)	TCAB	5.60	4.90	5.23	0.07
	TCAOB	2.91	5.35	1.17	0.02

Table 3. Percent recovery of TCAB and TCAOB in fish (ng/g), mussel (ng/g), sediment (ng/g), and water (ng/L) using GC-ECD and GC-NCI-MS.

Method	Target Compounds	Fish (ng/g) ± SD	Mussel (ng/g) ± SD	Sediment (ng/g) ± SD	Water (ng/L) ± SD
GC-ECD	TCAB	67.0 ± 4.0	69.8 ± 3.2	75.8 ± 3.2	83.9 ± 6.2
	TCAOB	64.9 ± 5.0	69.7 ± 3.0	71.0 ± 2.0	50.3 ± 9.1
GC-NCI-MS (SIM)	TCAB	47.5 ± 23.7	57.2 ± 2.4	72.4 ± 15.3	146.2 ± 55.9
	TCAOB	34.5 ± 17.3	47.5 ± 2.7	71.6 ± 7.9	114.7 ± 44.5

FIGURE LEGENDS

Figure 1. Sampling locations for the USGS Biomonitoring of Environmental Status and Trends (BEST) Program: Environmental Contaminants and Their Effects on Fish in the Mississippi River Basin project. The circled area denotes locations where samples were collected for this study. Fish, mussel, soil, sediment, PSD, and water samples were collected from the circled area where propanil is applied as the primary herbicide.

Figure 2. Chemical structurs of propanil, 3,4-DCA, TCAB, TCAOB, and TCDD. Propanil is a chloraniline herbicide which can break down to form 3,4-DCA. 3,4-DCA has been shown to condense to form TCAB and TCAOB in the environment. TCAB and TCAOB are structural analogs to TCDD, the most potent rat toxicant known to man.

Figure 3. Percent recovery for TCAB and TCAOB in fish (ng/g), mussel (ng/g), sediment (ng/g), and water (ng/L) using GC-ECD and GC-NCI-MS (SIM). Bars represent standard deviation of three or more samples extracted.

Figure 4. EROD activity (mol/mg/min) in fish collected from BEST and NCSU sampling locations where propanil is applied as the primary herbicide. TCAB was detected in the soil where chloraniline herbicides are applied.

Figure 5. Relationship between TCAB detected in mussel, sediment, and water at sites where propanil is applied in surrounding areas.

Figure 6. Estimated TCAB water concentrations (ng/L) from LDPE strips (♦) compared to those measured in grab samples (ng/L) (■). Propanil is measured in the water (ng/L) (▲) but degrades rapidly. As propanil degrades TCAB is detected in PSDs and water samples. Estimated average concentration of TCAB is 0.9 ng/L.

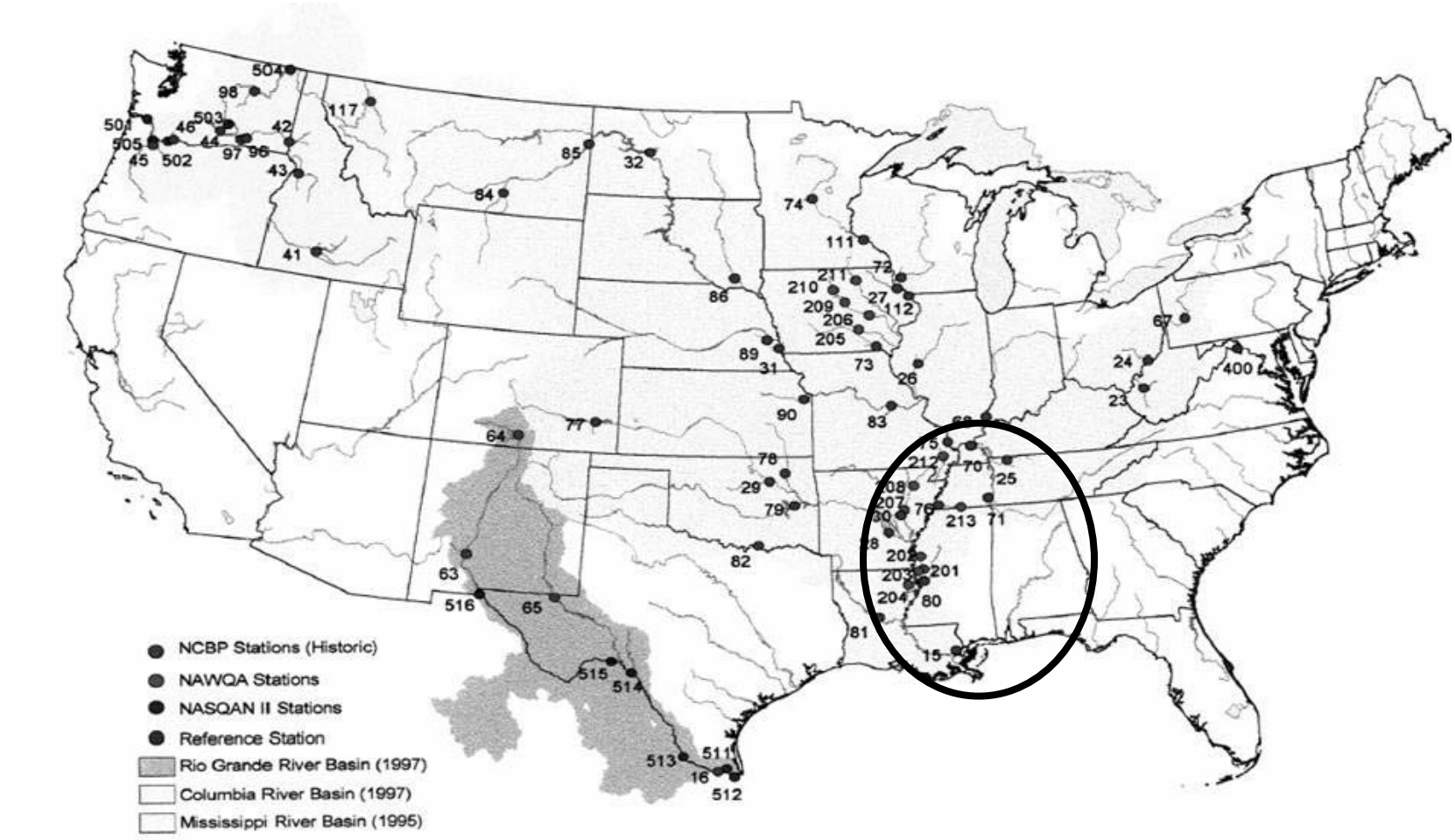
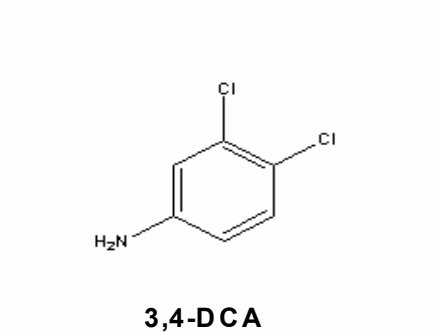
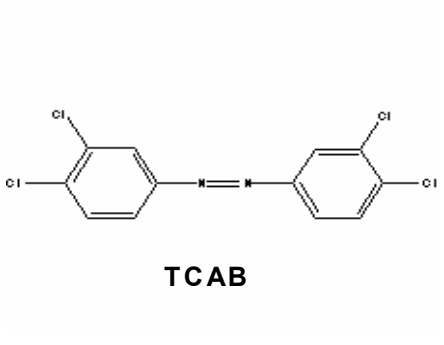

NCBP Stations (Historic)
NAWQA Stations
NASQAN II Stations
Reference Station
Rio Grande River Basin (1997)
Columbia River Basin (1997)
Mississippi River Basin (1995)

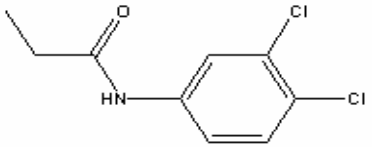
Figure 1



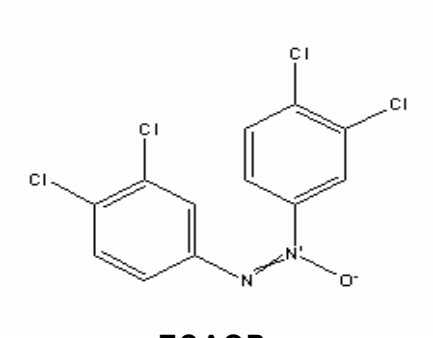
3,4-DCA



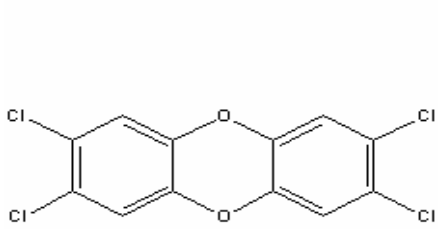
TCAB



Propanil



TCAOB



TCDD

Figure 2.

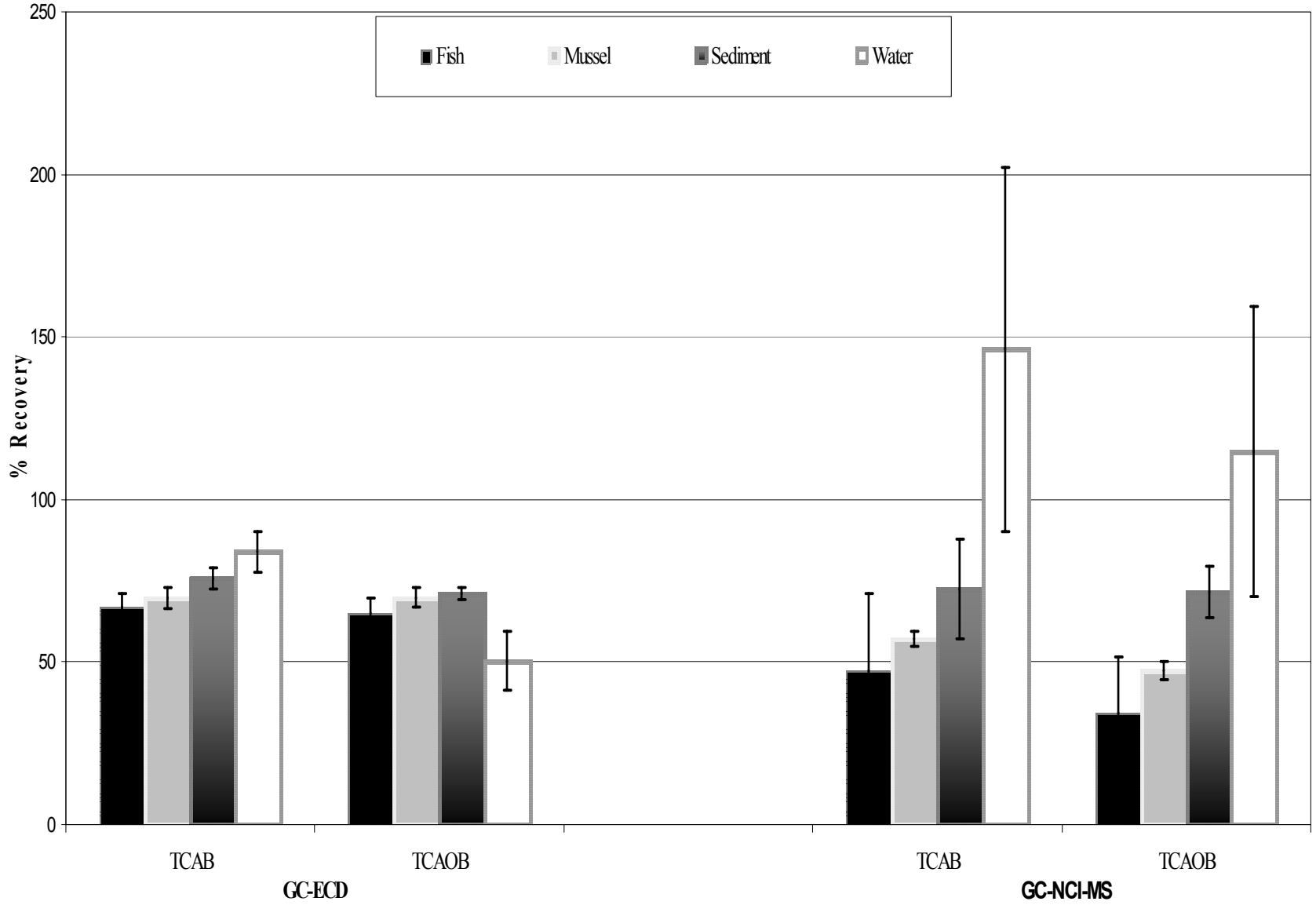

250
200
150
100
50
0
% Recovery
Fish
Mussel
Sediment
Water
TCAB
TCAOB
TCAB
TCAOB
GC-ECD
GC-NCI-MS

Figure 3

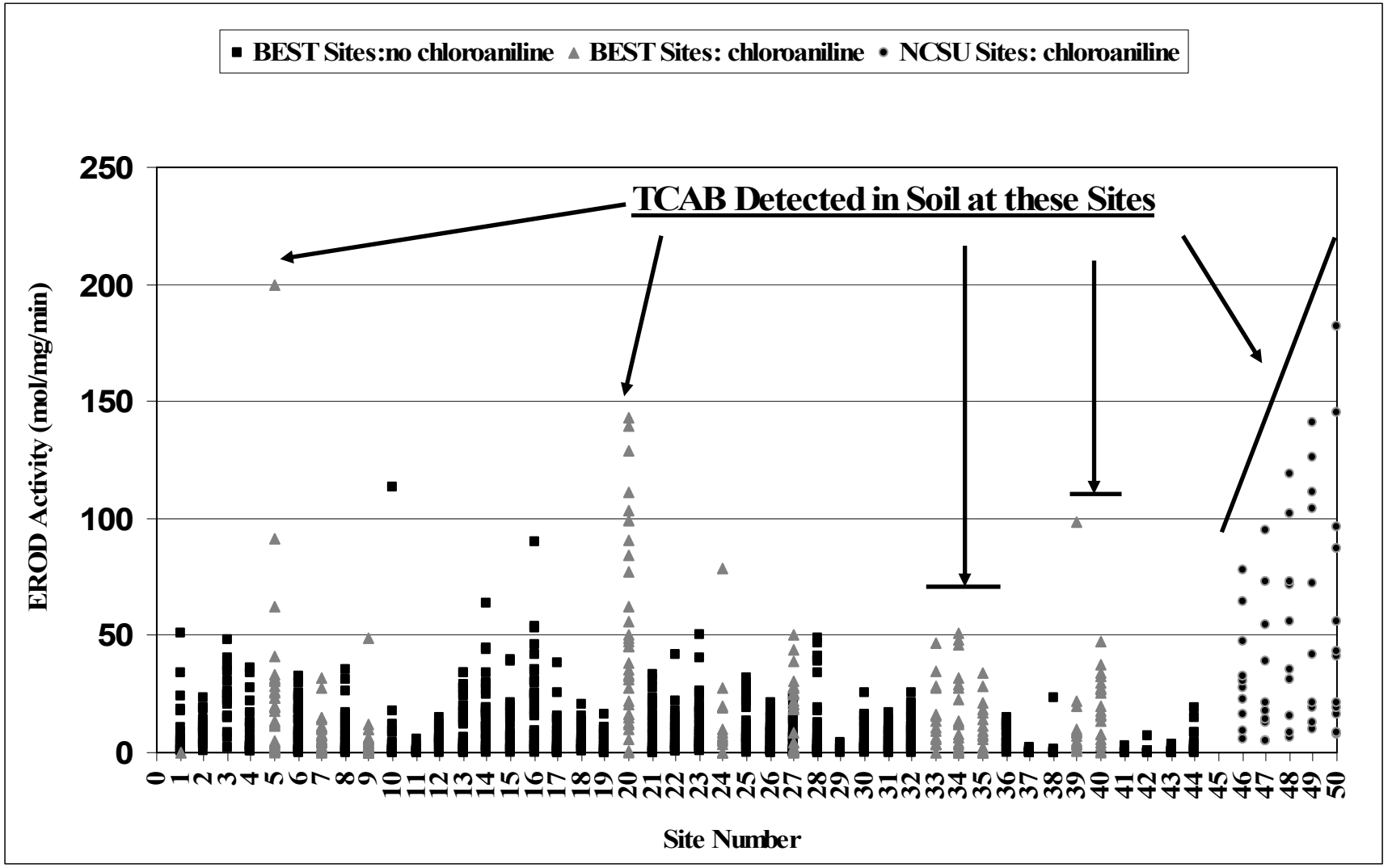

BEST Sites:no chloroaniline
BEST Sites: chloroaniline
NCSU Sites: chloroaniline
TCAB Detected in Soil at these Sites
EROD Activity (mol/mg/min)
250
200
150
100
50
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50
Site Number

Figure 4

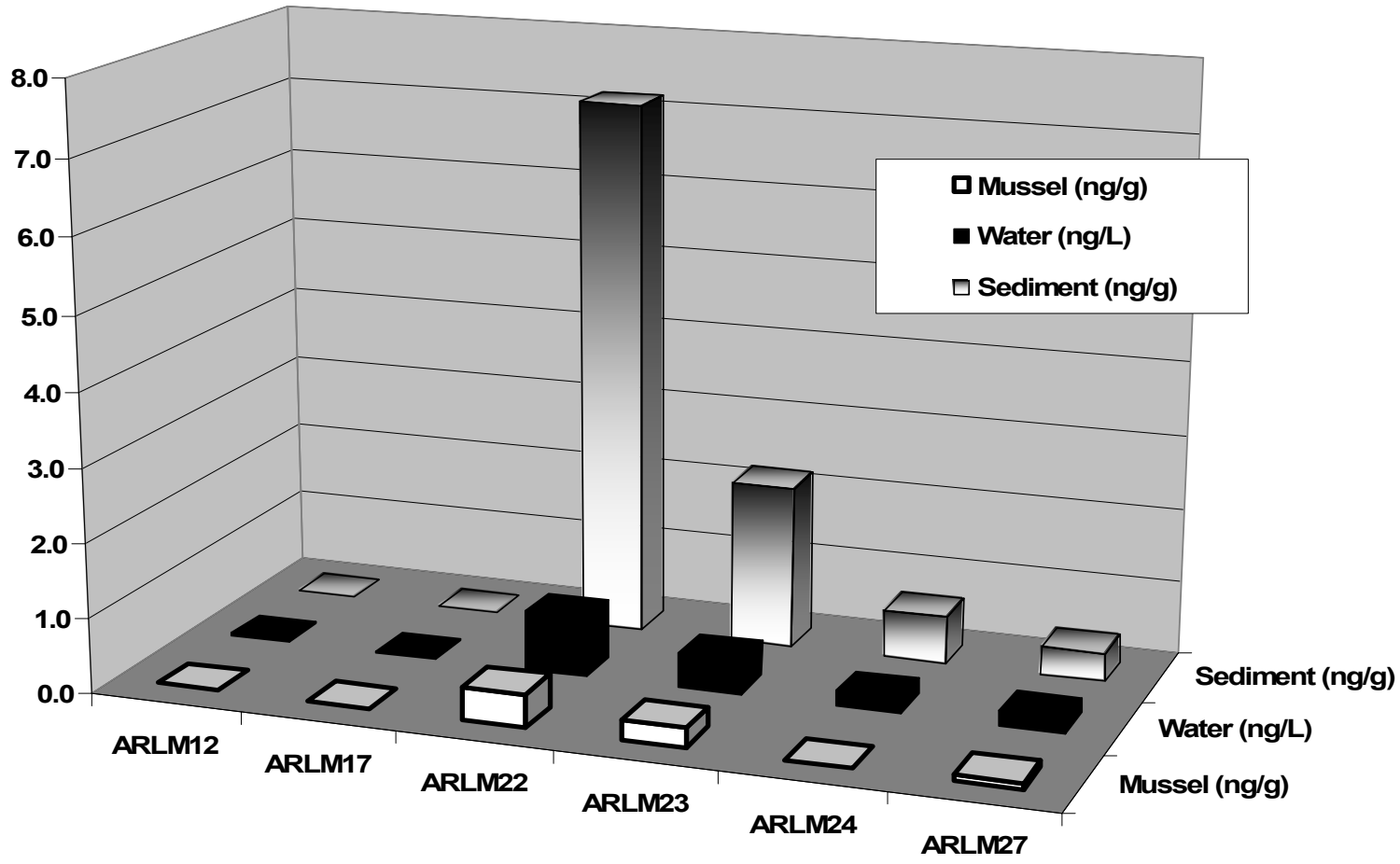

8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Mussel (ng/g)
Water (ng/L)
Sediment (ng/g)
ARLM12
ARLM17
ARLM22
ARLM23
ARLM24
ARLM27
Sediment (ng/g)
Water (ng/L)
Mussel (ng/g)

Figure 5

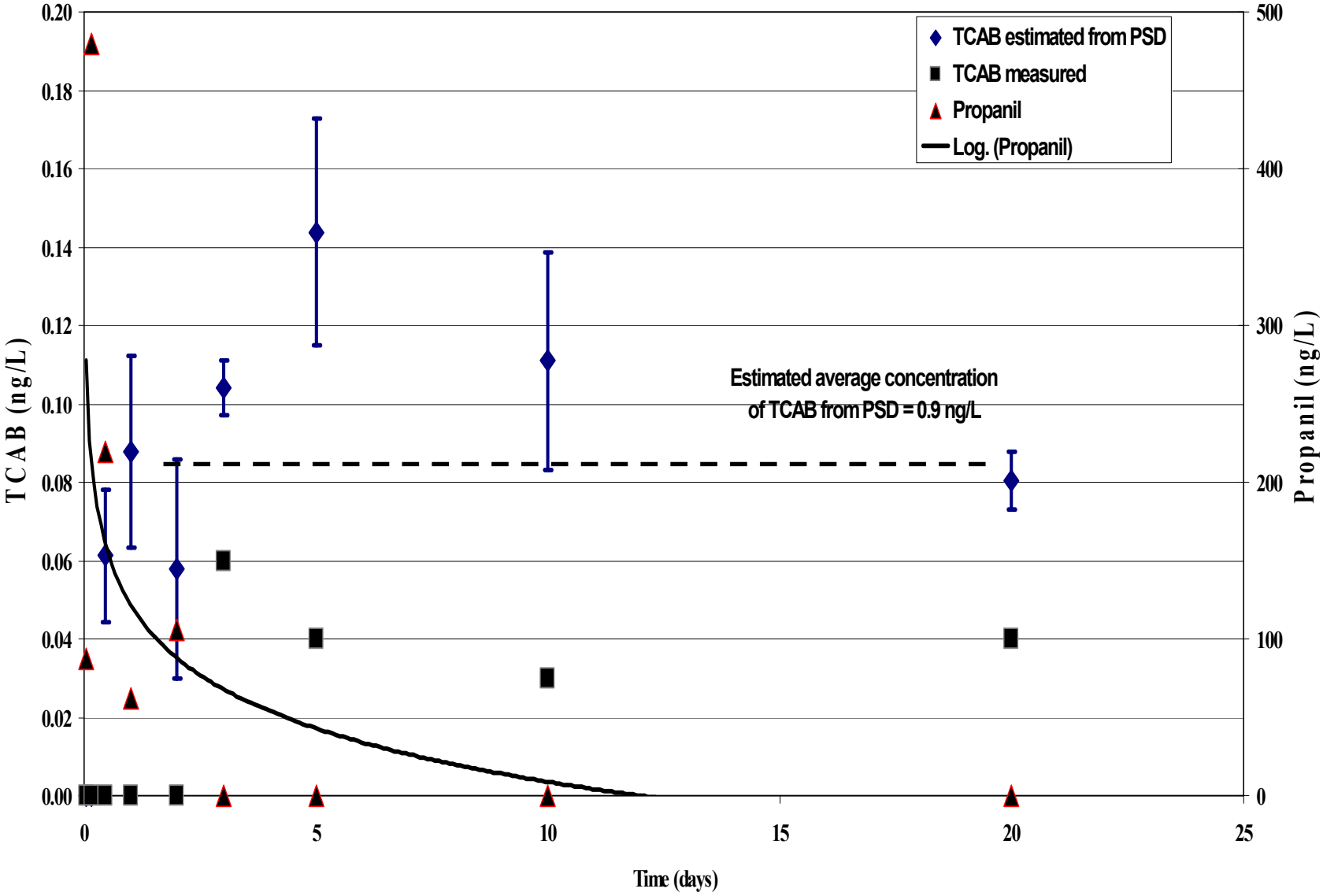

TCAB estimated from PSD
TCAB measured
Propanil
Log. (Propanil)
Estimated average concentration
of TCAB from PSD = 0.9 ng/L
TCAB (ng/L)
Propanil (ng/L)
Time (days)
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
500
400
300
200
100
0
0
5
10
15
20
25

Figure 6

Chapter 5. **Comparison of a Non-lethal Fish Tissue Sampling Technique to Mobile and Stationary Passive Sampling Devices for Assessing Organic Contaminants**

REBECCA M. HELTSLEY,*

W. GREGORY COPE,

DAMIAN SHEA,

North Carolina State University, Department of Environmental and Molecular Toxicology, Box 7633, Raleigh, North Carolina 27695-7633

THOMAS J. KWAK, AND

EDWARD G. MALINDZAK

U.S. Geological Survey, North Carolina Cooperative Fish and Wildlife Research Unit, Department of Zoology, Box 7617, North Carolina State University, Raleigh, North Carolina 27695-7617

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* Corresponding author telephone: 919-515-1960; fax: 919-515-7169; e-mail: rmheltsl@unity.ncsu.edu.

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Abstract

As concerns mount over the human health risks associated with consuming fish with high concentrations of persistent organic pollutants, there exists a need to better evaluate body burdens without the sacrifice of thousands of fish. In this paper, we investigated two novel methods to aid in monitoring organic contaminants that are a concern for both fish and human health. Adipose fin clips, commonly removed for mark re-capture studies, were evaluated as a non-lethal sampling technique to estimate concentrations of PCBs and OCPs compared to those found in fillets of flathead catfish *Pylodictis olivaris*, which require killing the fish to obtain the tissue. We also examined the prospect of using polydimethylsiloxane (PDMS) as a mobile passive sampling device (PSD) attached to the catfish for assessing location specific exposure of an organism to waterborne contaminants. Results of our study demonstrated for the first time that adipose fin concentrations were highly correlated (r^2 of 0.77) with fillet concentrations. These results suggest that the adipose fin can accurately estimate tissue concentrations without causing mortality. Mobile passive samplers used for the first time were capable of accurately estimating ultra-trace concentrations of waterborne PCBs and OCPs without any signs of harming the fish indicating there appears to be no physical barriers with the use of mobile samplers on fish.

Introduction

The tendency for fish and other aquatic organisms to concentrate hydrophobic organic contaminants (HOCs) in their adipose tissues at relatively high levels compared to those in the surrounding environment has long been recognized by environmental researchers. This phenomenon commonly known as bioaccumulation is a critical process, possibly a prerequisite, in a series of events that progresses to a toxic response in organisms to certain environmental pollutants. Measurements associated with bioaccumulation of HOCs, for example exposure concentrations and body burdens, offer valuable information that can contribute to human health and ecological risk assessments. However, obtaining this data is often labor intensive.

There are many limitations associated with sampling and measuring ultra-trace concentrations in surface waters and improvements to traditional methods such as grab sampling, have been met with limited success. Passive sampling devices (PSDs) offer a relatively new and less labor intensive approach to monitoring waterborne contaminants. PSDs function by passively accumulating HOCs in a polymeric (lipid-like) membrane in a time-integrated fashion. The most commonly used function of PSDs is to provide an estimate of exposure concentrations in water. This process requires additional kinetic information (uptake rate constants) that can be determined in controlled laboratory or field settings (1−3). Uptake rate constants have been determined for many environmental pollutants that have known toxicological consequences in aquatic organisms, including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and organochlorine pesticides (OCPs) for passive samplers such as, semi-permeable membrane devices (SPMDs) and

chemical exposure dosimeters (CEDs) (1, 2, 4, 5−7). Sampling rates can then be used to estimate freely dissolved contaminant concentrations in the field with the following equation

$$R_{S\text{-}cal} = N_{PSD} / C_W * t \quad (1)$$

where $R_{S\text{-}cal}$ is the effective sampling rate (Ld^{-1}) determined in calibration studies, N_{PSD} is the amount (ng) of chemical sorbed by the passive sampling device, C_W is the concentration of chemical in water (ngL^{-1}), and t is period of time the PSD is deployed (days) (2).

It has been established that uptake rates of PSDs are affected by environmental conditions, such as temperature, flow, and biofouling of the surface of the PSD membrane which in turn can effect the estimation of ambient water concentrations (3, 8). Because each sampling location is unique, it is impractical to determine uptake rates for each exposure scenario. Current applications of PSDs have used performance reference compounds (PRCs) to account for these environmental influences. PRCs are analytically, non-interfering compounds with water solubilities (K_{ow}s) similar to the target compounds of interest, which are added to the PSD prior to deployment. Elimination rates (k_e) of PRCs from PSD, determined in laboratory calibration experiments, are proportional to the uptake of target compounds and consequently the rate of PRC loss can be used to adjust sampling rates accordingly (3). The following equation developed by Huckins *et al*. (2002) can be utilized to determine site-specific elimination rates of PRC; this equation is used when PRC concentrations are measured prior to deployment and at the end of an exposure study

$$k_{e\text{-}PRC\text{-}f} = \ln (C_{PSD\text{-}0} / C_{PSD}) / t \quad (2)$$

where $k_{e\text{-}PRC\text{-}f}$ is the elimination rate of the PRC from the PSD in the field (d^{-1}), $C_{PSD\text{-}0}$ is the amount of PRC in the PSD at time zero (ng), C_{PSD} is the amount of PRC remaining in the PSD at the end of an exposure (ng), and t is the time period of deployment (days). The site-

specific PRC elimination rate can be used to calculate an exposure adjustment factor (EAF) defined by the following equation

$$EAF = k_{e\text{-}PRC\text{-}f} / k_{e\text{-}PRC\text{-}cal} \quad (3)$$

where EAF is the exposure adjustment factor, and $k_{e\text{-}PRC\text{-}cal}$ is the elimination rate calculated for PRCs in controlled laboratory settings (d^{-1}). The EAF is then used to determine the site-specific sampling rate by the following equation

$$R_{S\text{-}f} = EAF\ (R_{S\text{-}cal}) \quad (4)$$

where $R_{S\text{-}f}$ is the *in situ* PSD sampling rate (Ld^{-1}). The $R_{S\text{-}f}$ is then used to calculate the concentration in the water estimated from PSDs using equation 1 rearranged to solve for C_w. The application of PRCs to adjust sampling rates not only reduces the amount of controlled laboratory studies needed to use PSDs but also improves the overall error associated with estimating water concentrations from PSDs (3).

Because PSDs passively accumulate contaminants into lipid-like phases, not only do they function as a means to estimate environmental exposure concentrations, but they also serve as a biomimetic technique by mimicking the uptake of HOCs by aquatic organisms from the water phase. Many studies have examined the potential of using SPMDs to correlate uptake of HOCs with finfish (1, 9). The strength of this correlation depends upon the species examined, owing to the fact that different species of fish will be unique in their ability to metabolize xenobiotics, possess different lipid compositions, and have specific food habits and migratory patterns. The best correlations have been observed with persistent, lipophilic, compounds that have minimal dietary uptake in organisms (3).

Meadows *et al*. (1998) compared the uptake of PCBs between SPMDs and brown trout *Salmo trutta*. Results from their study indicated that uptake rates of the two matrices

were similar within a factor of two. In contrast, Ellis et al. (1995) observed a poor relation between concentrations of OCPs in SPMDs and carp *Cyprinus carpio*, sauger *Stizostedion canadense* and channel catfish *Ictalurus punctatus* in the Mississippi River. Sources of potential variation between residues accumulated in SPMDs and biota could be attributed to the role of dietary uptake or metabolism of targeted compounds in fish that are not accounted for in SPMDs. Overall, there is promising evidence that PSDs may serve as a surrogate for monitoring residues accumulated in biota. However, bioaccumulation is a difficult phenomenon to replicate in fish, even with techniques such as SPMDs. Nonetheless, implementing non-lethal sampling methods to evaluate body burdens are becoming increasingly important, especially when sampling threatened or declining populations or important sport fish populations where killing the fish for contaminant assessment is not feasible or permitted.

Non-lethal or non-invasive tissue sampling techniques for fish are an attractive alternative to the traditional lethal methods commonly employed. Concerns related to non-lethal sampling include survival of the fish post-sampling and if small quantity tissue extraction will yield detectable results comparable to that of whole fish or fillets. Recently, Baker *et al*. (2004) applied this concept successfully to the analysis of mercury (Hg) from lake whitefish *Coregonus clupeaformis* and northern pike *Esox lucius* biopsies and found that concentrations were comparable to that of analyzing whole fish. Following one year post biopsy, there were reportedly no differences in survival of the non-lethally sampled fish relative to non-biopsied fish (10). Other researchers have applied non-lethal sampling techniques to obtain valuable information regarding genetic profiling of fish populations

from low-risk tissues (erythrocytes, blood plasma, caudal, pelvic, and adipose fin, and scale-epithelial tissue) with little to no performance loss in individual fish (11, 12)

The adipose fins of fish, primarily belonging to the family Salmonidae, have been removed for mark-recapture studies and obtaining population estimates for many decades. For example, in 1984, over 6 million steelhead trout, *Oncorhynchus mykiss*, from a hatchery in Idaho were marked with an adipose fin clip to differentiate between wild and hatchery raised fish. Prior to releasing, the trout were examined for clip healing and mortality rates were determined. Complete healing of the excision was observed in less than 4 weeks and from the 6 million marked fish, 0.3% mortality was observed (13). Numerous other studies have reported that adipose fins clipped in varies species of catfish, trout, and salmon for marking purposes have resulted in no deleterious effects on growth, weight or condition of fish and the approximate cost of clipping an adipose fin is as low as 2 cents/fish (14−16).

Despite the remarkably low occurrence of adverse effects resulting from removal of the adipose fin, coupled with its relatively low-cost to remove the fin, there have been no reports to our knowledge, investigating the use of adipose fin clips as a non-lethal sampling technique to estimate concentrations of organic contaminants compared to those found in fillets, which require killing the fish to obtain the tissue. The opportunity to examine the use of adipose fin tissue as a non-lethal sampling technique was afforded in conjunction with a population study of flathead catfish, *Pylodictis olivaris*, in the Deep River, North Carolina during the summer of 2004. In addition to evaluating the non-lethal sampling technique, the concentration of various organic contaminants measured in the fin was compared to that mobile PSDs (PSDs that were affixed to the flathead catfish via floy tags), as well stationary PSDs deployed at sites in the river. The objectives of this study were to determine if adipose

fin tissue provides an accurate estimate of organic contaminant concentrations in fish axial muscle fillets and to determine if the mobile PSDs could be used as surrogates for monitoring organic contaminants in fish.

Experimental Section

Site Description. The study area was in an impounded reach of the Deep River in Moore and Lee counties of North Carolina. This reach is a medium-sized river in the upper Cape Fear drainage, between Highfalls Dam and Carbonton Dam. The upstream half of the reach (between Highfalls Dam and the Glendon-Carthage Road Bridge) is composed of fast flowing, shallow water with a series of riffles and pools. The downstream half of the site (between the Glendon-Carthage Road Bridge and Carbonton Dam) is impounded deep water with depths that may exceed 6 m at base flow. The area is approximately 25 km west of Sanford, North Carolina, and 220 km from the mouth of the Cape Fear River where it discharges into the Atlantic Ocean. This section of the Deep River has been shown to be relatively uncontaminated by organic pollutants (17) and thus, provided a rigorous test of the adipose fin and PSD sampling techniques for detecting and measuring low-level contamination.

Materials. Virgin Polydimethylsiloxane (PDMS) sheeting (0.16 cm thick) manufactured by Diversified Silicone Products Inc. (Santa Fe Springs, CA) was purchased from McMaster Carr (Atlanta, GA). PDMS disks were constructed by punching circles (14 mm diam.) from the PDMS sheet with a cork punch. The average weight of a PDMS disk was 0.3 g (n = 324 disks made). The PDMS disks were pre-extracted with acetone on a shaker table (350 rpm) for 30 min. Then solvent was changed and the procedure was repeated five times. The disks

were dried in a 60 °C oven for 10 min. PDMS disks were then fortified with PRCs (Cl(02) and Cl2(11)) prior to use following methods described by Booij *et al*. (2002) with the following two modifications; PRCs were spiked into a 50:50 MeOH:H_2O solution and PDMS disks were added to the solution and allowed to shake overnight (18).

Virgin low density polyethylene (LDPE) tubing (75 mm wide and 1 mm thick) was purchased from Brentwood Plastics Inc. (St. Louis, MO). Each LDPE sampler was 75 mm wide and 250 mm in length. LDPE strips were extracted in hexane overnight prior to use. To avoid contamination of samples, all glassware, aluminum foil, stainless steel wiring, and sodium sulfate were either baked at 300 °C overnight or rinsed three times each with acetone, DCM, and hexane, as appropriate. All solvents used in this study were Ultra Resi-Analyzed grade purchased from J.T. Baker Inc. (Phillipsburg, NJ). Polychlorinated biphenyls and organochlorine mixtures for use in spike recovery studies and residue analysis were purchased from AccuStandard Inc. (New Haven, CT).

Sample Collection. Between 12 May and 29 June 2004, a total of 108 flathead catfish were collected by standard electrofishing methods from the study reach of the Deep River. Once captured, the fish were promptly measured (total length in mm), weighed (wet weight in g), and implanted with floy tags carrying three PDMS disks, each separated by a 5 mm piece of polyethylene aquarium tubing as a spacer, in the axial muscle tissue of the fish near the insertion of the dorsal fin. The PDMS disks and spacers were threaded onto the floy tag immediately prior to tagging to minimize contamination and the free end of the floy tag was doubled over and held secure with a small polyethylene tie-wrap to prevent the PDMS disks from slipping from the floy tag while attached to the fish. The unique floy tag number associated with each fish was recorded and the fish was released back into the Deep River.

The replicate stationary PDMS and LDPE samplers were deployed at a site in the impounded section of the lower Deep River just upstream of Carbonton Dam in polypropylene cages that shaded the PSDs from sunlight and protected them from floating debris. All passive samplers (PDMS and LDPE) were to be deployed for a minimum 21 d exposure period.

After the minimum 21 d deployment period had been reached, four, one-day electrofishing outings were used for recovery of tagged flathead catfish. Upon re-capturing a PDMS tagged flathead catfish, the tag number was recorded and the fish was measured, weighed and held on ice in the field. The fish (with tag still implanted) were then transported to the Analytical Toxicology Laboratory in the Department of Environmental and Molecular Toxicology at North Carolina State University and stored frozen (-20 °C) until processed.

Sample Extraction and Analysis. At the time of processing, PDMS disks were removed from the floy tags of fish (mobile sampler) or stainless steel wire (stationary sampler) and rinsed with deionized water to remove any biofilm. Each of the PDMS disks were cut into four small pieces with solvent-rinsed stainless steel scissors, spiked with a surrogate internal standard (SIS) mix containing dibromooctafluorobiphenyl (DBOFB), Cl5(112), and Cl8(197), and serially extracted three times in 4.5 mL vials on a shaker-table with a total 10 mL of acetone. Total extraction time was approximately 14 h. Extracts were collected and concentrated to less than 4 mL with a gentle stream of nitrogen then filtered with a Whatman Uniprep® filter vial, 0.45 μm PVDF. The filtered extract was further concentrated with nitrogen to 0.5 mL and spiked with a recovery internal standard, tetrachloro-m-xylene (TCMX).

LDPE strips were rinsed with deionized water, cut into small pieces with solvent rinsed stainless steel scissors, spiked with SIS, and serially extracted three times in Teflon®

bottles on a shaker-table with a total of 150 mL methylene chloride (DCM). Extracts were combined, filtered with a Uniprep® filter and concentrated to 1 mL. Any waxes were then removed by gel permeation chromatography (GPC). The final extract was solvent exchanged to hexane and evaporated to 0.5 mL and spiked with TCMX.

Samples of adipose fin and axial muscle fillet (right dorsal) were dissected from each flathead catfish bearing a floy tag and extracted in the following manner. The adipose fin was removed with a stainless steel solvent-rinsed scalpel, weighed, cut into smaller pieces, spiked with SIS, mixed with sodium sulfate, and serially extracted three times in Teflon® bottles on a shaker-table with a total of 15 mL DCM. The axial muscle fillet was dissected with a stainless steel solvent-rinsed fillet knife, weighed, homogenized, spiked with SIS, mixed with sodium sulfate, and serially extracted in Teflon® bottles on a shaker-table with DCM. The ratio of fresh weight to solvent volume was 1:40. Total extraction times for the fish tissues were 24 h. Solvent from the three sequential muscle extractions was combined and reduced in volume by rotary evaporation at 35°C to 35 mL. A gentle stream of nitrogen further reduced both the muscle tissue and the adipose fin tissue extracts to less than 4 mL. Extracts were then filtered with 0.45 μm PVDF syringeless filters. GPC was used to separate lipids from the pollutant fraction. The fraction containing the target analytes was solvent exchanged to hexane and reduced in volume to 1.5 mL. The concentrated extracts were applied to the top of a hexane-conditioned silica column. PCBs and OCPs were eluted with hexane and DCM, solvent exchanged to hexane and concentrated to 0.5 mL and spiked with TCMX. Lipid fractions were determined by evaporating and weighing lipid in an aluminum pan.

PCBs and OCPs were analyzed using an HP6890 GC equipped with electronic pressure control connected to an HP 5973 MSD or an electron capture detector (GC-ECD).

For GC-ECD analysis, extracts were injected in the pulsed splitless mode and separated on a 30 m x 0.32 mm ZB-50 (0.25 μm film thickness) fused silica capillary (Phenomenex, Inc. Torrance, CA). The temperature was programmed as follows: Initial temperature 60 °C for 1.4 min, 20 °C/min to 210 °C, 1 °C/min to 250 °C, 10 °C/min to 300 °C, hold for 7 min. The injector and detector were set at 300 °C. For GC-MS analysis, extracts were injected in pulsed splitless mode and separated on a Restek 30 m x 0.25 mm Rtx-5 (0.25 μm film thickness) MS with Integra-Guard column. The pressure was ramped to 30 psi before injections with a 1–min hold time. The pressure was then dropped to a constant flow of 1 mL/min for the duration of the run. The temperature program was as follows: Initial temperature 50 °C for 1.0 min, 25 °C/min to 100 °C, 15 °C/min to 245 °C, 0.5 °C/min to 247 °C, 10 °C/min to 300 °C hold for 5 min. The injector and detector were set at 300 °C. Selected ion monitoring (SIM) was used for the analysis.

Response factors were generated using a four- or five-point calibration curve, and response was monitored using the mid-level calibration standard. The relative percent difference between the mid-level check was always less than 15% for all analytes. Sample concentrations were calculated using the generated response factors and were based on the known amount of TCMX injected. A rigorous quality assurance protocol that included the measurement of solvent blanks, procedural blanks, replicate analyses, matrix spikes, and SIS recovery was followed during all analyses. Matrix spike recoveries ranged from 45 – 114%, surrogate recoveries for muscle tissue were all > 70%, surrogate recoveries for adipose fin were all > 50%, laboratory replicate relative standard deviations were <15%, and method blanks were not detected. The data were not corrected for recovery. Method detection limits were as follows, PDMS ranged from, 0.004 – 0.08 ng/g disk (GC-ECD) and muscle and

adipose fin tissue ranged from 0.2 − 0.5 ng/g dry weight. Fish data were normalized for lipid content and are reported as ng/g lipid weight.

Results and Discussion

Of the 108 flathead catfish implanted with PDMS floy tags, a total of seven fish were recovered from the Deep River (Table 1), resulting in a 6.5% re-capture rate. This re-capture rate is slightly less than the 10% commonly expected from studies of floy tagged fish. Nonetheless, sufficient samples were obtained to assess the relation of contaminant uptake into PDMS passive samplers to that accumulated in fish tissue. The total length of the seven fish tagged and recovered averaged 304 mm (range 215-518 mm) and fish weighed from 68 to 1352 g wet weight (Table 1). The wet weight of adipose fin samples averaged 1.1 g and contained a mean lipid content of 13% whereas axial muscle samples had a mean wet weight of 20 g and a mean lipid content of 0.1% (Table 1).

PCB and OCP Concentrations in Adipose Fin and Muscle Tissue.

Although the flathead catfish collected in this study were all relatively small (probably all less than 3 years of age) and came from a relatively unpolluted system, they had accumulated measurable quantities of PCBs and OCPs in their tissues (Table 2). Moreover, there was a good linear relation ($R^2 = 0.77$) between PCB and OCP concentrations in adipose fin and muscle tissue (Figure 1), illustrating the great potential for analysis of the adipose fin to serve as an excellent non-lethal sampling technique to predict concentrations in muscle tissue. Because flathead catfish often attain a large size (e.g., 20-50 lbs.), are an apex predator in most systems, and are gaining value as a prized sport fish for human consumption, this

predictive relation may have human health implications as a method for monitoring organic contaminants for assessment of human health risk of fish consumption.

The adipose fin is a small fleshy fin located between the dorsal and caudal fins of fish primarily belonging to the families Ictaluridae (catfish) and Fidae (trout, salmon). Other families of fish like the Osmeridae (smelt) and Percopsidae (troutperch) also contain species that have adipose fins and may broaden the applicability of using adipose fin clips for measuring organic contaminants in other species. State and federal fish and wildlife management agency biologists commonly remove the adipose fin of fish tagging purposes; often to help identify hatchery reared trout and salmon from wild fish for population assessments. Because this fin performs no known vital function in fish and is easily accessible and removed in the field, the potential for it to serve as a non-lethal tissue sampling technique is very valuable. We found that to compare these two tissues, lipid normalization of concentrations was required due to the much greater lipid composition in the adipose fin versus the muscle tissue (Table 1). However, our data strongly suggest that concentrations of PCBs and OCPs measured in adipose fins, which can be non-lethally removed, provide an accurate estimation of concentrations obtained from traditional lethal fillet methods.

PCB congener profiles of the seven re-captured fish, expressed as logarithmic concentrations, were consistent between the muscle tissue (Figure 2a) and adipose fin (Figure 2b). Biotransformation of lower chlorinated congeners in fish has been documented (19−22), which may explain the lack of detection of di-CB congeners in both tissues. Congeners with five or more chlorines were the most abundantly detected, peaking at hexa-CBs and then decreasing as chlorine number increased (Figures 3a, b). This trend is similar to that

previously observed in striped mullet (*Mugil cephalus*) and spotted sea trout (*Cynoscion nebulosus*) which supports the hypothesis that heavily chlorinated PCBs may have restricted membrane permeability and serves to decrease their uptake in fish (23, 24). Interestingly, PCB congener 195 was detected in all adipose fin samples, whereas only one of the muscle tissue samples had detectable concentrations of Cl8(195).

Advances in analytical chemistry over the past decade have been critical to using non-lethal sampling techniques. Technology has paved the way for scientists to examine the concentration of contaminants in smaller tissue samples with accuracy that compares to traditional methods (10). The average ratio of muscle tissue wet weight extracted to adipose fin wet weight extracted was 50:1. Even the smallest adipose fin (0.13 g) analyzed in our study provided an adequate amount of tissue for determination of organic contaminants. This illustrates that accurate measurements of organic contaminants can be determined from small tissue samples that do not require invasive or lethal procedures. Moreover, the analytical advances coupled with smaller tissue sample sizes that are required has the added benefit of reduced solvent use; thereby decreasing the cost of extraction and solvent disposal.

Estimated Exposure Concentrations from Passive Sampling Devices.

To our knowledge, this study demonstrates for the first time the use of mobile PSD technology (i.e., PDMS floy tagged fish) for assessing real-time, location-specific exposure of an organism to waterborne contaminants. Using PDMS as a time-integrated passive sampling device is a relatively new technique, however, we found that this technology could detect and measure the presence of PCBs and OCPs (Table 2) in a relatively uncontaminated ecosystem over a 25 to 36 d deployment period. Another major benefit of this of this

approach was that the PDMS disks attached to the fish with floy tags showed no signs of harming the fish (e.g., lack of skin abrasion or inflammation) upon re-capture beyond the minimal impact of normal floy tagging techniques.

The estimated water concentrations of individual PCB congeners and OC pesticides from the PSDs (estimates obtained by re-arranging equation 1 and using PRC adjusted sampling rates (25, 26) were extremely low (0.2–2 ng/L) therefore we present the sum of the 21 PCB congeners and the 28 OCPs investigated (Table 2). The estimated sum concentrations of PCBs in water derived from PDMS floy tagged fish (mobile PSDs) ranged from 0.6 to 13.1 ng/L and the sum concentrations of OCPs ranged from 1.8 to 59.6 ng/L. The exposure concentrations estimated from both the mobile and stationary PSDs were similar to concentrations reported from a previous study in the same river and general study area that used stationary LDPE PSDs to measure organic pollutants (17).

The target analytes in this study are environmentally persistent and extremely lipophilic with log octanol-water partition coefficients ranging from 3.6 – 8.2 (27). Therefore, if the role of dietary uptake in the catfish has minimal effects, there should be a correlation between tissue concentrations estimated from PSDs and measured tissue concentrations; this was not the case in our study. Tissue steady-state bioconcentration models can be used to estimate the tissue PCB concentrations using a steady-state bioconcentration factor (BCF) for fish. This model does not account for consumption of contaminated food or clearance of chemicals (28). Using BCF, one can estimate tissue residues in the fish with the following equation

$$C_w = BCF^{-1} * C_{tissue} * (1-e^{-k_2 t})^{-1} \quad (5)$$

where C_w is the measured concentration of chemical in the water (ng/L), BCF is the bioconcentration factor (L/g), C_{tissue} is the measured concentration of chemicals in tissue (ng/g), k_2 is the depuration rate constant (d^{-1}) and t is the time in days (29). Flathead catfish BCF and k_2 values were not found in literature therefore, values from Fox *et al*. (1994) were used to estimate water concentrations from the measured tissue concentrations (30).

Because low concentrations of the target analytes were detected with the PSDs compared to that detected in the tissues (Table 2), it was unlikely that the water phase was the only source of uptake for the fish. This is further illustrated in Figure 3 which represents estimated and measured tissue concentrations from the three matrices examined in this study for fish sample 24163. PSDs are most commonly used as a tool to estimate freely dissolved water concentrations. From table 2, PSDs estimate that concentrations of PCBs in the water phase are extremely low if present at all. This corresponds to the low concentrations or lack of detection estimated for tissue concentrations from PSDs, as seen in Figure 3. This is consistent with the fact that PCBs have extremely low water solubilities and tend to partition to sediments, DOC, or POC (28). Therefore, PCBs may not be readily available to be sampled by the PSDs. Based on PCB characteristics in water and the similarities of water concentrations estimated from PSDs in this area from Howard (2003) and the current study, we feel confident in the PSD estimations. It was interesting to note that each of the PSDs deployed in this study detected Cl2(08) which was detected in only one of the dorsal tissues sampled. The absence of Cl2(08) in the majority of tissue sampled could potentially be due to the ability of fish to metabolized lower-chlorinated PCBs (24). PCB water concentrations estimated from PSDs were low, however, they were still detected. The detection of low concentrations of PCBs can be attributed to the premise behind passive sampling techniques

which allow PSDs to preconcentrate hydrophobic residues over time to concentrations greater than those achieved by traditional sampling techniques (e.g., grab water samples) that may not have detected the PCBs.

Being both predatory and bottom dwelling fish, bioaccumulation models may be better suited for the flathead catfish because they are susceptible to accumulating PCBs and OCPs from both contaminated sediment and ingestion of contaminated food. Most PCBs have a log K_{OW} greater than 5, therefore it is highly likely that bioaccumulation is occurring through trophic transfer in food which has been confirmed by field studies (31−33) or from DOC, POC, or sediment-associated congeners. This could result in the increased concentrations that are seen in both adipose fin and muscle tissue in Figure 3 compared to the estimated tissue concentrations from PSDs.

Permeable Reference Compounds.

Permeable reference compounds (PRCs) were added to the PDMS matrix prior to deployment to evaluate how environmental conditions affected the uptake of chlorinated contaminants. This proved to be especially useful when a mobile PSD is involved because increased shear flow across the membrane can significantly increase the uptake of hydrophobic contaminants with log $K_{OW} > 4.4$ into the PSD (8). In general, the change in initial concentrations of the PRCs should be between 20 – 80%. However, if no loss is observed then it can be assumed that all compounds with log K_{OW} > than that of the PRC are in the linear uptake phase. Conversely, if all of the PRC is lost during a deployment compounds with log K_{OW}s similar to that of the PRC will be at equilibrium (3).

The PRC loss from the mobile PSDs for PRC Cl(02) ranged from 77 – 99% indicating all compounds with log K_{OW}s less than 4.6 were at equilibrium. Therefore, an equilibrium model should be used for target analytes when applicable. The following equation is used to estimate water concentrations from PSDs when equilibrium has been achieved

$$C_w = C_{PSD\text{-}E}/K_{PSD} \quad (6)$$

where C_w is the analyte concentration in water (ng/L), $C_{PSD\text{-}E}$ is the concentration of analyte in the PSD at equilibrium (ng/g), and K_{PSD} is the equilibrium PSD-water partition coefficient (L/g). Out of the 50 plus compounds analyzed in this study, there were only 5 compounds with log K_{OW} values less than 4.6, and of those 5, lindane and alpha-BHC were the only two compounds that were detected in the PDMS that C_w was estimated using K_{PDMS} values (25). In this instance, the PDMS no longer provides a time-weighted-average concentration of lindane and alpha-BHC. The loss of PRC Cl2(11) ranged from 2 – 65% and the log K_{OW} of Cl2(11) is 5.3. This loss rate is indicative that most of the analytes of interest in this study were still in the linear uptake phase where integrative sampling occurs. Equation 1 with the substitution of the R_s that has been adjusted with the EAF, can be utilized to estimate the water concentration for all compounds with a log $K_{OW} > 5.3$.

The stationary PDMS disks deployed for this study had less than 20% loss for both of the PRCs analyzed in this study. Therefore, the linear uptake model can be used to estimate water concentrations from the stationary PDMS disks. These results illustrate the importance of knowing which model to use when estimating water concentrations from PSDs. Selection of the appropriate phase is dependent on physicochemical properties of target analytes, exposure duration, and exposure conditions (temperature, biofouling, hydrodynamics).

In summary, although a 10% or greater recapture rate was desired, the 7 re-captured flathead catfish in this study yielded encouraging results for using adipose fin removal as a non-lethal sampling technique. The validity of using adipose fin tissue as a non-lethal, non-invasive sampling technique can be evaluated based on the criteria developed by Morizot *et al*. (1990), which includes the key elements for any non-lethal sampling technique (34). These elements state that the procedures must indeed be non-lethal and that techniques employed should not result in adverse effects to the fish. Even though these two specific outcomes were not specifically examined in this study, the adipose fin removal procedure has been extensively used for mark-recapture studies for many years in fisheries science with little to no impact documented on the subjected fish. Another decisive factor concerning the use of non-lethal sampling techniques is regarding the amount of tissue extracted or removed from the fish--there should be ample tissue for the desired analysis. Even the smallest adipose fin sampled in this study was highly correlated with organic contaminant concentrations from the corresponding muscle tissue fillet. The last measure of any successful non-lethal sampling techniques is regarding its application to the taxa being studied. This procedure is obviously limited to species of fish that have an adipose fin, which include the Ictaluridae, Salmonidae, Osmeridae, and Percopsidae families of fish. Although limited to these several families of fish, this approach has wide implications and may be especially useful in studies assessing the restoration of declining salmon populations in rivers of the western United States where contamination of from of organic pollutants is a concern for both fish health and human health from consumption of contaminated fish. Overall, the analysis of adipose fin proved to be a powerful tool that can be used to estimate whole tissue concentrations of organic contaminants without killing the fish.

Results from this study also demonstrated for the first time that mobile PSDs made from PDMS disks and implanted on fish with floy tags can be a valuable tool for assessing real-time, location-specific exposure of fish to organic contaminants. The mobile PSD concept combined with radio telemetry studies offer the ability to monitor organic contaminant exposure while at the same time tracking the animal to try to determine the ultimate source of contamination (e.g., spawning grounds versus wintering grounds for migratory fish species). In view of this work, there appears to be no practical/physical barriers to the use of mobile PSDs on fish and other aquatic organisms. PDMS disks fortified with PRCs reflected diverse exposure conditions for mobile PDMS verses the stationary PDMS and allowed us to better estimate site-specific water concentrations. Based on the results obtained in this study, PRCs play a vital role in estimating water concentrations, especially for mobile PSDs. Additional research is needed to demonstrate our observed relation between contaminant concentrations in adipose fin and muscle tissue for flathead catfish and other applicable species as a non-lethal sampling technique and to further evaluate and test the mobile PSD concept with PDMS to measuring environmental pollutants.

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Table 1. Tagging and re-capture dates, total length, wet weight, wet weight extracted tissue, and lipid compositions of flathead catfish collected from the Deep River of North Carolina.

Fish Tag No./ Sample ID	Date tagged	Date recaptured	Length of PDMS tag deployment (d)	Whole fish wet weight (g)	Total length (mm)	Muscle Fillet wet weight (g)	Adipose fin wet weight (g)	Lipid Content of Muscle Tissue (%)	Lipid Content of Adipose Fin (%)
24627	12-May-04	9-Jun-04	30	120.0	242.0	13.6	0.3	0.03	27.5
24632	12-May-04	29-Jun-04	36	200.0	265.0	24.5	0.6	0.09	21.4
24666	3-Jun-04	29-Jun-04	25	350.0	341.0	24.5	1.5	0.02	12.8
24171	29-Jun-04	23-Jul-04	29	196.0	293.0	21.3	0.5	0.05	3.5
24158	29-Jun-04	23-Jul-04	29	231.0	260.0	21.7	0.2	0.02	10.0
24163	29-Jun-04	23-Jul-04	29	1352.0	518.0	24.9	4.2	0.46	9.5
24167	29-Jun-04	23-Jul-04	29	68.0	215.0	10.3	0.1	0.04	9.4
PDMS stationary	11-Jun-04	23-Jul-04	41	–	–	–	–	–	–
LDPE stationary	11-Jun-04	23-Jul-04	41	–	–	–	–	–	–

Table 2. Estimated sum water concentrations of PCBs and OCPs from passive sampling devices (PSDs) (ng/L) and corresponding concentrations measured in adipose fin (ng/g lipid normalized) and muscle tissue (ng/g lipid normalized).

		Fish Tag No./Sample ID								
	Concentration	24171	24666	24158	24163	24632	24167	24627	PDMS stationary	LDPE stationary
PCBs	PSDs	7.1	13.1	4.1	2.3	0.6	1.1	1.9	2.2	3.7
	Adipose fin	1749.9	230.4	2930.4	381.1	332.5	2537.5	680.1	–	–
	Muscle	259.7	768.2	3875.1	338.8	382.9	2069.0	777.1	–	–
OCPs	PSDs	29.0	30.7	59.6	5.4	1.8	14.8	14.5	1.5	7.3
	Adipose fin	660.1	430.3	1150.9	291.0	207.1	795.5	245.6	–	–
	Muscle	931.1	487.2	1270.7	240.7	237.2	665.4	373.8	–	–

Figure Legends

Figure 1. Concentrations of PCBs and OCPs in muscle tissue (fillet, ng/g lipid normalized) versus adipose fin (ng/g lipid normalized) from individual flathead catfish collected from the Deep River of North Carolina.

Figure 2. Concentrations of tri-deca PCB congeners in muscle tissue (a) and adipose fin (b) from seven individual flathead catfish collected from the Deep River of North Carolina.

Figure 3. Example of estimated PCBs tissue concentrations (ng/g) determined from polydimethylsiloxane disks (Ct-PDMS), adipose fin (Cfin), and muscle tissue (Cmuscle) from flathead catfish, 24163.

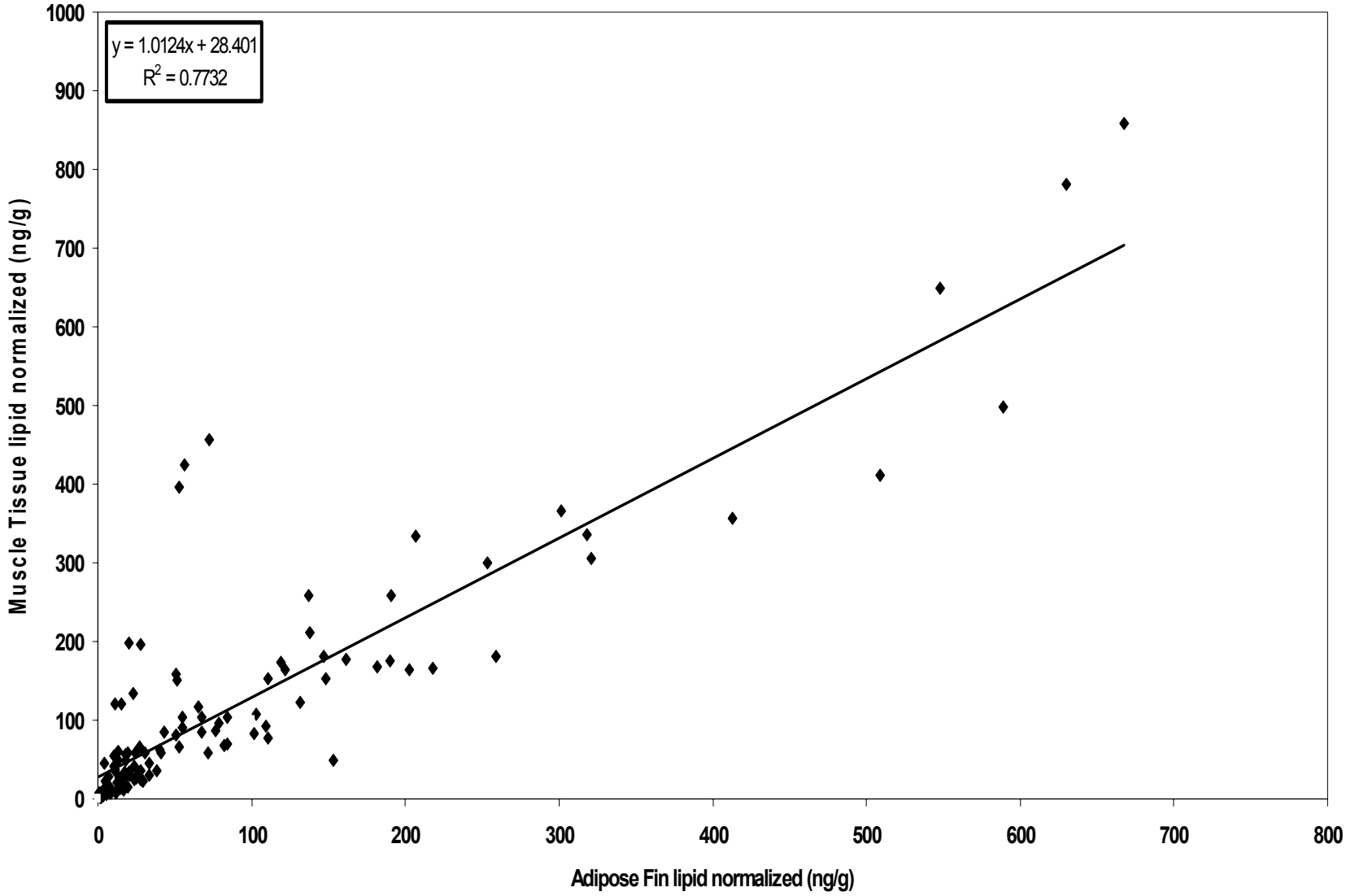
y = 1.0124x + 28.401
R2 = 0.7732
Muscle Tissue lipid normalized (ng/g)
Adipose Fin lipid normalized (ng/g)
0
100
200
300
400
500
600
700
800
900
1000

Figure 1

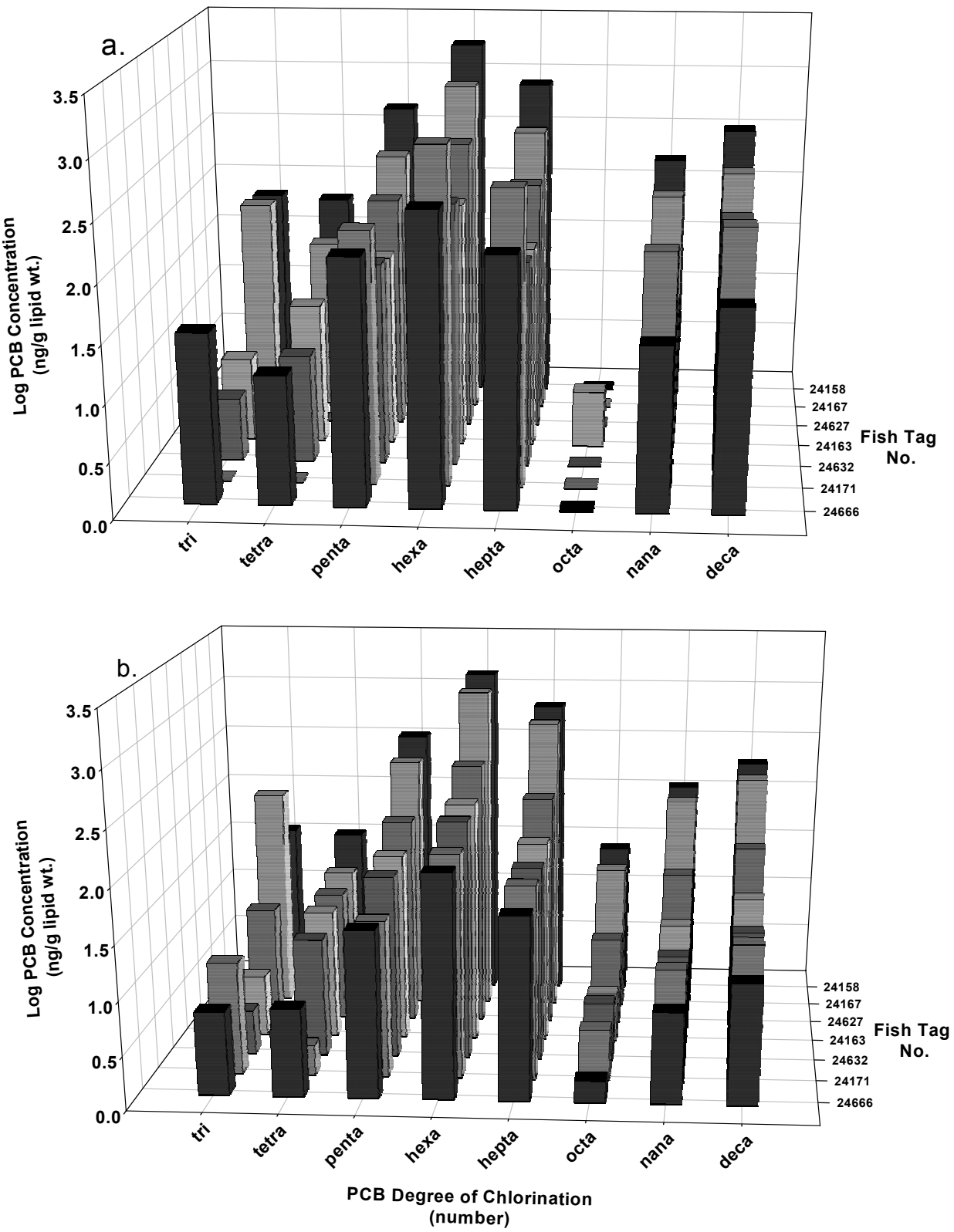
a.
b.
Log PCB Concentration (ng/g lipid wt.)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
tri
tetra
penta
hexa
hepta
octa
nana
deca
24158
24167
24627
24163
24632
24171
24666
Fish Tag No.
PCB Degree of Chlorination (number)

Figure 2

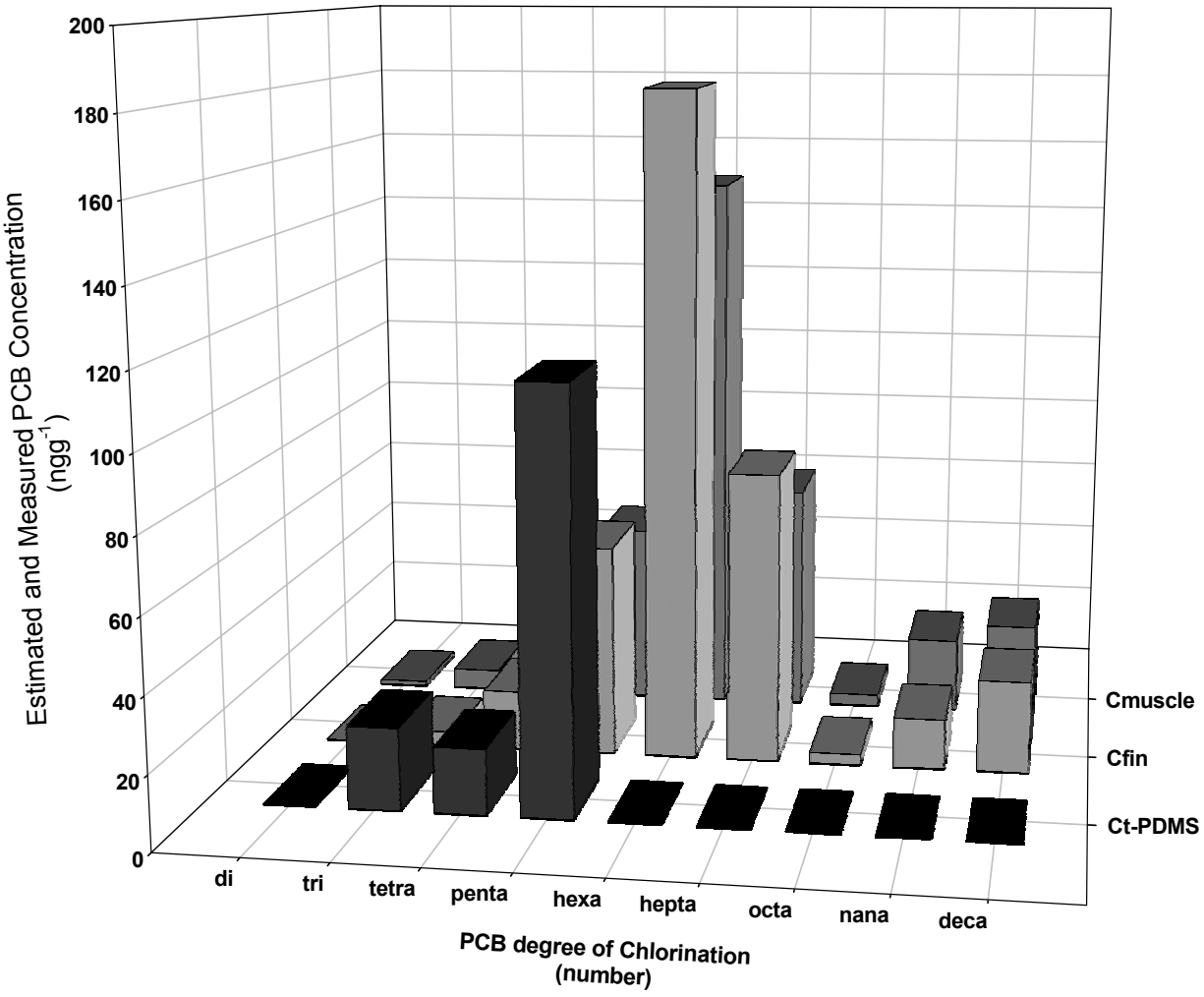
200
180
160
140
120
100
80
60
40
20
0
Estimated and Measured PCB Concentration
(ngg-1)
di
tri
tetra
penta
hexa
hepta
octa
nana
deca
PCB degree of Chlorination
(number)
Cmuscle
Cfin
Ct-PDMS

Figure 3