AN ABSTRACT OF THE THESIS OF

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Title: Development and Application of a New Passive Sampling Device: The Lipid-Free Tube (LFT) Sampler.

APPROVED:

Kim A. Anderson

Contaminants can exist in a wide range of states in aqueous environments, especially in surface waters. They can be freely dissolved or associated with dissolved or particulate organic matter depending on their chemical and physical characteristics. The freely dissolved fraction represents the most bioavailable fraction to an organism. These freely dissolved contaminants can cross biomembranes, potentially exerting toxic effects. Passive sampling devices (PSDs) have been developed to aid in sampling many of these contaminants by having the ability to distinguish between the freely dissolved and bound fraction of a contaminant. A new PSD, the Lipid-Free Tube (LFT) sampler was developed in response to some of the shortcomings of other current PSD that sample hydrophobic organic contaminants (HOCs). The device and laboratory methods were original modeled after a widely utilized PSD, the semipermeable membrane device (SPMD), and then improved upon. The effectiveness, efficiency, and sensitivity of not only the PSD itself, but also the laboratory methods were investigated. One requirement during LFT development was to ensure LFTs could be coupled with biological analyses without deleterious results. In an embryonic zebrafish developmental toxicity assay, embryos exposed to un-fortified LFT extracts did not show significant adverse biological response as compared to controls. Also, LFT technology lends itself to easy application in monitoring

pesticides at remote sampling sites. LFTs were utilized during a series of training exchanges between Oregon State University and the Centre de Recherches en Ecotoxicologie pour le Sahel (CERES)/LOCUSTOX laboratory in Dakar, Senegal that sought to build “in country” analytical capacity. Application of LFTs as biological surrogates for predicting potential human health risk endpoints, such as those in a public health assessment was also investigated. LFT mass and accumulated contaminant masses were used directly, representing the amount of contaminants an organism would be exposed to through partitioning assuming steady state without metabolism. These exposure concentrations allow for calculating potential health risks in a human health risk model. LFT prove to be a robust tool not only for assessing bioavailable water concentrations of HOCs, but also potentially providing many insights into the toxicological significance of aquatic contaminants and mixtures.

DEVELOPMENT AND APPLICATION OF A NEW PASSIVE SAMPLING DEVICE: THE LIPID-FREE TUBE (LFT) SAMPLER

by
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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Lucas W. Quarles, Author

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Development and Application of a New Passive Sampling Device: The Lipid-Free Tube (LFT) Sampler

CHAPTER 1 – INTRODUCTION

1.1 Background

The development of organic chemicals have proven to be exceptionally effective at remedying problems of humankind and have enabled our society to accomplish great technical advances and perform industrial processes with greater efficiencies and safety [1]. Over 100,000 chemicals are presently reported to be produced commercially in a range of quantities and used daily; an additional ~1000 are added each year [1-3]. Many of these chemicals have been released to the environment by human society for several decades, and most are organic in nature [1, 2]. Along with intentional releases, such as pesticides sprayed on to crops, it is obvious that even contained applications result in a certain level of unintentional discharge of these compounds to the environment [1] either through spills, leaks, or the like. It is not surprising to see that a large portion of chemicals produced have caused many problems [1] of which, maybe 1000 compounds are cause for significant concern to the environment [2], which is based on presence in the environment, toxicity, and a chemical's tendency to bioaccumulate in food chains [2, 4]. Environmental presence is, in part, largely dependent upon persistence in the environment, which also may facilitate long distance transport [2, 4]. Many of the contaminants of concern have been termed PBTs by agencies due to their persistent, bioaccumulative, and toxic behavior.

Human society as a whole maintains a stance of constant development and use of materials, energy and space in conjunction with an increased use of anthropogenic organic chemicals [1]. Holding to this stance, a potential result is further contamination of water, soil, and air with these compounds; potentially a major issue in environmental protection [1]. Understanding the distribution of chemicals in the environment is essential to assess potential exposures to humans and biota.

The distribution and fate of chemicals in the environment are governed by interactions between four groups of factors. First, are the conditions established in the environment: temperature, composition of media, flow and accumulations in air, water and solid matter, hydrodynamics, solar radiation, and pH. Also, the physicochemical properties of abiotic components, such as organic content of soil and sediment are involved. Second, are the physicochemical properties of a chemical such as partitioning coefficients, structure, solubility, vapor pressure, susceptibility to degradation as well as others. Third, are the composition, mass, and physiological, anatomical and behavioral characteristics of the organisms inhabiting ecosystems of interest. Fourth, are the patterns of use; how the substance and into which compartments it is introduced and whether introduction is episodic or continuous [2, 3].

Contaminants can exist in a wide range of states in aqueous environments, especially in surface water. They can be freely dissolved or associated with dissolved or particulate organic matter depending on their chemical and physical characteristics [4, 5]. These forms or states can be transported by water and dispersed throughout surface waters. The resulting concentrations may lead to adverse effects in aquatic organisms and on human health through multiple sources and pathways influenced by variable and often poorly understood factors. Development of protective actions for ecosystems and human health can be enlightened through monitoring of aquatic environments for contaminants: an understanding of sources, pathways and processes of contaminant fate is vital [4, 6]. Under legislative frameworks, directives, and governmental agencies such as the US Environmental Protection Agency (EPA) and the Water Framework Directive of the European Union, monitoring is required to ensure water-quality standards are met and maintained [6, 7].

1.1.1 Ecotoxicology and environmental chemistry

The field of ecological toxicology or ecotoxicology studies distribution and fate interactions of chemicals in the environment. More specifically, ecotoxicology is the study of relationships between organisms and environmental contaminants in

order to predict biological consequences. To understand the potential adverse response of organisms to environmental contaminants requires an understanding of exposure processes and contaminant dose [8]. For the purposes of this thesis, the term contaminant will be defined as a chemical that exists at a level judged to be above those that would normally occur (background level) in any particular component of the environment that may or may not be harmful. A very closely related field of study to ecotoxicology is environmental chemistry or "chemodynamics", which focuses on growing our understanding of how chemicals behave in our complex environment. It sets forth to measure, assess, and predict how chemicals will behave in the laboratory as well as local to global environments [2]. It is the intimate interaction between the two disciplines that strives to address the distribution and fate of chemicals in the environment. At the root of ecotoxicology and environmental chemistry is a need to monitor the environment. Without monitoring data of contaminants there would be no knowledge of relevant environmental concentrations or reasonable contaminant dose and thus little insight into distribution and fate. This is especially important with respect to PBTs discussed earlier and other contaminants that are of significant environmental concern.

1.1.2 Aquatic monitoring/sampling

The traditional and most common method for measuring contaminant levels in aquatic systems has been "active sampling". This requires a physical external energy input for sample collection and/or residue extraction [3]. Most commonly, this takes the form of discrete grab sampling, also known as spot or bottle sampling, where a large volume (liters) of water is taken from a site. The process is accepted for regulatory and legislative purposes, as it is well established and validated [6, 7]. Grab sampling only represents a single discreet location and point or "snapshot" in time [6, 7, 9-11]. When viewed from that perspective its usefulness is severely limited. Grab sampling also cannot readily detect pulses in contaminant concentrations which may result from episodic events. This type of sampling also collects the total fraction of a chemical in the environment not the

toxicologically relevant, truly dissolved, portion, unless the sample is further processed in the laboratory (i.e. filtration). Grab sampling produces different apparent concentrations of contaminants depending on the pre-treatment applied. Though grab sampling used to be the norm it has fallen out of favor due to the large volumes typically needed to meet detection limits. The total fraction, which this non-selective sampling approach collects, includes all waterborne phases: microorganisms and algae, particulate organic carbon (POC), dissolved organic carbon (DOC), inorganic particulates, and the freely dissolved phase. This could lead to potential inaccurate measurements. In addition, most grab sampling methods are plagued by sample preservation issues such as loss by volatilization, sorption to container media, and general chemical degradation [3, 7]. These issues are not trivial and there needs to be accuracy and confidence in results, as some of this data may be used in evaluating water quality guidelines.

To conduct large scale monitoring studies, required for many site assessments, where chemical concentrations can vary over time, a sample has to represent a longer duration of time. This can be done through manual or automatic means, where multiple individual samples pulled over time are combined, creating a pseudo time-integrated sampling regime. These methods are often impractical, prohibitively expensive, and time consuming [9, 11].

It is more typical for an aquatic monitoring program interested in biologically relevant data to either sample sediment or biota. Like all sampling methods, these too have drawbacks. For sediment, often benthic sediment concentrations will be measured then equilibrium distribution coefficient applied to calculate dissolved concentrations [6]. For biota, several test species can be used; which organism, depends on the specifics of the water body in question. These organisms, set out for an extended amount of time, passively bioaccumulate compounds of interest. The organism's tissue or lipid extracts are then analyzed giving and equilibrium concentration. Characteristics that influence sediment sorbent quality, degradation and elimination, bioturbation, re-suspension, and reliable biota condition and spatial information are all very difficult to asses and

have a major impact on observed concentrations [9]. Though biota sampling can give valuable insight into the bioavailable fraction of contaminants in the environment with the ability to bioaccumulate, it has many drawbacks. Biota can metabolize contaminants after uptake, generally there is a lack of spatial knowledge with respect to study site, a lack of available life history information, as well as sex, age, size and/or growth rate related differences that lead to contaminant load differences which impact bioaccumulation and dilution [6]. Care must be taken when interpreting results from such sampling methods because of the potential variability that may arise for hydrophobic organic contaminants (HOCs) concentrations. In biota, these lipophilic contaminants in a large volume of water move across organismal membranes such as fish gills and collect in an organism's fat [12]. This also, is the basic premise behind the passive sampling techniques discussed later. It is the freely dissolved form of the contaminant that is transported across biological membranes of aquatic organisms and potentially exerts toxic effects [5, 13, 14]. For toxic effects on the cell or organism to occur, most contaminants must pass through the bio-membrane [8]. A reduction in the freely dissolved concentration of the contaminant, such as contaminant association with dissolved organic material, results in a direct reduced bioavailability [5].

1.1.3 Bioavailability

Utilizing concepts from both environmental chemistry and ecotoxicology, bioavailability integrates contaminant concentration, fate, and organismal behavior in the environment [8] as can be seen in figure 1.1. The notion of bioavailability arises from the fact that the detrimental effects on organisms and ecosystems exposed to contaminants are not caused by the total amount of a chemical released to the environment, but rather only a specific bioavailable fraction [4, 8]. In an aqueous environment the most bioavailable fraction for hydrophobic organic contaminants is the freely dissolved fraction [4].

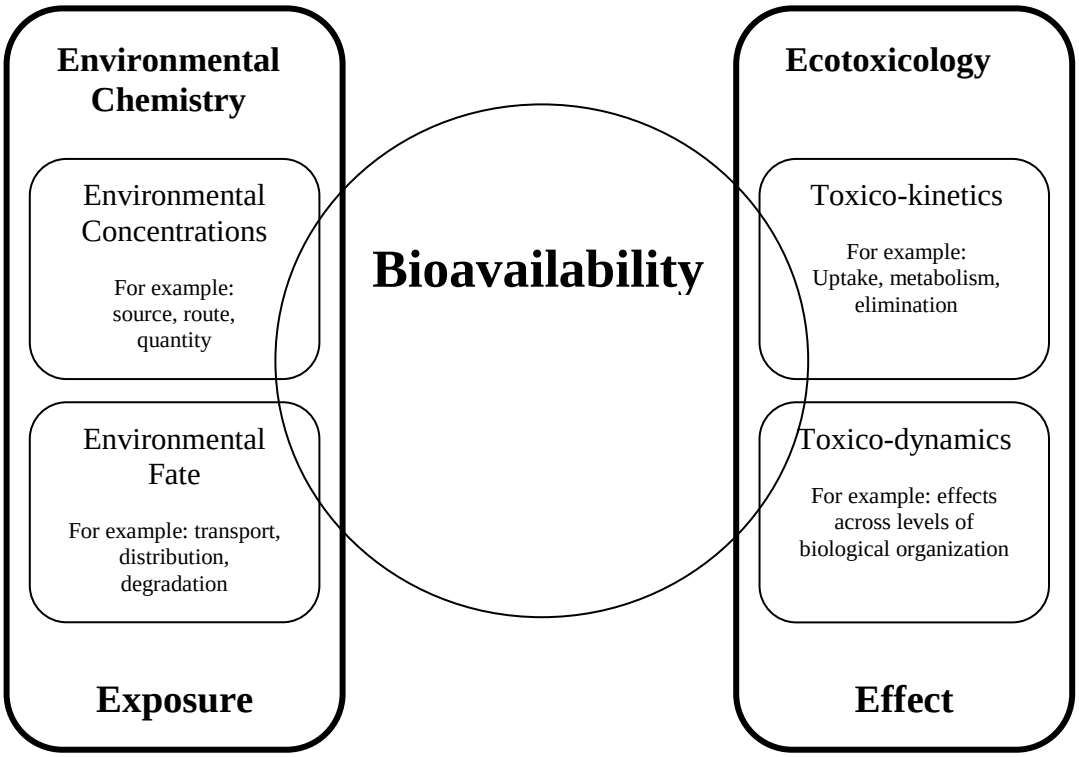

Figure 1.1 Bioavailability incorporates many processes from environmental chemistry and ecotoxicology. Modified from Anderson *et. al.* [8].

Figure 1.2 shows a simplified view of bioavailability. Process 1 represents the physical and chemical processes involved in the binding and unbinding of contaminants with other compartment components. Processes 2 and 3 illustrate transportation of bound and free contaminants to an organism's biological membrane. Unbound contaminants are subject to diffusion, dispersion, advection leading to transport by re-suspension, bioturbation, and diffusion [8]. Process 4 corresponds to the mechanisms involved during the transport of contaminants across and through bio-membranes. To exert toxic effects upon a cell or organisms, most contaminants require passage through the biologic membrane [8]. Passage can occur through passive diffusion, facilitated diffusion, or active transport. Process 5 represents those processes that occur after passage through the membrane. These may include metabolism, storage, and elimination. Another potentially serious environmental consequence of passage through a membrane is

the potential for a contaminant to bioaccumulate or biomagnify through the food web. Bioavailability describes many complex processes, such as the mass transfer and uptake of contaminants into organisms [8]. These processes are governed by the properties of the compounds and compartments mentioned as well as other factors. The processes work in concert rather than in isolation to produce a fraction representing the relevant exposure dose for an organism.

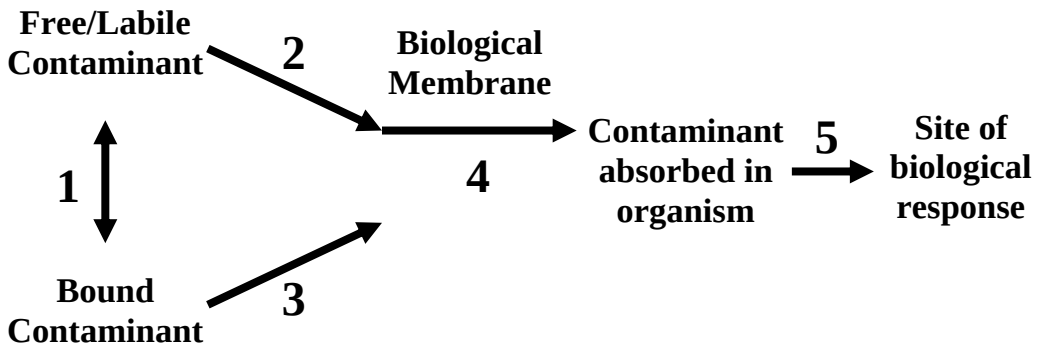

Figure 1.2 Bioavailability processes. Modified from *Anderson et al.* [8].

Identification and measurement of chemical forms that are, in fact, the bioavailable forms is essential to better understand physical, chemical, and biological mechanisms that control chemical fate and interaction in the aquatic environment. To reduce uncertainty and correctly predict the fate, interaction and ultimately the toxicity of environmental contaminants requires advanced understanding of bioavailable chemical distribution [8]. This knowledge can provide significant benefits for exposure assessments and water quality regulations. Identification and measurement can be completed easily through an appropriate passive sampling device discussed later.

1.1.4 Passive sampling techniques as surrogates for evaluating bioavailability

In broad terms, passive sampling is any sampling technique based on the free flow of analyte molecules from a sampled medium where chemical fugacity is high to a collecting medium where chemical fugacity is low; establishing a chemical potential difference of the analyte between the two media [15, 16]. This established net flow continues until either equilibrium is reached in the system or

the sampling session is terminated [15]. Passive sampling devices (PSDs) have gained wide acceptance and are being used broadly in air, water, and soil media to evaluate exposure and contamination [6, 17-19]. In this thesis, the focus will be on passive sampling of hydrophobic organic contaminants in the aquatic environment.

The past two decades have seen many iterations of passive samplers for the aquatic environment. Most of these consist of "membrane" samplers in which a semipermeable membrane, mediating diffusion, encloses a receiving medium that has a high affinity for organic contaminants [4]. An important and widely used sampler of this type is the semipermeable membrane device (SPMDs) introduced by Huckins and co-workers in 1990 [20]. SPMDs traditionally consist of additive free low-density polyethylene (LDPE) lay-flat tubing filled with triolein (a purified lipid) and heat-sealed at each end [10, 16, 21, 22]. Triolein, a high molecular weight lipid, is used to replicate lipid tissue of organisms as well as to act as a contaminant sink.

SPMD were originally developed as a three-compartment model that mimics cellular membranes and lipid tissues. The LDPE membrane employed by SPMDs, thought of as non-porous, does have transient cavities on the order of ~10 Å by thermal motion [23]. These cavities are very close to estimated cell membrane pore diameters (~9.8 Å) [24]. A visual representation of the similarities between biomembranes and the LDPE membrane is shown in figure 1.3.

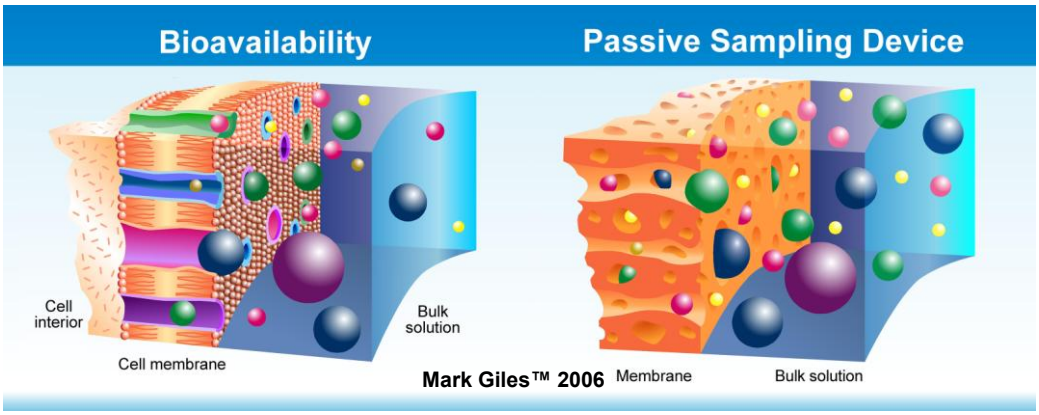

Figure 1.3 Cartoon showing similarities of biomembranes and a LDPE membrane

As stated earlier, a passive sampler is usually either allowed to reach equilibrium or the sampling terminated prior. When non-equilibrium sampling, the amount of analyte collected depends upon both its concentration in the sampled medium and the exposure time [15]. A time-weighted average (TWA) analyte concentration can be determined if the sampling rate and analyte concentration are known [15] and usually occurs over a period of weeks to months [4]. Water concentrations of contaminants can be back calculated from field deployed SPMD accumulations. Initially, SPMD uptake rates were estimated from laboratory experiments varying both flow and temperature using flow-through systems for target analytes and applied to the field accumulation data. A more accurate method for the determination of uptake rates was developed using performance reference compounds (PRCs) [16, 25-27]. PRCs are compounds with relatively high fugacity that have similar physico-chemical characteristics, such as K_{ow}, to target analytes, are analytically non-interfering, and not found in the sampled environment. Traditionally for SPMD, PRCs are spiked into the triolein, as it provides a convenient sink, before the triolein is inserted into the LDPE tubing. Measured PRC loss during an exposure can be used to estimate *in situ* sampling rates of analytes of interest [16]. Assumptions made in using PRCs are that all uptake and elimination rates of organic contaminants are governed by first-order kinetics

following Fick's first law of diffusion and uptake is isotropic. The data gathered from an exposed sampler, such as PRC dissipation and analyte accumulation is inserted into equations developed by Huckins *et al.* [16] and detailed in Chapter 4 to estimate bioavailable water concentrations.

1.1.5 Lipid-free polyethylene membrane tubing sampler (LFT)

Experiments by Booij *et al.* [25], and discussion in Huckins *et al.* [16], propose that for SPMD, the analyte accumulation in the LDPE membrane represents a significant portion of the total accumulation of the device; up to 70%. This is not surprising given that up to 76% of an SPMD on a volume:volume basis consists of LDPE and the water partitioning coefficient of the LDPE membrane ($K_{mw)}$ is very similar to that of triolein (K_{Tw}); $K_{mw} \approx 0.1 – 0.5\ K_{Tw}$ [16, 28]. Furthermore, in Anderson *et al.* [17] (included as Appendix A), we evaluated SPMDs and triolein- or lipid-free polyethylene tube samplers (LFTs) through side-by-side field trials and found few differences in performance.

For SPMDs, PRCs are conveniently introduced into triolein, however LFT do not contain this same hydrocarbon reservoir thus a new technique was need to incorporate PRCs into LFT. Techniques have been developed previously [25, 29] but do require significant time and labor to accomplish; upward of 90 day for one study. Anderson *et al.* [17] developed a quick convenient solution to this problem and was used in this study for both PRCs and analytical recovery determination during method development.

Continually elevating environmental quality criteria and the subsequent need to measure concentrations of contaminants at increasingly low concentrations have caused typical environmental analyses to become more expensive. There is an obvious need for rapid but effective low-cost integrated methods that would enable monitoring of the fate and concentrations of contaminants in the environment directly. Effects and assessment of the hazards these chemicals pose to the environment and to human health could be evaluated through the use of passive sampling devices [10]; LFT are particularly well suited to this task. Many PSD including SPMD require specialized equipment or techniques during sample

processing. With fewer variables as discussed above with biota and typical sampling methods, LFT provided an attractive alternative.

1.2 Thesis outline

Chapter 2 profiles the further development of a passive sampling device for hydrophobic organic contaminants: The Lipid-Free Tube (LFT) sampler. The effectiveness, efficiency, sensitivity, and applicability of the device and associated laboratory and analytical methods were evaluated as compared to a similar widely used PSD and a previous method utilized in our laboratory.

Chapter 3 includes excerpts from the final report generated after a six-week hands-on training event at the Centre de Recherches en Ecotoxicologie pour le Sahel (CERES)/LOCUSTOX laboratory in Dakar, Senegal as part of a project between OSU and the Food and Agriculture Organization of the United Nations (FAO). I led the on-site hands-on training at CERES from January to March 2009, which focused on equipping the laboratory with the means and expertise to carry out trace pesticide residue analysis of aqueous phase hydrophobic organic contaminants. LFT technology was utilized as a simple sampling device to carry out this work. I was also involved in two prior training events in 2006 and 2008 during which two scientists (for each event) from CERES visited OSU to gain knowledge and training to help prepare CERES to carry out trace pesticide residue analysis using passive sampling devices.

Chapter 4 provides a more in-depth discussion on the concepts and technology of the refined LFT sampler. The link between LFT technology and its application as a biological surrogate for predicting potential human health risk endpoints, such as those in a public health assessment, are presented.

Finally, Chapter 5 presents general conclusions and talking points for future work.

1.3 Literature Cited

1. Schwarzenbach, R. P.; Gschwend, P. M.; Imboden, D. M., *Environmental organic chemistry*. 2nd ed.; John Wiley & Sons, Inc.: Hoboken, NJ, 2003.

2. Mackay, D.; Shiu, W. Y.; Ma, K.-C.; Lee, S. C., *Handbook of physical-chemical properties and environmental fate for organic chemicals*. 2nd ed.; CRC Press: Boca Raton, 2006.

3. Huckins, J. N.; Petty, J. D.; Booij, K., *Monitors of organic chemicals in the enviornment semipermeable membrane devices*. Springer: New York, 2006; p 223.

4. Wennrich, L.; Vrana, B.; Popp, P.; Lorenz, W., Development of an integrative passive sampler for the monitoring of organic water pollutants. *Journal of Environmental Monitoring* **2003,** *5*, (5), 813-822.

5. Suffet, I. H.; Jafvert, C. T.; Kukkonen, J.; Servos, M. R.; Spacie, A.; Williams, L. L.; Noblet, J. A., Synopsis of discussion session:influences of particulate and dissolved material on the bioavailability of organic compounds. In *Bioavailability:physical, chemical, and biological interactions*, Hamelink, J. L.; Landrum, P. F.; Bergman, H. L.; Benson, W. H., Eds. Lewis Publishers: Boca Raton, 1994.

6. Vrana, B.; Allan, I. J.; Greenwood, R.; Mills, G. A.; Dominiak, E.; Svensson, K.; Knutsson, J.; Morrison, G., Passive sampling techniques for monitoring pollutants in water. *TrAC Trends in Analytical Chemistry* **2005,** *24*, (10), 845-868.

7. Madrid, Y.; Zayas, Z. P., Water sampling: Traditional methods and new approaches in water sampling strategy. *TrAC Trends in Analytical Chemistry* **2007,** *26*, (4), 293-299.

8. Anderson, K. A.; Hillwalker, W. E., Ecotoxicology Bioavailability. In *Encyclopedia of Ecology*, Elsevier Publishing Inc: Oxford, in press 2008.

9. Stuer-Lauridsen, F., Review of passive accumulation devices for monitoring organic micropollutants in the aquatic environment. *Environmental Pollution* **2005,** *136*, (3), 503-524.

10. Namieśnik, J.; Zabiegała, B.; Kot-Wasik, A.; Partyka, M.; Wasik, A., Passive sampling and/or extraction techniques in environmental analysis: a review. *Analytical and Bioanalytical Chemistry* **2005,** *381*, (2), 279-301.

11. Kot-Wasik, A.; Zabiegala, B.; Urbanowicz, M.; Dominiak, E.; Wasik, A.; Namiesnik, J., Advances in passive sampling in environmental studies. *Analytica Chimica Acta* **2007,** *602*, (2), 141-163.

12. Kot, A.; Zabiegala, B.; Namiesnik, J., Passive sampling for long-term monitoring of organic pollutants in water. *TrAC Trends in Analytical Chemistry* **2000,** *19*, (7), 446-459.

13. Alexander, M., Aging, bioavailability, and overestimation of risk from environmental pollutants. *Environmental Science and Technology* **2000,** *34*, (20), 4259-4265.

14. Escher, B. I.; Hermens, J. L. M., Internal Exposure: linking bioavailability to effects. *Environmental Science and Technology* **2004,** *38*, (23), 455A-462A.

15. Górecki, T.; Namiesnik, J., Passive sampling. *TrAC Trends in Analytical Chemistry* **2002,** *21*, (4), 276-291.

16. Huckins, J. N.; Petty, J. D.; Booij, K., *Monitors of organic chemicals in the enviornment: Semipermeable membrane devices*. Springer: New York, 2006; p 223.

17. Anderson, K. A.; Sethajintanin, D.; Sower, G.; Quarles, L., Field trial and modeling of uptake rates of *in situ* lipid-free polyethylene membrane passive sampler. *Environmental Science and Technology* **2008,** *42*, (12), 4486-4493.

18. Esteve-Turrillas, F. A.; Pastor, A.; Yusà, V.; de la Guardia, M., Using semi-permeable membrane devices as passive samplers. *TrAC Trends in Analytical Chemistry* **2007,** *26*, (7), 703-712.

19. Alvarez, D. A.; Petty, J. D.; Huckins, J. N.; Jones-Lepp, T. L.; Getting, D. T.; Goddard, J. P.; Manahan, S. E., Development of a passive, *in situ*, integrative sampler for hydrophilic organic contaminants in aquatic environments *Environmental Toxicology and Chemistry* **2004,** *23*, (7), 1640-1648.

20. Huckins, J. N.; Tubergen, M. W.; Manuweera, G. K., Semipermeable membrane devices containing model lipid: A new approach to monitoring the bioavailability of lipophilic contaminants and estimate their bioconcentration potential. *Chemosphere* **1990,** *20*, (5), 533-552.

21. Esteve-Turrillas, F. A.; Yusà, V.; Pastor, A.; de la Guardia, M., New perspectives in the use of semipermeable membrane devices as passive samplers. *Talanta* **2008,** *74*, (4), 443-457.

22. Huckins, J. N.; Manuweera, G. K.; Petty, J. D.; Mackay, D.; Lebo, J. A., Lipid-containing semipermeable membrane devices for monitoring organic

contaminants in water. *Environmental Science and Technology* **1993,** *27,* (12), 2489-2496.

23. Comyn, J., *Polymer Permeability*. Elsevier Applied Science LTD: London, England, 1985.

24. Opperhuizen, A.; van der Velde, E. W.; Gobas, F. A. P. C.; Liem, A. K. D.; van der Steen, J. M. D.; Hutzinger, O., Relationship between bioconcentration in fish and steric factors of hydrophobic chemicals. *Chemosphere* **1985,** *14,* 1871-1896.

25. Booij, K.; Smedes, F.; van Weerlee, E. M., Spiking of performance reference compounds in low density polyethylene and silicone passive water samplers. *Chemosphere* **2002,** *46,* (8), 1157-1161.

26. Booij, K.; Sleiderink, H. M.; Smedes, F., Calibrating the uptake kinetics of semipermeable membrane devices using exposure standards. *Environmental Toxicology and Chemistry* **1998,** *17,* (7), 1236-1245.

27. Huckins, J. N.; Petty, J. D.; Lebo, J. A.; Almeida, F. V.; Booij, K.; Alvarez, D. A.; Cranor, W. L.; Clark, R. C.; Mogensen, B. B., Development of the permeability/performance reference compound approach for *in situ* calibration of semipermeable membrane devices. *Environmental Science and Technology* **2002,** *36,* (1), 85-91.

28. Gale, R. W., Three-compartment model for contaminant accumulation by semipermeable membrane devices. *Environmental Science and Technology* **1998,** *32,* (15), 2292-2300.

29. Adams, R. G.; Lohmann, R.; Fernandez, L. A.; MacFarlane, J. K., Polyethylene Devices: Passive samplers for measuring dissolved hydrophobic organic compounds in aquatic environments. *Environmental Science and Technology* **2007,** *41,* (4), 1317-1323.

Contributions to Chapter 2

Amanda Ackerman developed the gas chromatographic instrument methods used and carried out some of the GC data analysis and interpretation. She and Kim Anderson provided instruction on and helped to develop the experimental design. Zebrafish developmental assay results data was generated, compiled, and provided by Margaret Corvi, Wendy Hillwalker and Sarah Allan. PAH analysis by HPLC was performed and data provided by Greg Sower. Greg Sower, Doolalai Sethajintanin, and Gene Johnson also contributed to the initial steps of method development.

CHAPTER 2 – LIPID-FREE TUBE (LFT) PASSIVE SAMPLER LABORATORY METHOD DEVELOPMENT

2.1 Introduction

Contemporary environmental analysis and monitoring methods have become increasingly expensive as an outcome of continually elevating environmental quality criteria such as measuring concentrations of contaminants at increasingly low levels [1]. Sampling and sample preparation has been estimated to account for 70 to 90% of analysis time [2]. Thus, often the largest gains in analytical efficiency can be realized by reducing sample-processing times. Given this, much effort has been focused on the development of reliable sampling and sample preparation (extraction) procedures characterized by the simplicity of both the operations and the devices involved in the process [1-3]. Passive sampling seems to be a promising alternative to contemporary methods, eliminating almost every disadvantage of active extraction and/or sample preparation techniques [1, 3]. There is also an obvious need for rapid but effective low-cost integrated methods that would enable monitoring of concentrations of contaminants in the environment directly. In turn, valuable insight could be gained on the fate and distribution as well as effects and assessment of the hazards these chemicals pose to the environment and to human health through the use of passive sampling [1].

A very important and widely utilized passive sampler for measuring aqueous concentrations of hydrophobic organic contaminants (HOCs) is the semipermeable membrane device (SPMD) introduced by Huckins and co-workers [4]. Publications involving PSDs, specifically SPMDs, have increased drastically in recent years further legitimizing their use as an effective means of sampling the environment. It has been suggested [5] that there are three main reasons for this increase in publications over the last decade. First, considerable modifications had been made to the standard SPMD devices. Second, new alternative laboratory methods were investigated as sample processing can be long and tedious especially

for both dialysis and cleanup. Lastly, novel compounds to be sampled by SPMDs were assessed. Most of the studies carried out concerning SPMDs were field studies though; few research groups [6-8] have investigated new applications or method improvements for these devices [5].

SPMDs traditionally consist of additive free low-density polyethylene lay-flat tubing filled with triolein and heat-sealed at each end [1, 5, 9, 10]. Triolein is a major non-polar lipid found in aquatic organisms and is commercially available as a synthesized product [5]. As a thin film, it produces intimate contact with the LDPE, but is large enough to resist permeating the LDPE. Nonporous LDPE lay-flat tubing is used due to its stability in organic solvents which is required for cleaning the membrane before use and for extraction of analytes [10, 11]. It resists abrasion and puncturing, making it ideal for use as a membrane.

Analysis of lipid-containing PSDs (SPMDs) may be hampered by interferences from polyethylene oligomers and lipid impurities [10, 12, 13]. One or more post-dialysis clean-up methods utilizing size exclusion chromatography, also know as gel permeation chromatography (GPC), silica gel, florisil or other methods can be used to remove co-dialyzed polyethylene oligomers and the bulk of lipid materials, but small amounts of methyl oleate and oleic acid can remain and present analytical problems [10, 13]. The post-dialysis methods that attempt to reduce interferences add cost and often sacrifice target analytes. Use of LDPE as a sampling device alone, without the addition of a phase such as solvent or triolein acting a reservoir, has been investigated previously with differing but promising results [6-8, 14, 15]. The ability to use LDPE strips or tubing without a central hydrocarbon reservoir is crucial, as it would result in a simpler less expensive sampling device with the same benefits, but without sacrificing target analytes. Lipid-containing PSD represent a three-compartment model and are more difficult to model compared to the two-compartment model of a lipid-free polyethylene membrane sampler. As discussed earlier the LDPE portion of an SPMD has been shown to sequester a significant portion of the total amount of solute accumulated by an SPMD [10, 16]. This is not surprising considering polyethylene has a solute-

water partitioning coefficient similar to triolein [16]. Out of these findings and ideas the lipid-free polyethylene membrane tubing sampler (LFT) was developed and introduced by Anderson *et al.* [7].

To accurately estimate bioavailable aqueous concentrations of hydrophobic organic contaminants performance/permeability reference compounds (PRCs) are introduced into a passive sampling device. Discussed more in depth in Chapters 1 and 4, measuring PRC loss during an exposure can be used to estimate *in situ* water sampling rates and eventually the data used to back-calculate water concentrations of analytes. With a typical polymeric film-based PSD such as SPMD the reservoir medium (triolein) serves as a convenient carrier for PRCs. For triolein-free, lipid-free or solvent-less PSD this proves problematic and a new approach was needed. Booij *et al.* [8] developed an effective, but seemingly laborious method of infusing PRCs into a PE membrane. It was based upon equilibration of the membrane in a spiked methanal:water solution. Using methods such as this, and one proposed in Adams *et al.* [6], produces large volumes of solvent waste and contaminated water, and in the later approach takes 90 days to complete. Anderson *et al.* [7] proposed a much simpler, less laborious and wasteful method and was used in this study. A micro volume, less than 100 μL, was inserted into the interior of the tubing with an autopipet then the tubing heat-sealed.

Huckins *et al.* [10] showed that SPMDs can be coupled successfully to many types of bioassays: biomarker or bioindicater tests, immunoassays, and classic toxicity tests. Typically in these studies, a composite of the extracts from several field deployed SPMDs were transferred into dimethyl sulfoxide (DMSO) then applied to the assay. Coupling of PSDs and compatible bioassays may provide information on the relative toxicological significance of exposure to contaminants present in actual environmental matrices [10]. However, there have been documented complications with assays exposed to SPMD extracts. Oleic acid (an impurity found in triolein) and elemental sulfur (sequestered from the

environment) have been shown to have a role in toxicity of extracts leading to complication in assays [17, 18].

With the ever-rising interest in and use of PSDs there seems to be several main trends toward future development [19]. One is a push towards simplification and miniaturization. Smaller simpler devices are less expensive to prepare, transport, implement and process. Beyond economics, there is a desire to decrease solvent and reagent use leading to greener practices. A second trend focuses on improving robustness by reducing the potential impacts of environmental factors as well as evaluating and implementing PRCs further. Lastly, bridging research gaps by coupling PSDs with biological analysis of samples collected, potentially providing many insights including the relative toxicological significance of real-world aquatic contaminant concentrations including contaminant mixtures.

2.2 Objective

The research discussed here follows well with the current trends and future development in passive sampling technology. The main purpose of this study was to further develop, adapt and validate a new PSD, the lipid-free tube (LFT) sampler, and analytical method for measuring hydrophobic organic contaminants. Some of this work was utilized previously in Anderson *et al.* [7] which can be found in Appendix A. For this new PSD and analytical method to be viable, significant advantages in technology or techniques over current and previous PSDs should be realized. As such, LFTs and accompanying laboratory methods were developed to be simple, cost effective, and robust, while maintaining sensitivity and accuracy comparable to current PSDs. We created a specific list of criteria listed below that if attained would legitimize the new PSD method.

The newly developed PSD and method must maintain a level of specificity and sensitivity at or surpassing that of other current PSD for hydrophobic organic contaminants. For pesticides and PCBs this would be 5 ng/mL or 5 parts per billion (ppb) and 1 pg/mL or 1 parts per trillion (ppt) for PAHs. The method should be efficient with respect to cost, time/labor, and solvent use. It should

exhibit efficient recovery of analytes as defined by ≥ 70% average recovery from LFT fortified at 100 ng/mL for target analytes consisting of certain pesticides, PCBs and PAHs. The method should significantly reduce the use of chlorinated solvents by no less than 50% of current polyethylene PSD methods in exchange for less toxic alternatives; leading to a greener approach to passive sampling. Finally, zebrafish (*Danio rerio*) embryos exposed to un-fortified (no PRCs added) LFT extracts in an *in vivo* zebrafish developmental toxicity assay should show no significant biologic response above that of a 1% dimethyl sulfoxide (DMSO) vehicle control. The specific criteria aims and goals are summarized briefly in Table 2.1.

Table 2.1 Criteria for a new PSD method

Criteria For a New PSD Method		
Aim	**Specific Goal**	
Good specificity/ low extract background noise	GC/ECD pesticide LOQ ≤ 5 ng/mL (ppb)	
	HPLC PAH LOQ ~1 pg/mL (ppt)	
Efficient method	Preparation	Time/ labor savings
		Decreased solvent use
	Processing	Time/ labor savings
		Decreased solvent use
		≥ 70% recovery at 100 ng/mL
Reduction of chlorinated solvent	Reduce by ≥ 50%	
Biological model exposure to extracts	Zebrafish assay: biologic response to extracts not significantly different from DMSO control	

2.3 Materials and methods

2.3.1 PSD materials, chemicals, and solvents

Low-density polyethylene (LDPE) lay-flat tubing made from 100% virgin resin, Barefoot® brand, was obtained from Brentwood Plastics, Inc, St. Louis, MO. The lay-flat tubing has an average width of 2.5 cm, a membrane wall thickness of 75-95 μm, and transient polymer cavities of ~10 Å. All solvents used for method

development were optima or pesticide grade purchased from Fisher Scientific (Fairlawn, NJ). Standards were purchased from both Chem Service and Accustandard for pesticides, polychlorinated biphenyls (PCBs) and polycyclic aromatic hyrdocarbons (PAHs). In brief, PCB's were widely used for many applications, especially as dielectric fluids in transformers and capacitors and coolants and are described as a class of organic compounds with 1 to 10 chlorine atoms attached to biphenyl, a molecule composed of two benzene rings. PAHs are composed of two or more carbon rings fused at two carbons. They are created naturally and anthropogenically as a result of either incomplete combustion of organic material, through intense geologic pressure, or produced by certain organisms.

2.3.2 Traditional PSD preparation and processing

PSD preparation, extraction, cleanup and fractionation methods based on those developed for SPMD by Huckins and other colleagues were used and modified slightly as necessary for adaptation to our facility's technical requirements. A description of the previously developed or "original" methods follows and has been described and reviewed multiple times in the literature [4, 9, 10, 20-22].

LDPE lay-flat tubing, 2.5 cm wide with a wall thickness of 75-95 μm, was rolled by hand with light force and cleaned by a two-stage Soxhlet extraction with dichloromethane (DCM) for a total of at least 24 h. After the second extraction, the solvent-cleaned LDPE was dried by pulling a vacuum through clean polyurethane foam plugs (PUFs). The apparatus was left under vacuum for approximately 48-72 h at room temperature or until the DCM was removed from the LDPE. After dried, the tubing was stored in clean, sealed paint cans, and placed into a freezer (-20°C).

PSDs were constructed on inverted L-shaped jigs set at a distance of ~1 m. One end of the cleaned LDPE tubing was folded over itself to form a loop approximately 2.5 cm long and several heat-seals applied to join the two LDPE layers together. The loop was placed on one jig and tubing drawn over the other and folded upon itself to create another 2.5-cm long loop and cut with scissors. If

the PSD was to contain a fortification or PRCs, the tubing was removed from the jigs and the fortification or PRCs were introduced into the tubing interior through this open end as a micro-volume injection (< 100 μL) with an automated pipet. The looped end of the tubing was placed back on a jig. While holding the open end with one hand, the micro-injection was disbursed evenly by running the tubing through two fingers. Removal of air from the interior of the tubing was achieved through the same procedure. The open end of the tubing was then pinched against the jig, folded over, and heat-sealed to form a fully assembled PSD.

PSD processing consisted of a dialytic extraction using hexanes followed by clean-up and fractionation using GPC. PSD were placed in 500-mL amber jars with Teflon® lined lids and a two-stage dialysis in hexanes was performed for 24 h (18 then 6 h) each using a volume of 250 mL for one PSD or 400 mL for a composite of five. Each dialysate was decanted off into a 1000-mL round bottom flask (RBF). Extracts were concentrated by rotary evaporation to a volume of 0.5-1 mL. The extracts were then quantitatively transferred to glass centrifuge tubes and evaporated under nitrogen flow using a TurboVap®, then transferred to amber sample vials and stored at -20 ^{o}C until analysis.

Briefly, a Waters® Gel Permeation Chromatography Cleanup System equipped with a 515 HPLC pump, 2487 UV detector, and fraction collector was used for extract cleanup and fractionation. Two Environgel™ GPC Cleanup Columns (19x150 mm and 19x300 mm) were used in series. A mobile phase of 100% DCM at a flow rate of 5 mL/min was used with an injection volume of 550 μL. UV detection was set at 254 nm and a 30-min run time was used. The fraction from ~14-20 min was collected. Confirmation and validation of fraction content were performed by injecting standards of corn oil, sulfur, and analytes of interest. Collected fractions, ~30 mL, were evaporated under nitrogen flow using a TurboVap®. Finally, PSD extracts were transferred to auto-sampler vials and stored at -20 °C until analysis.

2.3.3 Instrumental analysis

Pesticides, PCBs, and other compounds compatible with election capture detection gas chromatography identification and quantification (seen in the first two columns in table 2.2) was accomplished by an Agilent 6890N GC equipped with duel μ-ECDs (electron capture detector), columns, and inlets; operated with splitless injection. The inlets are set at a temperature of 250 °C. Splitless purge flow is 40 mL/min and the purge time is 0.75 min with a sample injection volume of 2 μL. The helium carrier gas is maintained at a constant pressure of 19.5 psi. DB-17MS and DB-XLB (J&W Scientific Inc., Palo Alto, Ca) 30 m x 0.25 mm ID x 0.25 μm film thickness fused silica columns are used for quantitation and confirmation purposes respectively. The oven temperature is initially set at 110 °C with a 0.5 min hold. The temperature is ramped to 180 °C at 20 °C/min with no hold. The temperature is then ramped to 320 °C at 10 °C/min with a 10-min hold. Detectors are operated at 330 °C with a nitrogen make-up gas flow of 60 mL/min.

PAH chromatographic analysis for 15 EPA priority pollutant PAHs in Table 2.2 was performed by a Hewlett Packard 1100 series HPLC equipped with fluorescence and diode array detection and a Phenomenex Envirosep PP (125 x 4.6 mm)) column. The temperature was held at 30 °C with a mobile phase of 18 MΩ•cm water and acetonitrile at a flow rate of 2 mL/min, and an injection volume of 10 μL. The mobile phase gradient initially is 40% acetonitrile for 2 min, then ramped up to 100% acetonitrile over 25 min and held for 5 min. Detection was accomplished by a fluorescence detector with an excitation wavelength at 230 nm and emission wavelengths at 332, 405, and 460 nm, as well as diode array detection absorbance at 254 and 230 nm.

2.4 Results and discussion

2.4.1 Method development: Overview

Table 2.1 above outlines the criteria created to guide the method development process. Method development initially focused on a broad array of pesticide compounds, PCBs, and PAHs, which are complied in Table 2.2.

Table 2.2 Complete list of pesticides, PCBs and PAHs used during various stages of method development

Pesticides and break down products	Polychlorinated biphenyl	Polycyclic aromatic hydrocarbons
a-BHC	PCB 8	Naphthalene (NAP)
Lindane	PCB 49	Acenaphthylene (ACY)
b-BHC	PCB 103	Acenaphthene (ACE)
Heptachlor	PCB 100	Fluorine (FLO)
d-BHC	PCB 37	Phenanthrene (PHE)
Aldrin	PCB 101	Anthracene (ANT)
Heptachlor expoxide	PCB 77	Fluoranthene (FLA)
g-Chlordane	PCB 118	Pyrene (PYR)
a-Chlordane	PCB 82	Chrysene (CHR)
Endosulfan I	PCB 153	benz[a]anthracene (BAA)
p,p'-DDE	PCB 114	benzo[b]fluoranthene (BBF)
o,p'-DDE	PCB 105	benzo[k]fluoranthene (BKF)
Dieldrin	PCB 138	benzo[a]pyrene (BAP)
Endrin	PCB 126	benzo[ghi]perylene (BPL)
p,p'-DDD	PCB 166	indeno[1,2,3-c,d]pyrene (IPY)
Endosulfan II	PCB 180	
p,p'-DDT	PCB 128	
o,p'-DDT		
Endrin aldehyde		
Endosulfan sulfate		
Methoxychlor		
Endrin ketone		
Chlorpyrifos		
Vinclozolin		
Dicofol		
o,p'-Dicofol		
Propanil		
Dimethoate		
Cypermethrin		
Fipronil		
Fenitrothion		

2.4.2 Removing GPC as a clean-up step

During the first stage of method development for LFT preparation and processing, the aim was to remove the use of GPC as a post-dialysis clean-up step from the original method previously described and assess its requirement for sample processing. GPC as well as other chemical enrichment and fractionation processes are typically employed as a critical step in the processing of polyethylene-based PSDs. These post-dialysis processes remove analytical interferences such as co-dialyzed polyethylene oligomers and lipid impurities, in the case of SPMDs, from the sample matrix. This is especially crucial with respect to GC/ECD analysis and removal is necessary before analyses can be performed. Discussed later, HPLC analysis was not hindered to the same degree by co-dialyzed interferences from LDPE, likely due to the selectivity of detection. Since LFT are comprised of a two compartment system, an aqueous boundary layer and a polyethylene membrane, without a third phase consisting of a lipid, it was thought that the use of GPC or other chemical enrichment and fractionation processes may not be necessary in processing LFT. Also, by eliminating this step, significant decreases in sample extraction and processing time as well as chlorinated solvent usage could be realized. GPC consumes approximately 175 mL of DCM per sample, then a sample transfer and solvent exchange has to be performed consuming additional quantities of organic solvent.

It is generally accepted that the increased handling and processing inherent in these processes can result in decreased recoveries of more volatile lower molecular weight HOCs. Decreased recoveries for low molecular weight compounds were observed using the original method with GPC. Increased handling and processing as well as several concentration and sample transfer steps associated with the use of GPC are a probable source for this. Even more, the application of GPC as a sample clean-up step is laborious, time intensive, and consumes a significant volume of DCM per sample. Consequently, it was removed to evaluate possible gains in analyte recovery. An overview of the criteria that potentially could be affected is shown in Table 2.3.

Table 2.3 Overview of criteria potentially affected by removing GPC

Experiment	**Aim**	**Specific goal**	
Removing GPC	Good specificity/ low extract background noise	GC/ECD pesticide LOQ ≤ 5 ng/mL	
		HPLC PAH LOQ ~1 pg/mL	
	Efficient method	Preparation	Time/ labor savings
			Decreased solvent use
		Processing	Time/ labor savings
			Decreased solvent use
			≥ 70% recovery
	Reduction of chlorinated solvent	Reduce by ≥ 50%	

Table 2.4 Average PAH recoveries ± 1 SD for LFT: With and without GPC (n=3)

Compound	**Original Method % Recovery**	**No GPC % Recovery**
NAP	42 ± 5	73 ± 1
ACY	54 ± 4	75 ± 3
ACE	56 ± 4	75 ± 3
FLO	66 ± 5	88 ± 3
PHE	66 ± 4	86 ± 3
ANT	79 ± 4	98 ± 3
FLA	71 ± 4	89 ± 1
PYR	68 ± 4	90 ± 3
CHR	87 ± 3	98 ± 3
BAA	80 ± 4	94 ± 3
BBF	84 ± 4	96 ± 3
BKF	85 ± 4	96 ± 3
BAP	84 ± 4	99 ± 2
DBA	91 ± 4	104 ± 4
BPL	92 ± 4	110 ± 11
IPY	97 ± 14	82 ± 6
Total AVG	**75 ± 2**	**91 ± 3**

Low-density polyethylene tubing was cleaned and processed using the "original" methods previously described in Materials and Methods and also with the GPC clean-up step omitted. After processing, the extracts were analyzed by GC/ECD and HPLC. It was determined, upon inspection of chromatograms from the GC/ECD analysis, that the removal of GPC was detrimental to GC sample analysis. Figure 2.1 shows a side-by-side comparison of LFT extracts that did and did not undergo clean-up with GPC. A higher extract background level is shown with the sample that did not undergo GPC clean-up. Quantitation of some target analytes was either impossible or unreliable at environmentally relevant concentrations as is seen when comparing the LFT extract chromatogram with a chromatogram of a typical pesticide composite standard at a concentration of 10 ng/mL; several tall wide peaks co-elute with analytes of interest. One goal was to maintain an average limit of reliable quantitation (LOQ) for the GC/ECD of at least 5 ng/mL. With many interfering peaks, several of which that were obviously well above our stated LOQ target, our goal was not met.

Analysis by HPLC for PAHs, however, was unaffected with respect to limits of quantitation and general background, most likely due to the selectivity of detection. In addition, average PAH recoveries increased by 16%, much of which is a direct result of increased recoveries of low molecular weight PAHs. As summarized below (table 2.5), most of our goals were achieved simply by removing one step in the processing method. The apparent success of HPLC analysis regardless of inclusion or exclusion of the GPC clean-up step, led us to focus the majority of subsequent experiments on improving the methods for GC/ECD analysis, more specifically, pesticide analysis.

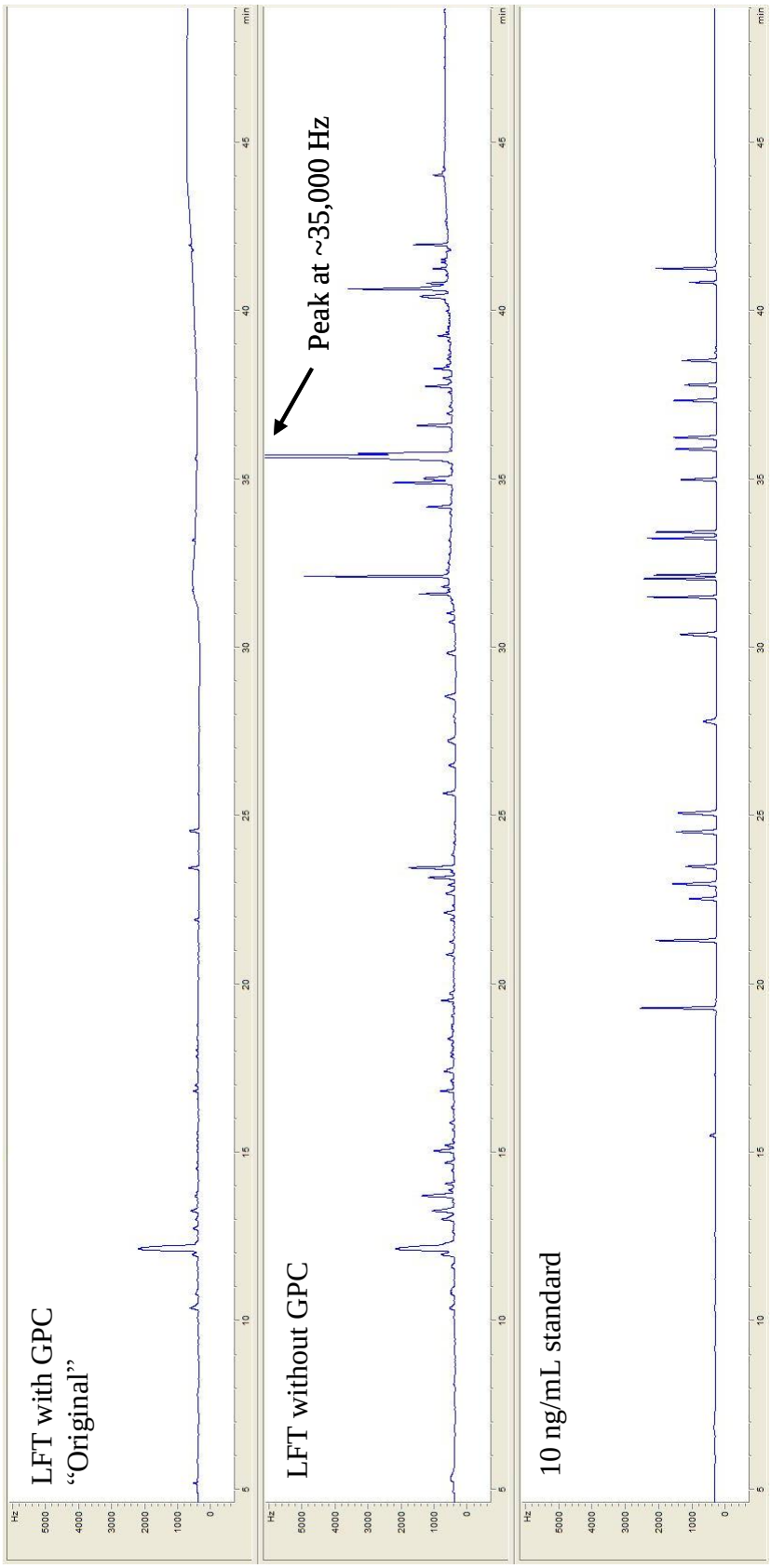

Figure 2.1 A side-by-side comparison of LFT extracts that did and did not undergo clean-up with GPC: A 10 ng/mL pesticide standard is included for additional comparison.

Table 2.5 Summary of achievement of goals through removing GPC

Criteria For a New PSD Method			Goal Met?		
Aim	**Specific Goal**		**SPMD**	**Original Method**	**Original Method: No GPC**
Good specificity/ low extract background noise	GC/ECD pesticide LOQ ≤ 5 ng/mL		Yes	Yes	No
	HPLC PAH LOQ ~1 pg/mL		Yes	Yes	Yes
Efficient method	Processing	Time/ labor savings	NA	No	Yes
		Decreased solvent use	NA	No	Yes
		≥ 70% recovery	NA	Yes*	Yes
Reduction of chlorinated solvent	Reduce by ≥ 50%		NA	No	Yes
* Low molecular weight PAHs did not meet this criteria					

2.4.3 LFT Preparation: Pre-extraction cleaning

With the potential of reducing chlorinated solvent usage, time, and labor while increasing analyte recoveries by removing the GPC, optimization and retooling of other procedures in the preparation and processing methods were investigated. The logical next step was to remove or at least decrease the chromatographic interferences hindering GC/ECD analysis. The best course of action was unclear at the time so the decision was made to start at the beginning: the pre-extraction or initial cleaning of the LDPE tubing. In the development of SPMDs, Huckins and others [10] placed careful attention to the pre-cleaning of all SPMD components, stating it "is a critical part of ensuring that potential interferences are at acceptably low levels."

Different organic solvents, solvent cleaning duration, solvent volumes and/or LDPE to solvent ratios, solvent temperature, as well as other cleaning techniques were investigated. For pre-extraction laboratory experiments, LDPE tubing was cut into ~1-m lengths, loosely rolled and placed into 250-mL RBFs, and each sample underwent two solvent exchanges for a total of three cleaning solvent volumes. The type of cleaning solvent was examined first. Hexanes and methanol were investigated: DCM was included as a reference, being used in the

original method. Previous studies [7, 10, 11] have examined the use of hexanes for cleaning LDPE for use as PSDs. Methanol was included because it was thought that it may be less aggressive towards or have a lower propensity to brake down the polymer. LDPE as well as other polymers swell while immersed in solvent. Rusina *et al.* [11] found that the more a polymer swelled the more breakable it became until the polymer was dried. Also, some solvents overtime, especially when hot, can break down the LDPE polymer matrix [10, 11]. It has been shown [11] that methanol is less absorbed (0.1%) into LDPE compared to hexanes or DCM (1.4 and 0.4%). The open-ended lengths of LDPE tubing were cleaned with three aliquots, 200 mL each, of solvent at room temperature for a total of 24 h. The RBFs were fitted with ground glass stoppers then placed on a wrist action shaker for the duration of the cleaning. Each length of tubing was subjected to a 24-h dialysis in hexanes as described earlier. Figure 2.2 shows a comparison between the three solvents. As indicated earlier and here, DCM was found ineffective at removing chromatographic interferences sufficiently. Some improvement versus early trials were seen though, which was probably a product of both a different cleaning method (previously using soxhlet extraction) and the ability for the tubing to move freely during cleaning; the benefits of which is discussed later in section 2.4.5. Cleaning with methanol yielded similar if not worse results than prior experiments. In figure 2.2, the large peak that begins at ca. 35.5 min was approximately 0.5 min long enveloping a major analyte, p,p' DDD, and reached a peak height (113,000 Hz) not seen in previous trials. Throughout method development, the peak at this retention time along with the few other peaks common to all three chromatograms below, proved challenging to reduce to acceptable levels. Compared to the other two solvents, hexanes-cleaned tubing showed marked improvement. Interfering peaks were significantly decreased in both peak area and total number with the use of hexanes; some of the larger peaks decreased by more than thirteen times verses DCM. In addition, general low-level background noise decreased.

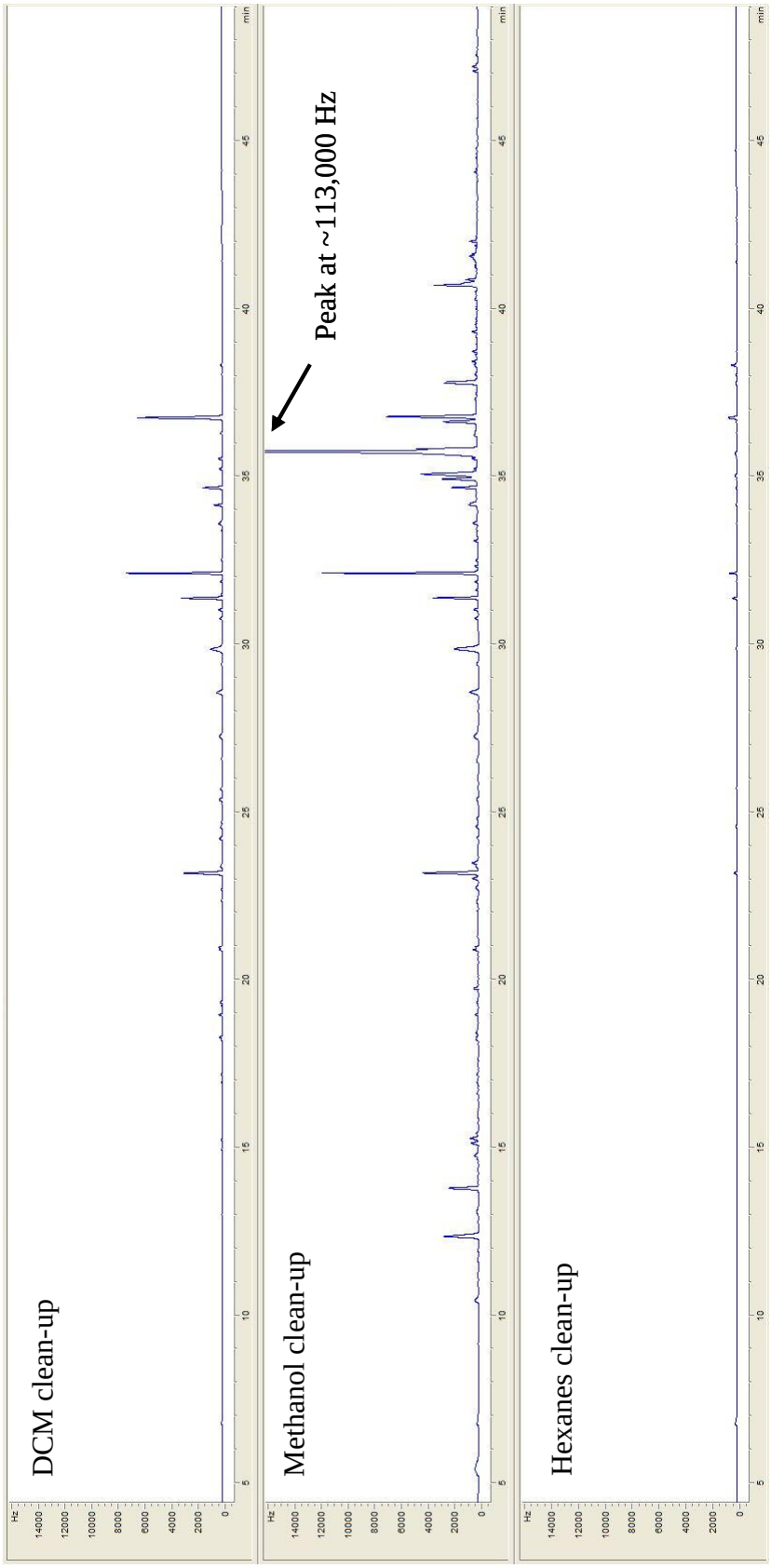

Figure 2.2 Side-by-side comparison of GC/ECD chromatograms for three cleaning solvents: DCM, methanol, and hexanes

Following these promising results, different pre-extraction cleaning techniques were investigated to improve analysis further. The first of which was to heat and agitate hexanes, possibly mimicking a Soxhlet type extraction, and then contrast it to a simple soaking in non-mixing (still) room temperature hexanes. Heated solvent, to clean LDPE prior to use as a PSD, has been used previously by other researchers [10, 11]. Tubing was either; (1) inserted into a flat bottom flask, suspended above the bottom and then placed on a magnetic stir hot plate for 24 hours with a Teflon coated stir rod inserted to agitate the solvent held at 25 to 28°C, or (2) placed into a RBF and left still besides two solvent exchanges. A dialysis was performed as described earlier. Tubing cleaned using heated and agitated hexanes showed a five-fold increase in the levels of interfering peaks, as compared to a simple soaking in still hexanes, chromatograms not shown. This and the previous results from the solvent comparison confirmed that a simple soaking in still or agitated hexanes was best for the pre-extraction cleaning of LDPE. Since it was easiest to implement in the laboratory for a large scale batch pre-extraction cleaning, a still soaking was chosen as the preferred method. Comparison of the chromatogram from a original method processed LFT (with GPC clean-up) from figure 2.1 to that of the hexanes clean-up in figure 2.2, noting the different y-axis range, shows similar matrix background levels. It turns out that some of our GC target analytes still had co-eluting interferences that were not low enough to maintain or achieve our desired sensitivity. Cleaning duration was investigated next as a means to further improve analysis.

A small-scale study was carried out to evaluate the optimum length of cleaning time. We looked at the trade offs between time savings and low background and at eliminating interfering peaks. RBFs were prepared as before with ~1 m of tubing using three total aliquots (two exchanges) of hexanes over a wide range of cleaning times. Total cleaning times of 1, 6, 12, 18, 24, and 48 h were considered; all other variables held constant. A cleaning time of 48 h provided the lowest background and decreased interfering peaks, thus it was chosen. This was confirmed by a second experiment containing several more

replicates each of the 24 and 48 h. There seemed to be an apparent general trend of decreasing background with increasing cleaning time; data not shown. Pre-extraction cleaning experiments and general results are summarized below in table 2.6.

Table 2.6 Summary of results from pre-extraction cleaning experiments

Experiment	**Goal met?**		
Pre-extraction cleaning solvent	Low extract background noise/ reduction of interferences for GC/ECD	Time/Labor savings	Decreased solvent use
DCM	No		
Methanol	No		
Hexanes	Yes (some co-eluting peaks)		
Pre-extraction cleaning techniques			
Agitation with elevated temperature	No	No	NA
Shaker agitation room temperature	Yes	No	NA
Still soak room temperature	Yes	No	NA
Pre-extraction cleaning duration			
1, 6, 12, 18, 24, and 48 h	48 h (best)	No	NA

Table 2.6 shows that we were able to achieve one of our major goals through optimizing the pre-extraction cleaning when GPC was removed for laboratory samples. For GC/ECD analysis, background was sufficiently low to quantify at relevant concentrations. Limits of detection and limits of quantitation were not determined until after most of the experiments were concluded and only qualified estimations of these parameters could be made at this time. Time and labor needed for pre-cleaning of LDPE was similar to other studies [10] which generally range from 24 to 72 h. Solvent usage could not be commented on here

but is discussed in section 2.4.5 in the context of a large-scale batch cleaning. The optimized method for pre-extraction cleaning thus far consisted of a 48-h cleaning in still hexanes with two solvent exchanges.

2.4.4 LFT Processing: Dialysis

Another main goal was to increase the efficiency of the processing method, in particular, extraction of analytes of interest from the sampler medium. Traditionally, this is performed through dialytic extraction of the PSD using a non-polar solvent. Other methods for extraction of analytes have been investigated and a summarized very well in Esteve-Turrillas *et al.* [5]. Microwave assisted extraction has garnered some attention with the ability to cut extraction time per sample to ~5-60 min. Yusá *et al*. [23] proposed the use of MAE for the extraction of HOCs from SPMDs. Microwave assisted extraction does suffer from analyte instability and high matrix co-extraction. A second method is accelerated solvent extraction, which we investigated previously [7] and was first used by Wenzel *et al.* [24] in 2004 for SPMD. It too uses less solvent than traditional methods and takes around 40 min per sample. Some drawbacks to this approach are analyte instability, matrix co-extraction, as well as a significant financial investment to acquire the equipment to carry out the extraction. Though this method only takes 40 min per sample, when conducting a monitoring study with many samples, very little if any time would be saved. Another method using sonication provides a more simple procedure than the previous two. Setková *et al*. [25] describes a method where SPMD were cut lengthwise and both the LDPE membrane and triolein were placed in an appropriate vessel submerged by solvent and placed in an ultrasound water bath for three extractions at 20 min each. Sonication does not significantly reduce solvent usage, has high sample manipulation and matrix co-extraction, but does decrease extraction time. For SPMD a clean-up step such as GPC is absolutely needed. While most of these alternative extraction methods have the potential to reduce solvent usage and extraction time, they all produce a high level of co-extracted matrix. Without a sample clean-up step, these methods are unacceptable and dialysis proved to be the best extraction method to use.

Previously the dialysis was 24 h, comprised of two stages; an 18-h stage for the bulk and a 6-h stage for the remainder. The soundness of this previous dialysis was investigated here. Dialysis duration was viewed as the most crucial step and as such was investigated first. An emphasis was place on maximizing analyte recoveries while minimizing chromatographic interferences and background.

Tubing, pre-cut to ~105 cm, was prepared individually using the optimized pre-extraction cleaning method. The tubing was heat-sealed 2.5 cm from one end, which had been folded to make a small loop. A standard suite of 18 pesticide compounds with a final concentration at the instrument of 100 ng/mL was inserted in the tubing interior as a micro-volume (20 μL) with an automated pipet. Air was removed from the tubing interior and the open end heat sealed like the other to form a completed LFT. Each LFT was then rolled up and placed in 50-mL glass amber jars with Teflon® lined lids. Each stage of the dialysis used 40-50 mL of hexanes, leading to a sample volume of 80 to 100 mL. It was determined previously [7], that dialytic recoveries for HOCs, especially low molecular weight volatile compounds, could be increased by cutting the LFT prior to dialysis. Several iterations of polyethylene passive samplers have used polyethylene strips [6, 8] instead of tubing with success, but none were processed without a sample clean-up step such as GPC. In light of those studies and our previous findings, LFT were cut prior to dialysis by grasping both looped ends, thus folding it in half, then folding it again to put both loops and the middle of the LFT together. A clean blade, rinsed in acetone and hexanes was used to cut each loop in half and the LFT in half, leaving two 0.5-m tubes. A small (~1-cm) cut was placed in the LFT just inside the heat seal on each end to ensure easy penetration of hexanes during dialysis throughout. This method was used for four different dialysis durations; 6, 12, 18, and 24 h. Individual analyte and total average percent recoveries for this experiment are listed in Table 2.7.

Table 2.7 Selected analyte recoveries for dialytic extraction duration

Compound	**Duration of dialysis**			
	6-hr	12-hr	18-hr	24-hr
a-BHC	67	68	72	70
Lindane	72	76	83	79
Heptachlor	69	71	82	80
Aldrin	66	67	79	76
Heptachlor epoxide	70	72	83	81
g-Chlordane	68	69	82	79
a-Chlordane	69	70	82	80
Endosulfan I	80	87	100	93
p,p'-DDE	74	76	85	83
Dieldrin	72	94	104	87
Endrin	86	87	97	96
p,p'-DDD	76	79	86	88
Endosulfan II	76	71	89	87
p,p'-DDT	72	66	79	80
Endrin aldehyde	69	73	85	82
Endosulfan sulfate	76	75	82	82
Methoxychlor	80	78	92	92
Endrin ketone	71	71	69	73
Total AVG	**73**	**75**	**85**	**83**

Recoveries alone are not as indicative as would be thought to the data analysis process as a whole. As the time of duration increased generally so did co-dialyzed interfering compounds leading to a rise in background. A 6-h dialysis in hexanes was chosen as it had the best mix of recoveries, time savings, and low background noise.

From the preceding small-scale experiments we were able to remove the use of chlorinated solvent entirely from the method and replace them with a better alternative solvent, hexanes. Soxhlet extraction for pre-extraction cleaning of LDPE was replaced by a simple method of soaking in hexanes. Analytical recoveries were improved and surpassed our goals while keeping co-dialyzed interferences to a minimum using a 6-h two-stage dialytic extraction in hexanes. Our goal of removing GPC without adversely affecting sample analysis was a qualified success for both GC/ECD and HPLC analysis.

2.4.5 LFT batch preparation and processing

The prior pre-extraction cleaning experiments dealt with relatively short lengths of tubing (~1 m); to be viable for real world applications such as monitoring studies and site assessments, large batches consisting of many meters of tubing need to be cleaned simultaneously. To assess batch LFT preparation or pre-extraction cleaning initially, LDPE tubing was rolled somewhat loosely until a diameter of 9 cm was achieved. A roll was placed in a 500-mL short wide-mouth glass jar and the jar filled with hexanes until the roll was sufficiently covered. The tubing sat uninterrupted besides two solvent exchanges. The roll was removed from the hexanes and dried under vacuum for ~72 h. A noticeable greasy film was deposited inside the glass jar; on the bottom and ~1 cm high on the sides, suggesting that compounds, likely residual uncured oligomers and polyethylene waxes, were being extracted from the tubing and deposited on the inside of the jar and potentially re-deposited on the tubing itself. Significant increases in both background and chromatographic interferences can be seen compared to the small-scale study. Originally, it was thought that these interferences were caused by re-deposition of extracted compounds. As is shown later, the increase in interferences were rather likely a product of inadequate cleaning.

A different approach was investigated which was previously incorporated into the method development: agitation. The basis behind this was to keep the interfering compounds extracted from the tubing dissolved in the hexanes, thus limiting possible re-contamination of the LDPE. Also, a platform was placed inside the jar that the tubing roll was placed upon. The platform was constructed from steel wire mesh cut into a square then the corners bent at 90° angles to create legs as seen in Figure 2.3. The platform allowed the tubing to remain suspended above the jar bottom as a further contamination prevention measure. The tubing was prepared as before in 500-mL wide-mouth jars, but was placed on an orbital shaker at ~130 rpm for the duration of the cleaning. This approach did not show the same greasy film on the bottom of the jar, but also did not significantly improve chromatography over the previous batch experiment (chromatograph not

shown). This may indicate that the hexanes were extracting interfering compounds and retaining them, but solvent was not penetrating and cleaning the entire roll thoroughly. Evidence is provided by the previous smaller scale experiments where the tubing was loosely rolled and placed in RBFs. In the small-scale experiments, sample extracts show a much lower number of interfering peaks as well as lower peak heights.

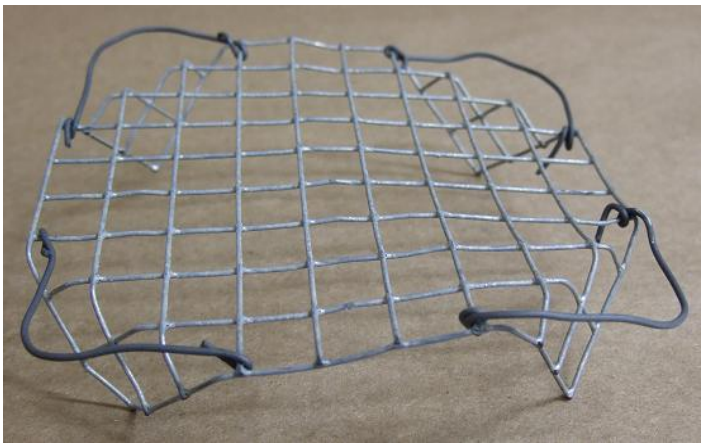

Figure 2.3 Platform for pre-extraction cleaning

As a result of these failed attempts, attention shifted to how tightly the tubing was rolled, the amount of free space the roll was allowed to expand and/or unravel into the jar, and to increasing the solvent to tubing ratio. Tubing was loosely rolled until ~9-cm in diameter (approximately 20 m). A roll was placed into a 2-L glass jar on top of a platform, hexanes added to submerge the roll, and the jar placed on an orbital shaker. As the tubing unraveled more hexanes was added as necessary to keep the tubing submerged. Following a 48-h cleaning with two solvent exchanges, tubing was removed and dried under vacuum. After drying, a white cloudy residue was observed in an irregular fashion on the inside of the tubing. After analysis by GC/ECD, chromatography was not improved, but similar to other batch experiment results. A significant amount of hexanes (actual volume not known) enters into the tubing interior either through diffusion or through the open ends of the roll during this process. In retrospect, as the solvent

evaporated from the tubing interior, likely extracted compounds were concentrated and re-deposited on the interior producing the residue.

Subsequently, hexanes was excluded from the interior of the tubing, by sealing the ends of the tubing being rolled, and the LFT was not cut prior to the dialysis. It was thought that possibly these analytically interfering compounds from the interior of the tube would not be co-dialyzed when processing the LFT. The compounds may be too large to diffuse through the entire polymer membrane thus excluding them for being co-dialyzed. Tubing was prepared exactly as the previous experiment dictated, except the ends of the roll were heat sealed to prevent hexanes from entering the interior of the tubing and depositing a residue. During pre-cleaning of the tubing roll, which was ~15 to 20 m in length, the interior of the tubing accumulated about 200 mL of hexanes. Either the tubing's transient cavities allowed absorption and penetration of hexanes through the membrane, which has been observed previously [10], or there were holes in the tubing itself, which has been occasionally observed in the past. The heat-sealed ends were clipped off with scissors after pre-extraction cleaning and the tubing was re-rolled tightly to remove hexanes. Upon drying, no noticeable residue was left on the tubing interior. Chromatographic analysis showed similar results as the previous experiments: significantly high background and large co-eluting peaks. This implies that these compounds are small enough to navigate through the LDPE polymer matrix and into the sample extract during dialysis, resulting in poor chromatography.

Next, tubing was loosely rolled, left open on each end and was placed on a platform in a glass jar. Hexanes were added as before and the jar was set on an orbital shaker for 48 h at ~130-170 rpm. It was observed throughout these experiments, that during the cleaning procedure, the center of the roll would not always unravel. This may have lead to non-uniform cleaning of the rolls. At the completion of the hexanes soak, tubing was re-rolled backwards very tightly as before to remove most of the solvent residing inside the tubing. Analysis by GC/ECD of extracted tubing showed promising results for some samples, as the

major interfering peaks were either absent or significantly reduced and background noise decreased as well. Chromatograms for several replicates were inconsistent though. The degree to which interferences were reduced appeared to be dependent upon the section of the LDPE roll the LFT was made from either outside, middle, or inside and was investigated further.

Two replicates were constructed each from either the original outer edge, halfway into, or the middle of the roll. Considerably less contamination (possibly side by side chromatogram figure) was seen with the outer and half-way-in sections of tubing when compared to the middle of the roll. This confirmed the earlier suspicions of non-uniform cleaning. For subsequent batch pre-extraction cleanings, the tubing rolls were re-rolled loosely backwards after the first pre-extraction volume of hexanes (at ~18 to 24 h) to invert the roll to combat this. Analysis of extracted samples prepared by inverting the roll during the cleaning revealed background noise had decreased to levels present in the previous single tube experiments using the optimized method. The large interfering peaks were also decreased significantly.

2.4.6 Detection and quantitation limit determination

Analytical detection limits for GC/ECD analysis were determined by overspiking a low-level standard into non-spiked LFT extracts (n=7). Instrument generated areas for each compound were multiplied by their respective response factors and averaged for each analyte. The standard deviation of each average was multiplied by 3.143 (t-distribution at 99%) to give the limit of detection and multiplied by 10 to give the limit of quantitation. The limits of detection and quantitation are shown below in table 2.8. Average limit of detection was 0.7 ng/mL and average limit of quantitation was 2.4 ng/mL and all but three analytes are at or below the goal of 5 ng/mL. Associated with these analytes are low level chromatographic interferences.

Table 2.8 Limits of detection and quantitation for GC/ECD analysis

Compound	Limits of detection (ng/mL)	Limits of quantitation (ng/mL)
Tetrachloro-m-xylene	0.1	0.5
a-BHC	0.1	0.4
Pentachloronitrobenzene	0.4	1.4
Lindane	0.2	0.8
b-BHC	0.4	1.4
Heptachlor	0.3	0.9
Vinclozolin	1.0	3.0
d-BHC	0.4	1.2
Aldrin	0.5	1.6
Propanil	0.9	2.8
Chlorpyrifos	0.6	2.0
PCB 103	0.5	1.6
PCB 100	0.8	2.5
Heptachlor Epoxide	0.2	0.5
g-Chlordane	0.5	1.5
a-Chlordane	0.7	2.3
Endosulfan I	0.8	2.6
DDE	1.5	4.7
Dieldrin	1.3	4.2
Endrin	1.8	5.8
DDD	1.9	6.2
Endosulfan II	1.4	4.4
DDT	1.8	5.7
Endrin aldehyde	0.9	2.8
Endosulfan sulfate	0.6	2.0
Dibutylchlorendate	0.8	2.4
Methoxychlor	0.5	1.5
Endrin ketone	0.3	0.9
Decachlorobiphenyl	0.3	0.9
Deltamethrin	0.7	2.3
Average	**0.7**	**2.4**

2.4.7 Addition of PRCs

For application to a field sampling regime using LFT or similar PE-based PSDs, PRCs are added to assess the time-weighted average water concentrations. Potential PRCs (shaded in grey in table 2.9 below) were included along with a large number of pesticides to evaluate extraction recoveries with the current optimized method detailed above. The initial results can be seen in the column titled "Normal" in table 2.9. A goal of 70% recovery indicated in table 2.1 was not reached for all components. Unfortunately, several of the potential PRCs especially those with higher log K_{ow} values were not near the ideal 100% recovery level. Since water concentration calculations, which are discussed in depth in Chapter 4, assume 100% extraction of LFT accumulations or in this case, spikes, significant derivation from 100% would result in a misrepresentation of the actual values unless values are recovery corrected.

Three simple modifications to the dialysis method were investigated to potentially increase extraction efficiency. The fist modification used LFT that were prepared and cut as described previously but instead of a dialytic extraction in still hexanes, LFT submerged in hexanes in an amber jar were placed on an orbital shaker (~100 RPM) for the duration of the 6-h two-stage dialysis. The second, was to take the LFT after it is cut in half to take both halves and cut along one of the creases to create "V". These LFT remained non-agitated for the entire dialysis experiment. It was thought that perhaps the hexanes could access the entire surface of the LFT better and during the decanting stage would allow more of the solvent to be removed from the jar and interior of the tubing. A third approach took both previous modifications and combined them. Briefly, LFT were cut along one seam and submerged by hexanes in a glass jar then placed on an orbital shaker. Modified dialysis results are shown in table 2.9. Focusing on the total average recoveries, we see that both methods involving cutting the LFT lengthwise along one seam to create a "V" showed marked improvement over the previously optimized method; 25 and 24% as an average for still and orbital shaker respectively. Seen previously during the preparation pre-extraction cleaning method development, agitation

produced with an orbital shaker can increase co-dialyzed interfering compounds. Since appreciable increase in analyte and PRC recoveries were not realized with the addition of the orbital shaker, as shown when comparing the last two columns in table 2.9, the decision was made to exclude it from the final dialysis method. A few compounds show greater than 100% recovery for the three modifications. When d-BHC and Endrin are removed from the overall averages, little change in recoveries is observed (82, 96 and 96% for modifications 1, 2 and 3 respectively).

Table 2.9 Analyte recoveries for dialysis modification experiments. PRCs are shaded as grey.

Compound	Normal	Modification (1) Orbital shaker normal cut	Modification (2) Still length-wise cut	Modification (3) Orbital shaker length-wise cut
Tetrachloro-m-xylene	83 ± 2	87 ± 1	89 ± 4	95 ± 2
a-BHC	73 ± 1	80 ± 3	80 ± 2	84 ± 1
Pentachloronitrobenzene	81 ± 4	82 ± 2	88 ± 4	93 ± 2
Lindane	79 ± 1	87± 0	90 ± 5	89 ±
b-BHC	82 ± 2	88 ± 3	89 ± 3	92 ± 2
Heptachlor	72 ± 3	84 ± 0	102 ± 7	99 ± 1
Vinclozolin	75 ± 2	83 ± 3	89 ± 3	90 ± 2
d-BHC	91 ± 3	121 ± 33	142 ± 33	133 ± 28
Aldrin	65 ± 4	76 ± 0	91 ± 5	89 ± 1
Propanil	87 ± 2	95 ± 1	93 ± 2	95 ± 1
Chlorpyrifos	81 ± 3	94 ± 1	110 ± 4	111 ± 2
PCB 103	84 ± 2	88 ± 5	102 ± 5	104 ± 2
PCB 100	85 ± 2	89 ± 3	101 ± 3	104 ± 2
Heptachlor Epoxide	68 ± 3	80 ± 1	94 ± 4	93 ± 2
g-Chlordane	66 ± 4	78 ± 1	92 ± 4	91 ± 1
a-Chlordane	66 ± 4	78 ± 0	92 ± 3	91 ± 2
Endosulfan I	76 ± 4	88 ± 7	115 ± 13	104 ± 5
DDE	76 ± 3	85 ± 0	93 ± 3	93 ± 2
Dieldrin	70 ± 3	83 ± 1	100 ± 3	96 ± 1
Endrin	84 ± 2	91 ± 6	125 ± 3	112 ± 6
DDD	76 ± 2	87 ± 1	97 ± 2	97 ± 2
Endosulfan II	72 ± 3	84 ± 1	98 ± 3	97 ± 2
DDT	75 ± 4	85 ± 1	95 ± 2	96 ± 1
Endrin aldehyde	49 ± 4	61 ± 0	81 ± 0	80 ± 6
Endosulfan sulfate	77 ± 2	89 ± 1	107 ± 1	108 ± 2
Dibutylchlorendate	57 ± 6	53 ± 15	95 ± 1	99 ± 2
Methoxychlor	76 ± 3	88 ± 0	109 ± 3	108 ± 2
Endrin ketone	72 ± 3	86 ± 3	97 ± 4	98 ± 5
PCB 209	71 ± 2	74 ± 10	99 ± 3	101 ± 0
Deltamethrin	65 ± 6	74 ± 3	104 ± 4	108 ± 1
Total AVG	**74 ± 3**	**84 ± 3**	**99 ± 5**	**98 ± 3**

2.4.8 Final LFT method overview

The final optimized LFT laboratory method consisted of the following: (1) A pre-extraction cleaning of LDPE rolls in hexanes on an orbital shaker for 48 h with 3 total solvent volumes (two solvent exchanges). (2) After LFT are either exposed in the environment or laboratory experiments are carried out the LFT are cut lengthwise along a seam to create a "V" and extracted by dialysis with hexanes for 6 h (two-stage 4 h then 2 h) in still hexanes. Dialysates are combined and evaporated to a desired volume and analyzed by chromatographic means.

2.4.9 LFT Exposure to a biological model

The following data presented was generated by Margaret Corvi, Wendy Hillwalker, and Sarah Allan and used with permission.

A morphological assessment using zebrafish embryos was employed for our biological model. Laboratory procedures have been published and described previously [26]. The embryos were dechorionated prior to exposure. Exposures were started prior to organogenesis (before ~6 hours post fertilization). Fish water was used as a control as was DMSO (a vehicle control) at a concentration of 1%. DMSO at this concentration in fish water has been shown to not adversely affect zebrafish development [27].

Non-spiked (blank) LFT extracts (composite of 5LFT extracts) and controls were evaluated for morphological effects to verify that embryo exposure to LFT extracts did not cause significant biological response compared to controls. Several replications of an initial (before exposure) 24 fish per replication were performed for LFT extracts (n=15) as well as fish water (n= 16) and DMSO controls (n=5). Morphological effects determination included many endpoints but only a mortality, yolk sac defect, pericardial edema, and curved body axis are discussed here. Figure 2.4 shows the results of these exposures. We saw no significance difference between in biological response between non-spiked LFT extracts and controls. These graphs and results are representative of the biological response of all endpoints.

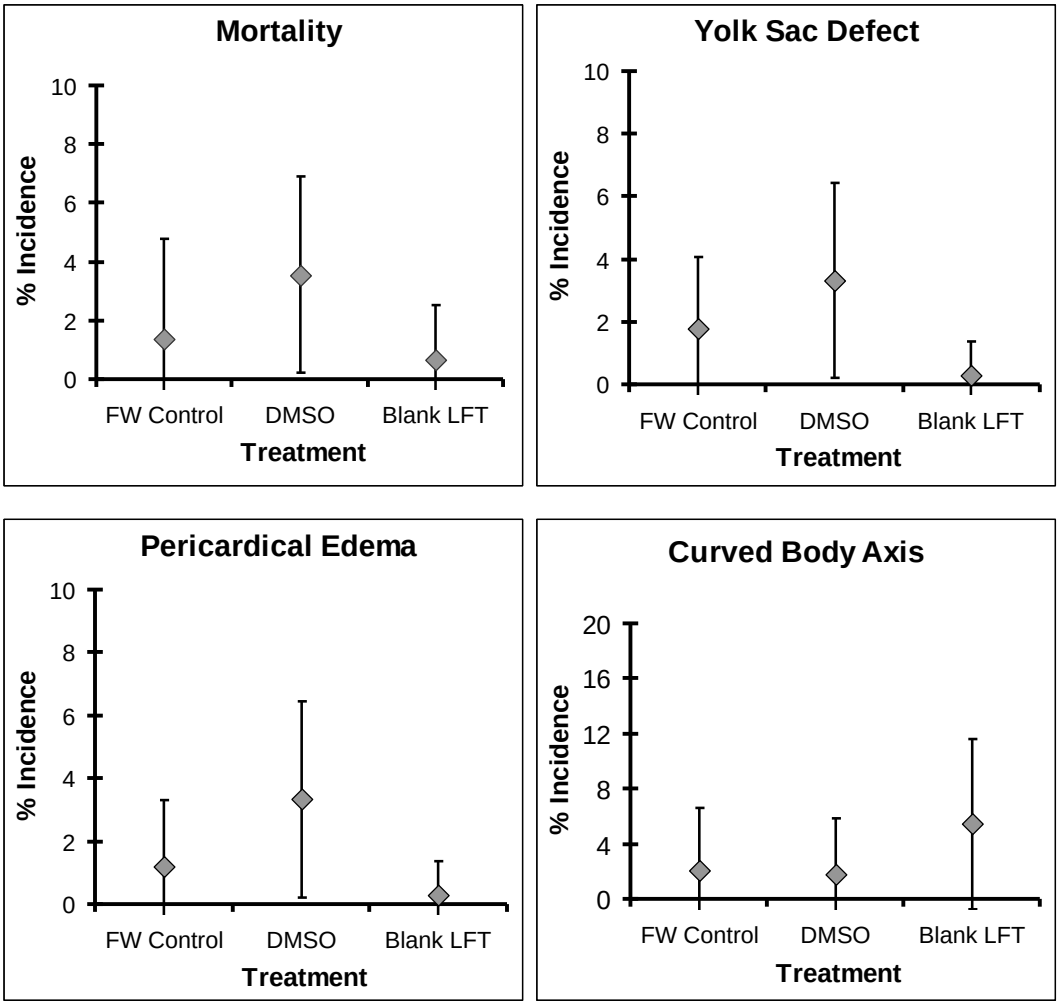

Figure 2.4 Percent incidence of biologic response for four toxicity endpoints of three different treatments

2.5 Conclusions

There is and ever-increasing interest in and use of passive sampling devices. Several current and future trends have been established as result: simplification (of devices and laboratory methods), miniaturization, increasing robustness, and bridging research gaps by coupling PSDs with biological analysis of samples. Our work here follows well with these trends. LFT and the laboratory preparation and processing methods developed provide an attractive and simpler alternative to current and traditional PSD. Though our preparation method for LFT

is similar in efficiency to other PSD with respect to time and solvent use, DCM, a chlorinated solvent, used previous was replace by a more favorable solvent, hexanes. All other goals set forth in the method development process were either met or exceeded. LFTs also show promise in the evaluation of real-world aquatic contaminant mixtures by coupling them with a zebrafish developmental toxicity assay. A summary of our criteria for a modified PSD laboratory and analytical method is seen below in table 2.10. Also listed in the table are the general results from the method development process.

Table 2.10 Summary of criteria and goal outcomes for developing a new PSD method

Criteria For a New PSD Method			**Goal Met?**		
Aim	**Specific Goal**		**SPMD**	**Original Method: No GPC**	**New LFT Method**
Good specificity/ low extract background noise	GC/ECD pesticide LOQ ≤ 5 ng/mL		Yes	No	Yes
	HPLC PAH LOQ ~1 pg/mL		Yes	Yes	Yes
Efficient method	Preparation	Time/ labor savings	NA	No	No
		Decreased solvent use	NA	No	No
	Processing	Time/ labor savings	NA	Yes	Yes
		Decreased solvent use	NA	Yes	Yes
		≥ 70% recovery	Yes	Yes*	Yes
Reduction of chlorinated solvent	Reduce by ≥ 50%		NA	Yes	Yes
Biological model exposure to extracts	Zebrafish assay: biologic response to extracts not significantly different from DMSO control		NA	Yes	Yes

* Low molecular weight PAHs did not meet this criteria

2.6 Literature Cited

1. Namieśnik, J.; Zabiegała, B.; Kot-Wasik, A.; Partyka, M.; Wasik, A., Passive sampling and/or extraction techniques in environmental analysis: a review. *Analytical and Bioanalytical Chemistry* **2005,** *381*, (2), 279-301.

2. Górecki, T.; Namiesnik, J., Passive sampling. *TrAC Trends in Analytical Chemistry* **2002,** *21*, (4), 276-291.

3. Kot-Wasik, A.; Zabiegala, B.; Urbanowicz, M.; Dominiak, E.; Wasik, A.; Namiesnik, J., Advances in passive sampling in environmental studies. *Analytica Chimica Acta* **2007,** *602*, (2), 141-163.

4. Huckins, J. N.; Tubergen, M. W.; Manuweera, G. K., Semipermeable membrane devices containing model lipid: A new approach to monitoring the bioavailability of lipophilic contaminants and estimate their bioconcentration potential. *Chemosphere* **1990,** *20*, (5), 533-552.

5. Esteve-Turrillas, F. A.; Yusà, V.; Pastor, A.; de la Guardia, M., New perspectives in the use of semipermeable membrane devices as passive samplers. *Talanta* **2008,** *74*, (4), 443-457.

6. Adams, R. G.; Lohmann, R.; Fernandez, L. A.; MacFarlane, J. K., Polyethylene Devices: Passive samplers for measuring dissolved hydrophobic organic compounds in aquatic environments. *Environmental Science and Technology* **2007,** *41*, (4), 1317-1323.

7. Anderson, K. A.; Sethajintanin, D.; Sower, G.; Quarles, L., Field trial and modeling of uptake rates of *in situ* lipid-free polyethylene membrane passive sampler. *Environmental Science and Technology* **2008,** *42*, (12), 4486-4493.

8. Booij, K.; Smedes, F.; van Weerlee, E. M., Spiking of performance reference compounds in low density polyethylene and silicone passive water samplers. *Chemosphere* **2002,** *46*, (8), 1157-1161.

9. Huckins, J. N.; Manuweera, G. K.; Petty, J. D.; Mackay, D.; Lebo, J. A., Lipid-containing semipermeable membrane devices for monitoring organic contaminants in water. *Environmental Science and Technology* **1993,** *27*, (12), 2489-2496.

10. Huckins, J. N.; Petty, J. D.; Booij, K., *Monitors of organic chemicals in the enviornment: Semipermeable membrane devices*. Springer: New York, 2006; p 223.

11. Rusina, T. P.; Smedes, F.; Klanova, J.; Booij, K.; Holoubek, I., Polymer selection for passive sampling: A comparison of critical properties. *Chemosphere* **2007,** *68*, (7), 1344-1351.

12. Gustavson, K. E.; DeVita, W. M.; Revis, A.; Harkin, J. M., Novel use of a dual-zone restricted access sorbent: normal-phase solid-phase extraction separation of methyl oleate from polynuclear aromatic hydrocarbons stemming from semi-permeable membrane devices. *Journal of Chromatography A* **2000,** *883*, 143-149.

13. Lebo, J. A.; Almeida, F. V.; Cranor, W. L.; Petty, J. D.; Huckins, J. N.; Rastall, A.; Alvarez, D. A.; Mogensen, B. B.; Johnson, B. T., Purification of triolein for use in semipermeable membrane devices (SPMDs). *Chemosphere* **2004,** *54*, (8), 1217-1224.

14. Booij, K.; Hofmans, H. E.; Fischer, C. V.; Van Weerlee, E. M., Temperature-dependent uptake rates of nonpolar organic compounds by semipermeable membrane devices and low-density polyethylene membranes. *Environmental Science and Technology* **2003,** *37*, (2), 361-366.

15. Carls, M. G.; Holland, L. G.; Short, J. W.; Heintz, R. A.; Rice, S. D., Monitoring polynuclear aromatic hydrocarbons in aqueous environments with passive low-density polyethylene membrane devices. *Environmental Toxicology and Chemistry* **2004,** *23*, (6), 1416-1424.

16. Gale, R. W., Three-compartment model for contaminant accumulation by semipermeable membrane devices. *Environmental Science and Technology* **1998,** *32*, (15), 2292-2300.

17. Sabaliunas, D.; Ellington, J.; Sabaliuniene, I., Screening of bioavailable hydrophobic toxicants in surface waters with semipermeable membrane devices: Role of inherent oleic acid in toxicity evaluations. *Ecotoxicology and Environmental Safety* **1999,** *44*, 160-167.

18. Sabaliunas, D.; Lazutka, J.; Sabaliuniene, I., Acute toxicity and genotoxicity of aquatic hydrophobic pollutants sampled with semipermeable membrane devices. *Environmental Pollution* **2000,** *109*, 251-265.

19. Vrana, B.; Allan, I. J.; Greenwood, R.; Mills, G. A.; Dominiak, E.; Svensson, K.; Knutsson, J.; Morrison, G., Passive sampling techniques for monitoring pollutants in water. *TrAC Trends in Analytical Chemistry* **2005,** *24*, (10), 845-868.

20. Esteve-Turrillas, F. A.; Pastor, A.; Yusà, V.; de la Guardia, M., Using semi-permeable membrane devices as passive samplers. *TrAC Trends in Analytical Chemistry* **2007,** *26*, (7), 703-712.

21. Huckins, J. N.; Petty, J. D.; Lebo, J. A.; Almeida, F. V.; Booij, K.; Alvarez, D. A.; Cranor, W. L.; Clark, R. C.; Mogensen, B. B., Development of the permeability/performance reference compound approach for *in situ* calibration of semipermeable membrane devices. *Environmental Science and Technology* **2002,** *36*, (1), 85-91.

22. Booij, K.; Sleiderink, H. M.; Smedes, F., Calibrating the uptake kinetics of semipermeable membrane devices using exposure standards. *Environmental Toxicology and Chemistry* **1998,** *17*, (7), 1236-1245.

23. Yusà, V.; Pastor, A.; Guardia, M. d. l., Microwave-assisted extraction of OCPs, PCBs and PAHs concentrated by semi-permeable membrane devices (SPMDs). *Analytica Chimica Acta* **2005,** *540*, (2), 355-366.

24. Wenzel, K. D.; Vrana, B.; Hubert, A.; Schuurmann, G., Dialysis of persistent organic pollutants and polycyclic aromatic hydrocarbons from semipermeable membranes. A procedure using an accelerated solvent extraction device. *Analytical Chemistry* **2004,** *76*, (18), 5503-5509.

25. Setková, L.; Hajslová, J.; Bergqvist, P.-A.; Kocourek, V.; Kazda, R.; Suchan, P., Fast isolation of hydrophobic organic environmental contaminants from exposed semipermeable membrane devices (SPMDs) prior to GC analysis. *Journal of Chromatography A* **2005,** *1092*, (2), 170-181.

26. Usenko, C. Y.; Harper, S. L.; Tanguay, R. L., In vivo evaluation of carbon fullerene toxicity using embryonic zebrafish. *Carbon* **2007,** *45*, (9), 1891-1898.

27. Hallare, A.; Nagel, K.; Köhler, H.-R.; Triebskorn, R., Comparative embryotoxicity and proteotoxicity of three carrier solvents to zebrafish (Danio rerio) embryos. *Ecotoxicology and Environmental Safety* **2006,** *63*, (3), 378-388.

Contributions to Chapter 3

Guidance was provided by Kim Anderson, Paul Jepson, Jeff Jenkins and Wendy Hillwalker. Kim Anderson and Wendy Hillwalker developed and provided many of the training tools utilized during the training exchange. Much of the training and transfer design was previously developed by Kim Anderson and Greg Sower then modified as necessary.

CHAPTER 3 – LIPID-FREE TUBE TECHNOLOGY ON-SITE TRAINING AND TRANSFER: UTILIZING LFT TECHNOLOGY IN A WEST AFRICAN LABORATORY FOR USE IN MONITORING PESTICIDES AT REMOTE SAMPLING SITES

3.1 Introduction

Beginning the summer of 2006, our laboratory started a project between Oregon State University and the Food and Agriculture Organization of the United Nations (FAO). During this project, a number of training events and exchanges were conducted between our laboratory and the Centre de Recherches en Ecotoxicologie pour le Sahel (CERES)/LOCUSTOX laboratory in Dakar, Senegal. The overall aim of the project is to develop a system for monitoring the effects of pesticides on human and environmental health in the West African sub-region. These activities also have relevance to a Global Environmental Fund co-financed project titled "Reducing Dependence on Pops and other Agro-Chemicals in the Senegal and Niger River Basins through Integrated Production, Pest and Pollution Management".

I was intimately involved in three of four training exchanges [1, 2]: two at OSU and one at CERES. During a 9-week training event from April 10 to June 11, 2008, I was closely involved in training two CERES staff technicians that visited our laboratory. During that training exchange, concepts of quality assurance, passive sampling devices, gas chromatography performance, and data processing were stressed. I assisted with all of parts of the training and was heavily involved in the laboratory training effort, quality assurance, field sampling, and data processing.

Most recently, from January 27 to March 6, 2009, I conducted a six-week hands-on training at the CERES/LOCUSTOX laboratory in Dakar, Senegal. Following, is an excerpt of the final report developed at the conclusion of this most recent training exchange. Listed in Appendix 2 is a detailed daily log that was adapted from an online blog developed to allow involved parties insight into my

daily activities, accomplishments, and challenges in the laboratory at CERES and the field. My goals through this training event were (1) to complete an initial demonstration of competency by the CERES/Locustox laboratory in the use of a new passive sampling device, the LFT, and the use and maintenance of key laboratory equipment used in pesticide residue analysis. (2) To develop a sampling strategy for the pilot exercise. And (3) To complete chemical analysis of laboratory and field (duplicate) split samples between CERES and OSU.

3.2 Purpose of visit

To fulfill prior LOA objectives, OSU has approved the standard analytical method for trace pesticide analysis and completed a validation packet generated at the OSU laboratory. OSU has successfully established a number of communication pathways for technical assistance including telephone, e-mail, and online technology such as Skype, blogs and document transfer. A first draft manual outlining quality assurance protocol and PSD deployment and analysis in West Africa was created and given to CERES and UN personnel. We've also completed an initial demonstration of competency by CERES in the use of a new "passive sampling device" (PSD) and the use and maintenance of key laboratory equipment used in pesticide residue analysis during 3 separate training events; two at OSU, one at CERES. A number of critical infrastructure challenges at the CERES laboratory have been addressed, including the purchase of a gas chromatograph (GC) model and configuration identical to the OSU FSES laboratory, location and storage of GC gases and laboratory consumables and reliable sources for numerous pieces of laboratory equipment and consumables. The above tasks are essential components to the creation of a portable lab package necessary for technology transfer and capacity building and their completion highlight the success of the GEF/PRM goals to date.

The loss of critical knowledge due to key personnel at the CERES laboratory leaving and other infrastructure challenges need to be addressed before critical goals can be achieved, including 1) demonstration of calibration,

demonstration of accuracy and precision, demonstration of system background, and development of method detection and detection limit calculation at the CERES laboratory, 2) verification of laboratory blanks and contamination checks at the CERES laboratory, and 3) collaborative laboratory blind and split field sample and quality control (QC) sample analysis at OSU and the CERES laboratory.

Two objectives towards the implementation of the PSD for monitoring the effects of pesticides on human and environmental health in the West African sub-region were defined in the Letter of Agreement (LOA):

3.2.1 Objective 1

To continue technical capacity building at the CERES Laboratory, Dakar, Senegal. Collaborative validation of the PSD technology at trace pesticide analysis levels by both the OSU and CERES labs can only be achieved by repetitive and reciprocal training of quality assurance (QA) protocols, instrument maintenance and use, and PSD preparation, deployment, processing and analysis. It was the goal of OSU that this training would only occur after all the necessary consumables, materials, GC gases, PSD cages and a glassware bake-out oven were purchased or acquired to avoid any delay on delivery time which could affect the analytical process.

3.2.2 Objective 2

To deploy and validate PSD at one of the GEF Project PDF-B field sites. The continued QA, instrument maintenance and PSD training of CERES staff coincide with PSD deployment at Pont Gendarme. PSD field and QC samples deployed in duplicate will be split between CERES and OSU for laboratory split analysis. The data acquired from this pilot sampling exercise and analysis will be used in the development of the final QAPP DQO.

3.3 Summary

During a 6-week training event from 26 January to 6 March 2009, OSU continued technology transfer of the aquatic passive sampling device (LFT) and capacity building at the Centre de Recherches en Ecotoxicologie pour le

Sahel/LOCUSTOX laboratory in Dakar, Senegal. As the OSU trainer, my goal was to evaluate the laboratory facilities and infrastructure and continue to train the staff on general laboratory techniques and QA as well as field and analytical techniques with respect to the theory and practice of aquatic passive sampling as a method for integrated environmental monitoring and chemical analysis. These goals address the objectives in the LOA for the GEF/PRM project.

Like previous training events this one had two major components: field and laboratory exercises. As was seen previously, the CERES staff was familiar and comfortable with field tasks, but was less proficient with laboratory performance, instrument care and maintenance and quality control documentation. CERES continues to be plagued by an unreliable electrical supply and limited availability of laboratory supplies. Health hazards, such as poorly working fume hoods, are critical problems that need to be addressed immediately. With respect to the gas chromatograph, it became apparent that improper use of the GC/ECD for projects other than the GEF/PRM project has negatively impacted the instrument resulting in decreased performance and delays during training. To successfully perform trace pesticide analysis, CERES needs to continue to develop and work on infrastructure, personnel training and laboratory equipment maintenance and use.

With each training event, the CERES staff has continued to show improvement in the efficiency of constructing, deploying and retrieving the passive sampling technology, but it is evident that without consistent and repetitive training that knowledge would be lost. Section 3.4 of this report addresses the specific goals, current status and summary of the training. Training issues and recommendations are in Section 3.5. Self-assessment evaluation surveys were administered before and after the training event to assess the success of the training. A summary of and comment on the results from these are shown in Section 3.6. An edited version of the daily training log posted to an online blog location during the training process is included in Appendix 2. Also, a training schedule is included at the end of Appendix 2.

The CERES laboratory has the ability to implement the PSD technology for monitoring the effects of pesticides on human and environmental health in the West African sub-region but the continuing problems with equipment mismanagement, infrastructure challenges and loss of skilled personnel without sufficient knowledge transfer is not adequate for immediate implementation of trace pesticide analysis under quality assurance protocol. It is also evident that someone who is knowledgeable in analytical methods and quality assurance be either hired as permanent staff or identified among the current staff and sufficiently trained to fulfill those duties. If the recommendations in Section 3.5 are followed, particularly the appointment of a laboratory director that will enforce laboratory quality control protocols, cross-training of personnel on GC use and maintenance, is knowledgeable of fundamental laboratory basics, and will locate key laboratory supplies or build relationships with local suppliers; CERES will be capable of performing the field and laboratory work necessary for successful completion of the GEF/PRM project.

Table 3.1 List of abbreviations

BPL	Bonne Pratiques Laboratoire
CERES	Centre de Recherches en Ecotoxicologie pour le Sahel/LOCUSTOX
DQO	Data quality objective
EST	Environmental Sampling Technologies
FAO	Food and Agriculture Organisation
FCFA	Franc Communauté financière d'Afrique
FSES	Food Safety and Environmental Stewardship
GC	Gas chromatograph
GC-ECD	Gas chromatograph-electron capture detector
GEF	Global Environmental Fund
GLP	Good Laboratory Practices
GPS	Global Positioning System
HCl	Hydrochloric acid
I.S.	Internal standard
LDPE	Low-density polyethylene
LFT	Lipid-free tubing
OC	Organochlorine
OCP	Organochlorine pesticide
OSU	Oregon State University
PRC	Performance reference compound
PSD	Passive sampling device
QA	Quality assurance
QAPP	Quality assurance program plan
QC	Quality control
RBF	Round bottom flask
SAM	Standard analytical method
SOP	Standard operating procedure
USD	United States dollars

3.4 Training objectives, status and conclusions

3.4.1 Training objectives

The following objectives were established for the 6-week training period:

1. Determine the ability of unsupervised CERES staff to meet the data quality objectives for trace pesticide analysis (at the parts per billion level) as outlined in the appropriate SOPs and SAMs with both laboratory and field samples.

2. Demonstrate and evaluate CERES staff competency on the relevant laboratory equipment with respect to operation and maintenance as determined by OSU personnel and senior CERES staff.

3. Develop and implement a PSD sampling strategy and pilot field exercise at a GEF Project field site to acquire data to be used in the development of the QAPP DQOs.

4. Completion of laboratory and field (duplicate) split sample analyses between CERES and OSU. This final training objective will be presented in a follow-up report.

3.4.2 Status

Some of the training was not addressed due to a number of factors. At the onset of this training event much of the laboratory and field sampling equipment and supplies used specifically for this project were either missing, in unsatisfactory condition, or non-operational. This is reflected in the training log, Appendix B, and was at odds with what was communicated by CERES to OSU prior to training. Some of these issues were not fully at the fault of CERES, such as the delay in the acquisition of a suitable oven for glassware bake-out. Significant delays were caused by the poor electrical service despite having critical laboratory equipment attached to an uninterruptible power supply (UPS). The UPS did and has allowed critical laboratory equipment to continue operation during brown-outs and brief black-outs, but the diesel generator located at CERES did not function during extended periods of power loss. Table 3.2 lists activities necessary for achieving Objectives 1 through 4 in Section 3.4.1 and their current status. Because important aspects of the work are pending, Objectives 1, 3 and 4 could not be fully evaluated until that work is completed, but certain aspects can be assessed. Objective 2 however was evaluated.

3.4.3 Conclusions

As was seen in previous training events, the field work was a success and the chosen pilot field site, Pont Gendarme, well suited to the task. Unfortunately, we were not able to duplicate all sites from the previous training event due to a malfunctioning pumping station. This limited our available sampling sites to two of the original sites, the inlet and outlet pumping stations, and one new site. Also, due to time constraints exacerbated by the equipment issues discussed throughout this report and the PSD sampling cages not being completed until the beginning of the third week, we only undertook two sampling events. The repetitive retrievals from these and past training events have had a clear positive impact on the staff's comprehension and retention of the training. However, the duration of training was insufficient to adequately address the laboratory work. Complications with equipment, non-routine maintenance and repair of equipment coupled with strict time constraints did not allow for in-depth sample analysis, data processing and review of samples collected from the field. The results of the CERES staff self-evaluation surveys in Section 3.6 compare very well to this assessment.

CERES staff members, as in the past, demonstrated competency in basic laboratory technique, however, in general they still lack the experience and advanced training necessary for rigorous analytical work at the trace pesticide analysis level. Advanced training is a prerequisite to successful completion of the project and thus, Objectives 1 and 2. CERES had an active GLP (termed Bonne Pratiques Laboratoire or BPL) program that went dormant with the loss of senior staff, as well as their ISO certification. From observations of current SOPs in place, routine maintenance logs have not been filled out for much of the equipment for a number of years. The program should be reactivated to help track progress and maintain analytical integrity; however, current staff will require additional training as few of them were present while the active GLP program was enforced. It is recommended that CERES thoroughly review and update current SOPs, potentially using those provided by OSU as a template and model. As stated earlier, someone who is knowledgeable in analytical methods and quality

assurance should be either hired as permanent staff or identified among the current staff and sufficiently trained to fulfill those duties. This could be in the form of a dedicated QA officer or the duties assigned to a lab manager.

In conclusion, some laboratory issues, a few of which are health concerns, should be addressed to ensure CERES can successfully complete all the tasks brought forth in the current LOA and future GEF/PRM work. Recruiting a lab manager and/or QA officer to facilitate quality assurance protocols should be a priority when addressing these issues. Other specific issues and the recommended solutions are listed in Section 3.5. Success also hinges upon CERES receiving further training, relatively inexpensive additional or replacement laboratory equipment, and improved electrical service. It is recommended that several staff, three or four, potentially including a member in a manager or director position participate in an additional training exchange at OSU. CERES has the foundation of a quality laboratory. Staff members are mostly amenable to training, the instrumentation when properly maintained is adequate, and the facilities are well maintained. Given the significant strides made in LFT preparation, processing and field work, and to help training retention CERES may proceed with sampling events, provided the analyses on retrieved samples are postponed until the recommendations are completed.

Table 3.2 Activities and status for the six-week training event

Activity	Status			
	Acceptable	**Unacceptable**	**Pending**	**Unable to complete**
Training evaluation survey "before"	X			
Practice construction of PSD	X			
Construction of spiked practice PSD	X			
Processing of spiked practice PSD	X			
GC analysis of spiked practice PSD				X
Data analysis of spiked practice PSD				X
Construction of field and QC PSD for deployment event	X			
Field deployment of PSD	X			
Field retrieval 1	X			
Processing of field retrieval 1 samples	X			
GC analysis of field retrieval 1 samples	X			
Data analysis and calculation spreadsheet of field retrieval 1 samples		X	X	
Field retrieval 2	X			
Processing of field retrieval 2 samples	X			
GC analysis of field retrieval 2 samples	X			
Data analysis and calculation spreadsheet of field retrieval 2 samples		X	X	
Data consolidation and review			X	
QC summary			X	
Training evaluation survey "after"	X			
Split sample delivery to OSU	X			
OSU analysis of split samples and QC			X	

3.5 Issues and recommendations

The following issues impede successful project completion and future work on the GEF/PRM project. Issues are listed in order of priority within their respective section; however, it is important to note that all recommendations will need to be implemented to achieve the project's stated goals. An * indicates an item important to laboratory safety and health and these are always considered priority.

3.5.1 General lab issues

1. * Fume hood motors either do not work or are underpowered.

Recommendation: Replace all motors with or without a new ducting system with a more efficient and powerful design.

2. * Emergency shower access is blocked by a refrigerator.

Recommendation: The refrigerator should be moved and a no obstruction area designated on the floor with paint or tape. The shower should be periodically checked for correct operation.

3. * Paint on wall and ceiling in laboratory is pealing, flaking, and falling onto sensitive laboratory workspace and the analytical balance. This is a potential ongoing source for sample contamination.

Recommendation: The current paint treatment does not address the obvious problem of moisture seeping through the concrete walls of the laboratory. CERES at the very least should strip the current paint from all affected walls and other surfaces and apply a sealer (moisture barrier) to the cement then paint over the sealer with an appropriate resilient paint.

4. Laboratory working surfaces such as countertop space and hoods serve shared purposes, such as preparing several different types of samples for differing analyses.

Recommendation: Designate and set-aside a laboratory countertop and the adjacent hood for trace pesticide analysis tasks. Working surfaces should be covered preferably in a fairly inert material such as stainless steel as discussed with the staff during the training. These laboratory spaces should be dedicated to the GEF/PRM project and for trace pesticide residue analysis work only. Pesticide formulation work should not be done near these designated laboratory spaces, as was observed during the training event. Preferably, formulation and residue analysis work would be done in separate laboratories to minimize potential cross-contamination.

5. Insufficient emphasis and reluctance for record keeping and labeling was seen. Instrument and equipment SOPs are present, but not enforced or followed. Maintenance logs are available in the SOP folders but are not updated regularly.

Recommendation: SOPs should be thoroughly reviewed and updated as necessary. SOPs should be assigned to new equipment. CERES should use the SOPs made available by the OSU laboratory and modify them as needed or use them as a template for developing new SOPs. Maintenance logs should be filled out regularly as outlined in the SOPs and reviewed periodically. A log for analytical gas cylinders should also be instated. Further information can be found in Section 3.5.3.1.

6. * Waste organic solvent stored in an unsafe manner (stored in either an unmarked large round bottom flask or large open beaker).

Recommendation: Waste solvent should always be disposed of and stored in appropriate, designated containers and facilities.

7. * Organic solvents stored in an unsafe manner.

Recommendation: The organic solvents should be moved to a flammable liquids locker (see Section 3.5.2.1).

8. * Acids stored in an unsafe manner.

Recommendation: If acids are to be stored in a hood then the hood sash should be closed when hood not in use. Leaking acid containers should be disposed of properly.

9. * The fume hood sashes are generally stored open. There are only two available positions for the hood sashes; fully open or fully closed.

Recommendation: The sashes should be lowered at the end of the workday and when not in use.

10. Insufficient supplies for effective glassware cleaning were present.

Recommendation: Purchase and install a stainless steel sink of sufficient size to facilitate efficient throughput of glassware. Purchase brushes specific to cleaning laboratory glassware (volumetric flasks, round bottom flasks, funnels, beakers, and graduated cylinders). Define and/or demarcate glassware cleaning areas for dirty and clean glassware and keep separate. Follow OSU laboratory SOP 2110 for laboratory container cleaning or develop one similar for use at CERES.

11. Poor electrical service causes instrument failures and the current backup system insufficiently addresses the problem. The UPS in place can only run vital equipment for about 40 minutes before it shuts down. The diesel generator which should provide supplemental electricity until service can be restored did not run or operate correctly during the 6 weeks of the training despite several extended power outages.

Recommendation: As the quality of incoming electrical signal is beyond CERES' control, a professional electrician should inspect the system and recommend the appropriate solution to compensate for poor service. This was also recommended in a previous training event. The diesel generator should operate during an extended power outage if critical equipment is to be used during that time period. The UPS did seem to work properly, but did not perform as well as indicated by the staff prior to the outage event.

12. Difficulty obtaining general laboratory supplies.

Recommendation: As this appears endemic to the region, CERES may need assistance in developing relations with good suppliers and shipping services. Additionally, it will be incumbent upon CERES to purchase large quantities of supplies when the opportunity presents itself to ensure their availability for future work.

3.5.2 Equipment issues

1. * No flammable liquids locker in the organic lab.

Recommendation: A flammable liquids locker sized for the laboratory with proper ventilation should be obtained.

2. Laboratory working surfaces such as countertop space and hoods are made of tile with grout. The grout is difficult to clean adequately and therefore a potential source of contamination.

Recommendation: One of the countertops and the adjacent fume hood's working surface should be covered with high-grade stainless steel (1.5 mm thick and 316 grade or better). If this is not available CERES should use the adhesive backed Teflon® sheets shipped from OSU previously to cover these surfaces. This approach could be applied to other

parts of the laboratory, especially if used for the GEF/PRM project, and will also benefit other analytical work performed at CERES.

3. High purity water (HPLC grade or 18 MΩ·cm) is not available in the lab. The water distillation apparatus and purified water storage container not well maintained.

Recommendation: Several SOPs and SAMs demand high purity water for producing quality analytical data required by the project; therefore, a high purity water system should be obtained. This equipment will also be required for other analytical methods at CERES. If this is not feasible and/or water purified through distillation is only needed, then periodical maintenance should be performed on the water distillation apparatus to prevent potential failure. This maintenance should include but not be limited to flushing of the system with an appropriate solution to remove solids deposition and build-up. The purified water storage container should be periodically cleaned and flushed out. It should also be stored with the lid screwed on tight to minimize potential contamination.

4. Given the size and scope of the UN-GEF project, many samples are expected and the current lab capacity of 10 to 15 PSD samples per batch is insufficient.

Recommendation: CERES should obtain solvent evaporation equipment with a much higher throughput and capacity such as a Caliper Life Sciences TurboVap LV evaporator (capacity = 50 samples), Organomation Associates N-EVAP, or equivalent. CERES should also obtain replacement parts for the three working rotary evaporators. The rotary evaporators have a different ground glass fitting size as the round bottom flasks ordered and shipped by OSU. Short ground glass adapters should be purchased and used to allow higher throughput of samples since only one adapter was located in the laboratory.

5. Inadequate number of refrigerators and freezers that work properly. Most will not hold a programmed temperature.

   Recommendation: Have a technician diagnose the problem and repair equipment as needed.

3.5.3 Personnel issues

1. Some CERES staff exhibited either unfamiliarity with or failure to apply techniques required for advanced analytical work. Specific issues include improper or inconsistent pipet use, inefficient or compromising bench organization, inadequate labeling of samples, standards, and reagents, and reluctance to employ adequate record keeping (with respect to sample log-in, pipet verification, standard solutions, procedural notes, and error correction). Bench sheets were inadequately filled out on a consistent basis. With some of the staff there was unwillingness to finish filling out or correcting errors in bench sheets after being asked. These findings mirror those of previous training events.

   Recommendation: To build on the technical and analytical skills already developed, CERES staff members should obtain further advanced training that encompasses all aspects of bench work including, but not limited to, sample collection and preparation, analysis, instrument maintenance, record keeping and laboratory safety. Ideally, three or four technicians, potentially including a member in a manager or director position, would train at the OSU FSES laboratory in Corvallis, Oregon for a period of no less than one six weeks, preferably longer, and return to CERES to train the remaining staff. In general, the staff needs to pay more attention to detail when filling out paperwork.

2. Loss of staff and inadequate transfer of knowledge from previous staff has led to a low number of well trained staff in chromatographic analysis. Due

to time and logistical constraints only one staff member received sufficient training on the GC for the GEF/PRM project during the six week training. This staff member is the only one capable of adequately running trace pesticide analysis of numerous target analytes.

Recommendation: All CERES staff personnel responsible for advanced chromatographic analysis should obtain further advanced training at OSU or equivalent institution or hire an expert chromatographer to be stationed on-site for an extended period of time. The institution or expert would be responsible for developing CERES' analytical capabilities, training on performance of routine GC sample analysis duties and training CERES staff in advanced chromatography skills. Additionally, a contract maintenance program may be beneficial.

Note: A CERES technician with basic chromatography and chemistry skills would be well suited to the training recommended in Section 3.5.3.1.

3. A hierarchy in the laboratory is not evident.

Recommendation: Hire or appoint a laboratory manager who is knowledgeable about general analytical methods and PSD technology; who will also enforce QA protocols and emphasize cross-training of personnel on GC use and maintenance. Assign the SOP, SAM, and QA protocol and responsibility to the laboratory manager or appoint a QA officer to fulfill this need, and define the responsible person for the GC (the staff member with the most training; see Section 3.5.3.2).

3.5.4 Pending work issues *(see Section 3.4.2. and Table 3.2)*

Recommendations: CERES should obtain clean LFT from OSU for the construction, extraction, analysis and evaluation of PSD until it is determined by OSU that potential laboratory contamination is no longer an

issue. This determination would come from cooperative sharing of GC data between OSU and CERES. Before future projects go forth, laboratory PSD samples should be constructed, extracted, analyzed, and evaluated by CERES and OSU to ensure no contamination is present and that the instruments involved are working properly. Blind samples (PSD prepared and spiked by OSU) may also be provided to assess chromatography skills.

3.5.5 Training issues

The duration of training was insufficient to adequately address the LOA objectives. Complications with equipment, non-routine maintenance and repair of equipment and the strict time constraints did not allow for in-depth sample analysis, data acquisition and data work-up and review of samples collected from the field.

Recommendations: If this type of training is attempted with another laboratory the length of service should be extended to at least eight weeks to account for unforeseen complications similar to those seen in this and other training events. The required equipment and supplies should be on site and operating correctly before the arrival of the trainer(s). This has been an issue during these training events.

3.6 Analysis, summary, and comment of training surveys completed by CERES staff

This section serves as a summary and discussion for the surveys that were completed by the CERES staff before and after they had participated in the PSD training. The purpose of the survey was to determine the strengths and weaknesses of the program and training through participant self-assessment. It is important to note that not every member of the staff was able to participate in every training event thus increasing score variability. In addition, one staff member was a trainee and thus had very little to no knowledge of PSD technology or techniques prior to the training, potentially influencing survey averages negatively.

The surveys that were administered before the training indicated initial unfamiliarity with performance reference compound (PRC) function, and some aspects of quality control (QC) samples: As well as gas chromatography basics, maintenance, and method validation procedures. This is likely an indication of the lack of knowledge transfer with the recent staff departures. Ability in using LFT accumulations to calculate estimated bioavailable water concentrations was also low, which was a skill that was not addressed effectively in previous training events. However, the surveys indicate proficient knowledge before training of LFT construction, processing, deployment, and retrieval procedures in accordance to SOPs.

When comparing the before and after surveys, the least improvement was related to QC procedures such as instrument logs and using laboratory and field QC samples for contamination and background analysis and determination. Preparation and cleaning of LDPE for LFT construction was not addressed in this training event and thus did not show appreciable improvement. Due to time constraints and unforeseen challenges at the onset of training, we did not develop a method validation packet for the GC GEF/PRM project method. Furthermore, satisfactory time was not available to provide thorough training on using a spreadsheet to calculate estimated bioavailable water concentrations from LFT concentrations.

The strongest improvements were seen in using SAMs as part of a QA procedure, selection of appropriate sampling sites for LFT, incorporating and using internal standards in the GC data analysis, and using the GC software to produce and compile raw LFT data. Comprehension in use and theory of PRCs saw marginal improvement, as did knowledge of internal standards function and implementation and understanding of general GC procedures.

As you can see by looking at the improvement column in the figure below titled “min” or minimum there are several negative entries. These numbers were posted by the same respondent. In previous training events both the before and after training surveys were printed side by side on the same sheet of paper

allowing respondents to see their previous answers. During this training event, they were deliberately separated to more accurately evaluate self-assessed improvement in skill and ability. It appears this respondent had an over-confidence in their skill and ability in many fields before the training. These results mirror my experience with the CERES staff in the laboratory. During discussions prior to activity, the staff's stated level of confidence and competency with skills or lab tasks often were overstated compared to what was observed.

Question 30 on the survey asked respondents to list the skills and abilities for which they did not receive sufficient training. Several of those echo those skills that were seen as having the least improvement. In addition to these, they listed skills pertaining to using the GC software to perform calibration curves, peak verification, and quantifying and reporting raw data from the GC. This is likely a result of the focused training on one individual rather than all personnel due training constraints. Conversely, question 31 asked respondents to indicate skills and abilities for which they felt best trained. The skills listed fall inline with the results shown above. The respondent who received the most training on the GC listed those skills as such.

The personnel were asked to suggest parts of the training that could be changed to better meet their needs and those of the staff. They suggested increasing the length of the training and spending more time on calculating estimated bioavailable water concentrations from LFT concentrations. Also, they indicated that translating SOPs, SAMs, bench sheets, and other items into French would assist the training. Question 33 asked for other general comments. Answers included the purchase of GC supplies, mainly columns, the need to continue deployments for solidification of training, and the purchase of new laboratory supplies and equipment; nitrogen assisted blow-down device (similar to a TurboVap), GC grade solvents, and gas regulators.

Recommendations for future training would include extending the length of training to account for unforeseeable setbacks. The after-training surveys should be administered before the end of the training allowing sufficient time to address

and follow up on potential inadequacies of the training. CERES personnel responsible for advanced GC analysis should receive further training in data (raw data and chromatograms) analysis and interpretation. This may well be accomplished through another training event at OSU as discussed in Sections 3.4.3 and 3.5.3. It is obvious that the repetitive training in PSD construction, processing, deployment and retrieval has resulted in good retention of the training and knowledge and should be seen as a major success. CERES is able to competently deploy and retrieve LFTs and thus initiate project sampling.

Table 3.3 CERES/Locustox on-site training evaluation: 2009. This table is a representation of a survey administered before and after the 2009 training event. A summary of the before and after responses as well as improvement is shown on the right hand portion of the table. A response of "1" is equivalent to no ability or skill and a "7" is equivalent to very able and skilled.

Questions	**Skill or Ability Before Training**			**Skill or Ability After Training**			**Improvement**			
	Avg	Min	Max	Avg	Min	Max	Avg	Min	Max	S.D.
1. Understand and demonstrate the use of Standard Operating Procedures (SOP) as Quality Assurance (QA) procedures.	4.3	1	7	5.8	3	7	1.5	0	3	1.3
2. Understand and demonstrate the use of Standard Analytical Methods (SAM) as QA procedures.	4.0	1	6	5.8	3	7	1.8	1	3	1.0
3. Understand and demonstrate the use of bench sheets as sample processing quality control (QC) procedures.	4.5	1	7	5.8	3	7	1.3	0	3	1.5
4. Understand and demonstrate the use of equipment and instrument logs as QC procedures.	4.5	1	7	5.0	3	7	0.5	-3	3	2.6
5. Understand the preparation (cleaning) of LDPE tubing for LFT construction.	4.3	1	7	4.5	3	7	0.3	-2	3	2.1
6. Demonstrate the preparation of LDPE tubing for LFT construction.	4.5	1	7	4.5	3	7	0.0	-3	3	2.4
7. Understand the purpose of LFT fortification/spiking of performance reference compounds (PRCs).	4.3	1	6	5.8	4	7	1.5	0	3	1.3
8. Understand the selection of appropriate compounds and concentrations for fortification/spiking of PRCs.	3.3	1	6	4.8	4	6	1.5	-2	3	2.4

Table 3.3 (Continued)

9. Understand and prepare laboratory blank and LFT blank samples as QC procedures for background contamination analysis.	5.0	1	7	5.8	5	7	0.8	-1	4	2.2
10. Construct an LFT according to the SOP.	5.3	1	7	6.3	5	7	1.0	-1	4	2.2
11. Prepare deployment hardware including cages and supporting equipment.	5.3	1	7	6.5	5	7	1.3	0	4	1.9
12. Understand LFT sampling sites selection.	4.0	1	7	5.8	5	6	1.8	-1	4	2.1
13. Understand and demonstrate LFT deployment requirements.	4.8	1	7	6.3	4	7	1.5	0	3	1.3
14. Understand and complete field samples as QC procedures for field background contamination analysis.	4.8	1	7	5.5	3	7	0.8	-2	2	1.9
15. Retrieve and clean LFTs in field according to SAM	5.3	1	7	6.5	5	7	1.3	0	4	1.9
16. Understand and complete benchsheets for LFT field deployment and retrieval.	5.0	1	7	6.0	3	7	1.0	0	2	1.2
17. Perform LFT extraction according to SAM.	5.3	1	7	6.8	6	7	1.5	0	5	2.4
18. Understand and complete QC LFT extraction samples for background contamination analysis	4.8	1	7	5.8	5	6	1.0	-1	4	2.2
19. Understand and complete QC overspike/ fortification samples for LFT extraction and analysis.	3.3	1	6	4.8	4	7	1.5	-2	4	2.6

Table 3.3 (Continued)

20. Understand and perform quantitative transfer procedures according to the SAM.	3.8	1	6	4.8	4	7	1.0	-2	3	2.2
21. Understand, perform and report verification of quantitative transfer devices (e.g. pipettes).	4.3	1	7	6.8	6	7	2.5	0	5	2.4
22. Demonstrate the preparation of analytical standards according to QC procedures.	4.3	1	7	5.8	4	7	1.5	0	3	1.7
23. Understand and develop a Method Validation packet for a select analyte list on the gas chromatograph (GC).	2.8	1	5	3.5	3	4	0.8	-2	2	1.9
24. Understand and perform column and inlet troubleshooting procedures on the GC.	3.5	1	6	5.0	4	6	1.5	-1	3	1.7
25. Perform analysis of LFT extracts on the gas chromatograph (GC) according to SAM.	3.8	1	6	5.3	3	7	1.5	0	2	1.0
26. Understand and perform an appropriate calibration curve according to a method validation procedure.	4.0	1	6	5.0	2	7	1.0	0	2	0.8
27. Understand and perform peak verification according to appropriate internal standards.	3.0	1	5	4.8	2	7	1.8	0	3	1.5
28. Understand, quantify (using the calibration curve), and report instrument raw data (e.g., chromatograms).	3.3	1	5	5.0	3	7	1.8	0	3	1.3
29. Use a spreadsheet to calculate estimated bioavailable water concentrations from the LFT concentration.	2.8	1	4	3.0	2	5	0.3	-2	2	1.7

3.7 Literature Cited

1. Sower, G. J. *Lipid-free tube passive sampling device technology transfer: On-site laboratory and field training*; Oregon State University: 2007.

2. Anderson, K. A.; Hillwalker, W. *Lipid-free tube passive sampling device technology transfer: On-site quality assurance, laboratory and field training*; Oregon State University: 2008.

Contributions to Chapter 4

Spreadsheets utilized for calculating LFT derived water concentrations of contaminants were developed with the help by Greg Sower. Greg Sower first adapted the ATSDR PHA used in Chapter 4 for PAHs.

CHAPTER 4 – UTILIZING LFTS TO ESTIMATE AQUEOUS CONTAMINANT CONCENTRATIONS AND EXTENDING LFT USE AS A BIOLOGICAL SURROGATE TO ESTIMATE HUMAN HEALTH RISKS

4.1 Introduction

Aquatic ecosystems are used through diverse avenues by human activity ranging from recreational purposes such as boating, sport and subsistence fishing to agricultural practices, collection of water for cooking and drinking, bathing, and where domesticated animals are watered. Many of these aquatic ecosystems contain highly polluted areas. Public health assessments (PHAs) are developed in an attempt to inform the public about potential risks during such activities. They provide information about possible exposures and the probability that such an exposure could lead to current or future adverse health effects without any action taken [1], as well as estimate exposure dose and exposure concentration. The exposure dose is based on site and contaminant data, existing regulatory standards, and an evaluator's professional judgment. A baseline risk assessment provides a basis to determine whether remedial actions are required [2].

Risk assessments generally consists of four steps; hazard identification, toxicity assessment, exposure assessment, and risk characterization [2]. Hazard identification and toxicity assessment are science-based characterizations, while exposure assessment and risk characterization generally utilize a scientific database to predict risk. Interpretation of contaminant levels in environmental samples such as water, air or organismal tissues are complicated by environmental and biological processes, and the risk to society can be real or perceived, both of which can affect regulatory decisions [2, 3].

Determining and incorporating route of exposure can be very complex in PHAs and thus for our purposes we simplify the approach and focus only on exposure due to consumption of fish tissue. The risk model approach proposed is based on an existing PHA [4] conducted by the Agency for Toxic Substances and Disease Registry (ATSDR) for the Portland Harbor Superfund site on the

Willamette River, Oregon. The exposure route used in the Portland Harbor PHA used the same fish tissue consumption exposure route.

Evaluation of exposure due to consumption is typically based on concentrations found in harvested resident fish and/or shellfish tissues [1]. Significant concentration variation in sample tissues can be found though and highlight many of the issues that plague this sampling approach. Variation arises from a number of factors such as, species, sex, age, growth rate, range, health, diet, and internal degradation processes. Often, there is difficulty in collecting fish/shellfish and producing usable data. Another risk assessment approach utilizes computer modeling programs, but like most assessment tools many assumptions have to be made. An approach used to reconcile some of the issues plaguing environmental biota sampling is to expose and analyze caged fish or shellfish also termed biomonitoring organisms (BMOs) [1, 5]. Accumulated residues in BMOs are by nature bioavailable and are based on bioaccumulation factors (BAFs). A BAF is found by dividing the equilibrium concentration of chemicals in an organism by the concentration of those same chemicals in the surrounding medium.

However, some physiological, anatomical and behavioral characteristics of a species in conjunction with site-specific conditions contribute to variability of tissue HOC concentrations for BMOs as well, requiring field data or modeling to compare across studies [5]. This suggests that BAFs do not necessarily represent thermodynamic equilibrium, where residues in tissues are proportional to environmental exposure concentrations [6]. A more suitable assumption of constant tissue concentration may be steady state. In addition, BMO tissue concentrations may not correlate well with HOC residues in species of concern [1]. Given these complications, the applicability of aquatic BMOs can be very limited for the prediction of exposure concentrations and contamination sources [1].

Many studies have compared, side-by-side, accumulations of HOCs by PSDs and BMOs [1]. With a primary goal of determining commonalities in the type and concentration of HOCs accumulated, these comparisons showed both

similarities and differences in accumulation patterns [1]. Inconsistencies are expected between the two monitoring matrices, as one is inanimate and the other a living organism

As the Portland Harbor PHA focused on exposure to chemicals through consumption, the BMO approach is not needed. Some PSDs, such as LFT, represent not only exposure concentrations but also possible tissue concentrations, from which an assessor can make informed decision on health hazards. Using PSDs instead of fish/shellfish tissue concentrations offers many advantages, leading to less variability in concentrations and more efficient and simpler laboratory methods. A single PSD configuration can be used in many different environments (fresh, saline, riverine, lakes, oceans, sewage, cold, hot…etc.) and phases (air and water). This level of flexibility and adaptability simply is not attainable with living organisms. A further advantage is that PSD can be used to estimate a waterborne contaminant concentration, which usually cannot be accomplished with fish/shellfish [7]. With the ability to compare across sites and between separate studies, PSD allow for a finer level of spatial and temporal comparison of concentrations and ultimately risk [8, 9].

PSDs can underestimate mass concentrations and consequently perceived exposure if not at equilibrium. However, with the use of PRCs, the calculated water concentrations are unaffected. The freely dissolved water concentrations of hydrophobic contaminants can be calculated from LFT using equations provided in Huckins *et al*. [1] and discussed here. Polyethylene-based PSDs such as LFTs mimic biomembranes and organismal lipid tissues measuring the bioavailable fraction of lipophilic contaminants. Thus, LFT can be a very useful tool when conducting a public health assessment. Often there is difficulty in collecting fish/shellfish and the producing usable data. Other risk assessment approaches have been proposed such as modeling, but these too have shortcomings and many assumptions have to be made.

The purpose of this section is to demonstrate how estimated bioavailable water concentrations can be calculated from deployed LFT analyte accumulations,

and to describe how LFT can be used in a human health risk model; serving as an effective screening tool as well as helping communicate potential human health risks. The risk model approach was based on an existing PHA for the Portland Harbor Superfund site on the Willamette River, Oregon. Fish tissue concentrations normally used in calculating health risks from consumption were substituted by using mass-to-mass concentrations of contaminants in LFTs.

4.2 Discussion

4.2.1 Calculating PSD based water concentrations

Estimated bioavailable water concentrations are calculated using equations provided in Huckins *et al*. [1]; the process is described here and is shown in brief in figure 4.1. PRCs, incorporated into a PSD as an *in situ* calibration method, are used to determine site-specific sampling rate constants. This process is based on the hypothesis that the same mechanism that determines the rate constant for the release process (k_e) also applies to the uptake process rate constant (k_u) [10, 11]. Site-specific *in situ* sampling rate values (R_s) can then be calculated. Utilizing PRC sampling rates, we can account for physico-chemical factors as well as environmental factors including temperature and potential bio-fouling of the sampling device. With the concentration of PRC before deployment (N_{PSD-0}) and duration time (t) known, measuring PRC loss during an exposure (concentration after exposure) (N_{PSD-e}) provides an *in situ* clearance rate constant or $k_{e,\,PRC}$ (analogous to release process rate constant) [11].

$$k_{e,PRC} = \ln\left(\frac{N_{PSD-o}}{N_{PSD-e}}\right) \bullet t^{-1} \qquad \textbf{Eq. 4.1}$$

The site-specific R_s of a PRC for an exposure may be determined from:

$$R_{s,PRC} = \frac{\left(K_{mw,PRC} \bullet k_e \bullet V_m\right)}{1000} \qquad \textbf{Eq. 4.2}$$

Where k_e is calculated from equation 1, K_{mw} is the compound-specific polyethylene membrane-water partitioning coefficient, V_m is 5.9 cm^3 for LFT and R_s is then reported in L/day. K_{mw} is calculated currently using an empirical model previously published by Booij *et al.* [12] of which the derived equation is seen below as equation 4. Since LDPE is a non-polar organic phase, partitioning is expected to be driven by hydrophobic interactions: Meaning, there will be a strong correlation between K_{mw} and K_{ow} values. Huckins *et al.* [1] plotted experimental log K_{mw} values against corresponding log K_{ow}. The plot of this data was best described by a quadratic function of log K_{ow}. It was found that a better fit of the data resulted from using a different intercept (a_o) for moderately polar and non-polar compounds.

$$\log K_{mw} = a_o + 2.321(\log K_{ow}) - 0.1618(\log K_{ow})^2 \qquad \textbf{Eq. 4.3}$$

Where a_o = -2.61 for non-polar compounds such as PCBs, PAHs, and 4,4'-DDE and a_o = -3.2 for polar pesticides[1]. The sampling rates (R_s) for specific target analytes (*i*) in different exposure conditions (*j*) may be estimated from:

$$R_{s,i[j]} = R_{s,PRC[j]} \bullet \left(\frac{\alpha_{i[j]}}{\alpha_{PRC[j]}} \right) \qquad \textbf{Eq. 4.4}$$

If isotope labeled surrogates are employed equation 4.2 is used directly. Without labeled surrogates, PRC-derived sampling rates can be used to calculate reasonably accurate relative sampling rates for similar compounds by employing the empirical relationship developed by Huckins *et al* [1] shown as equation 4.5. The unitless compound-specific effect term, α, is determined by equation 4.5. According to Huckins *et al.* [1] this equation was born out of observations in 19 calibration experiments; where log transformed sampling rates were fitted to a the 3rd order polynomial in log K_{ow}. It had been noted that experimental sampling rates

had a similar dependence on log K_{ow}, but were offset in different studies. The plot showed quite good fit considering the large differences in exposure conditions [1].

$$\log \alpha = 0.0130(\log K_{ow})^3 - 0.3173(\log K_{ow})^2 + 2.244(\log K_{ow}) \quad \textbf{Eq. 4.5}$$

To calculate the estimated bioavailable water concentration (C_w in ng/mL) of a compound (x_1) from measured accumulations in exposed LFT ($C_{LFT, x1}$) we use equation 4.6. The measured accumulations are, of course, instrument generated data.

$$C_{w,x_1} = \frac{C_{LFT,x_1}}{\left(V_m \bullet K_{mw}\left(1 - \exp\left(-\frac{R_{s,x_1} \bullet t}{V_m \bullet K_{sw}}\right)\right)\right)} \quad \textbf{Eq. 4.6}$$

The preceding equations can be imported into a spreadsheet to calculate estimated water concentrations for many analytes quickly similar to figure 4.1. A sample calculation is modeled here using 2,2',4,4'6-pentachlorobiphenyl (PCB-100) as the PRC and p,p'-DDT as our analyte of interest. Shaded in grey are the various inputs that can be variable depending on exposure duration and PRC concentration.

Input data and calculations Adapted from Huckins *et al.* [1]

LFT V_m = 5.90 cm^3

exposure duration in days (E) = 21 Days

PRC amount in PSD,					Log K_{ow}
PRC at t_0 =	100.0	ng/LFT	PRC	PCB-100	6.23
PRC at t_E =	80.000	ng/LFT	Analyte	p,p DDT	6.36

$\text{Log } K_{mw} = a_0 + 2.321(\log K_{ow}) - 0.1618(\log K_{ow})^2$

a_0 = -2.61 for PCBs, PAHs, 4,4'-DDE

a_0 = -3.2 for polar pesticides

(from Huckins page 54 equation 3.28)

Step 1. Calculation of PRC R_s

$k_{e, PRC} = -\ln (t_E / t_0) / E$ = 0.011 day^-1

$\log K_{mw, PRC}$ = 4.676

$R_{s, PRC} = (V_s \cdot 10^{\log Kmw, PRC} \cdot k_e)$ = 2972.8 cm^3/day 3.0 L/day

Step 2. Calculation of the relative Rs of analyte and PRC

$\log \alpha = 0.0130 \log K^3_{ow} - 0.3173 \log K^2_{ow} + 2.244 \log K_{ow}$

(from Huckins page 60 equation 3.35)

Step 3. Calculate the Rs of the analyte

$R_{s, PRC} \cdot (\alpha_{x1} / \alpha_{PRC}) = R_{s, x1}$

Step 4. Calculate the aqueous concentrations

$C_{w, x1}$ = x1 accumulation/ ($V_m \cdot K_{mw}$ (1 - exp (-(R_s (mL/d) · E)/ ($V_m \cdot K_{mw}$))) = ng/mL

Compound	Accumulation (ng)	log α	α	Log K_{mw}	R_s (L/day)	Estimated water concentration (ng/mL)
PRC	na	4.81	64304.8	5.57	23.29	na
p,p DDT	10	4.78	60472.07	5.61	21.90	2.4E-05

Figure 4.1 Calculation of sampling rates (R_s) from PRCs

4.2.2 Expanding the use of passive sampling devices as biological surrogates in human health risk models

Estimated bioavailable water concentrations from exposed LFT accumulations are calculated using the equations and methods listed above. Mass:mass concentrations for LFT are calculated from average LFT mass and instrument generated accumulation data. Rather than calculate back from LFT water concentrations to mass and introduce unnecessary complexity and potential uncertainty, LFT concentrations are based on the mass of a contaminant collected versus the mass of the sampler. LFT are handled as direct biological surrogates when mass:mass concentrations are used, representing the amount of contaminant an organism would be exposed to through partitioning assuming steady state without metabolism.

An approach set forth in the Portland Harbor PHA [4] can be used for calculating exposures and human health endpoints. Contaminant concentrations in fish tissue are replaced by the LFT analyte concentrations using all other variables as stated in the ATSDR PHA. The PHA used an ingestion rate (IR) based on local sport and subsistence angling populations that correspond to the 90^{th} and 99^{th} percentile to help answer study-specific questions, thus the rates may not be applicable elsewhere for direct comparison and could be modified as needed.

The equation for chemical intake is:

$$Exposure = \frac{C \bullet CF \bullet IR \bullet EF \bullet ED}{BW \bullet AT} \qquad \textbf{Eq. 4.7}$$

Where exposure represents the chronic daily intake of a specific chemical (mg/kg-day), C is the concentration in an LFT (mg/kg), CF is a conversion factor (kg/g), IR is the ingestion rate (g/day), EF is the exposure frequency (days/year), ED is the exposure duration (years), BW is the body weight of the consumer (kg), and AT is the averaging time or period over which exposure is averaged (days). A table of parameters and values for calculating exposure and health endpoints is located in Appendix 3 and in the PHA [4].

For non-cancer health effects, a single contaminant's exposure concentration is divided by a reference dose (RfD) or minimum risk level (MRL) as a comparison. Giving a hazard quotient as seen in equation 4.8. The reference dose was introduce by the EPA and is an estimate of daily oral exposure to the human population, which includes sensitive subgroups, that is likely to be without considerable risk of harmful effects during a lifetime. Minimal risk level is a term used by the ATSDR to describe and estimate daily human exposure, taking into account specific routes and length of time of an exposure, to a dose of chemical that is likely to be without a measureable risk of adverse, non-cancerous effects. These are chemical specific estimates, which are intended to serve as screening levels, used by ATSDR health assessors and other responders to identify contaminants and potential health effects that may be of concern at hazardous waste sites [4].

In the ATSDR PHA hazard quotients presented were based on single compounds or compound classes such as PCBs that were associated with the most risk for consuming a particular species. Depending on tissue concentrations, this approach could critically underestimate the risk associated to exposure to mixtures of a large number of contaminants.

Each contaminant's exposure value is added to produce a sum of hazard quotients ratio (equation 4.8). When the ratio exceeds one, an unacceptable risk and a potential for adverse health effects is present. The higher the number the greater the risk, but a ratio above one does not necessarily indicate a health effect will occur. This would be done for all analytes of interest that are not considered carcinogenic by the EPA.

$$\Sigma\, Hazard\, Quotient = \frac{Exposure}{RfD\ or\ MRL} \qquad \textbf{Eq. 4.8}$$

For cancer risk, a contaminant's exposure is multiplied by its oral slope factor; these values are summed to provide an estimated excess cancer risk. The

oral slope factor is the upper bound of an approximate 95% confidence limit from the increased cancer risk produced by a lifetime oral exposure to a contaminant. Oral slope factors for many carcinogenic compounds can be found using the EPA's Integrated Risk Information System (IRIS) online database. In the PHA an excess cancer risk was considered unacceptable if the calculated occurrence was greater than one in ten thousand (1×10^{-4}). The unacceptable excess cancer risk frequency can be adapted to suit other project needs. Parameters are the same for cancer risk as for non-cancer. Carcinogenic endpoints would be assessed for adults only, assuming 30 years of exposure.

$$Excess\ Cancer\ Risk = Exposure \bullet Slope\ Factor \qquad \textbf{Eq. 4.9}$$

The risk model can be evaluated by using US EPA, state guidelines, or similar water quality or guidance values as inputs. Values are available for a number of pesticides for the protection of human health based on either water ingestion, water and fish combined, and fish consumption only. For a non-cancer endpoint a hazard quotient value that exceeds 1 would likely indicate that either the water quality value provides insufficient protection for human health or the model overestimates the risk associated to a compound. The later result would be preferred as it is preventative.

The approach of using LFT as biological surrogates in human health risk assessments has been applied for PAHs previously in our group [9, 13]. Use of LFTs in an ecological risk assessment was also evaluated previously [9, 13] based on an approach developed by Neff *et al.* [14]. A similar approach for pesticides and PCBs potentially may be applied, but has yet to be evaluated.

4.3 Conclusions

The principal role of membrane-based passive samplers is to provide easy yet powerful analytical tools for determining dissolved HOC concentrations in ecosystems. Following the steps shown in this chapter, PSDs can also be useful as

risk assessment screening tools based on steady state assumptions. PSDs, in this way, allow for estimation of organismal exposure to HOCs. Studies have demonstrated [1, and studies listed there in] that PSD concentrations can be linked to organismal exposure, but may not necessarily connect the PSD concentrations to human health risk. Some PSDs, however, can reveal exposure as well as potential tissue concentrations. An assessor of risk could use such information as a standard to evaluate health hazards.

For a PSD such as LFT to be viewed as biomimetic and to correlate with BMOs, equilibrium has to be attained. This is not typically how LFT are utilized, as it is ideal for the sampler to remain in the linear or curve-linear uptake phase. LFT are well suited for reliable determination of HOC sources, concentration gradients and concentration estimates [13], but for the estimation of bioaccumulation factors in species of concern their usefulness is still under debate [1]. The approach described above however, looked at using LFTs in public health risk assessments not the link between BMOs and LFTs

The human health risk model proposed here may not be applicable for some situations as contaminant exposure focused on subsistence angling, but the ingestion rate parameter as well as other parameters could be adjusted to fit the needs of a particular project. Taking that into consideration, this is not necessarily a shortcoming of the model as it can be catered to special people groups of interest. The model, however, assumes that all exposure comes from consumption and that the toxicities of individual compounds are additive. This may lead to either over or underestimations of toxicity. Literature indicates synergistic, additive, and antagonistic effects can occur in mixtures, but predicting the character of the mixture interaction is often difficult and uncertain [15].

Sower et al. [9, 13] utilized this same human health assessment for PAHs. They found that LFTs demonstrated the ability of PSD to provide excellent spatial and temporal data used in conducting ecological and human health assessments and provided increased resolution in risk analysis. Even though our study did not test the model with real-life data values, Sower et al. [9, 13] applied three years of LFT data for PAHs to the model successfully.

4.4 Literature Cited

1. Huckins, J. N.; Petty, J. D.; Booij, K., *Monitors of organic chemicals in the enviornment: Semipermeable membrane devices*. Springer: New York, 2006; p 223.

2. Cockerham, L. G.; Shane, B. S., *Basic Environmental Toxicology*. CRC Press, Inc.: Boca Raton, Florida, 1994.

3. Roberfroid, M. R. S. M. I. A. D. D. M., The risks of risk assessment in foods. *Food and Chemical Toxicology* **1999,** *37*, (2-3), 183-189.

4. DHHS, U., Public Health Assessment for Portland Harbor. In ODHS; ATSDR, Eds. 2006.

5. Burkhard, L. P.; Cook, P. M.; Mount, D. R., The relationship of bioaccumulative chemicals in water and sediment to residues in fish: A vizualization approach. *Environmental Toxicology and Chemistry* **2003,** *22*, (11), 2822-2830.

6. Huckins, J. N.; Prest, H. F.; Petty, J. D.; Lebo, J. A.; Hodgins, M. M.; Clark, R. C.; Alvarez, D. A.; Gala, W. R.; Steen, A.; Gale, R.; Ingersoll, C. G., Overview and comparison of lipid-containing semipermeable membrane devices and oysters (*Crassostrea gigas*) for assessing organic chemical exposure. *Environmental Toxicology and Chemistry* **2004,** *23*, (7), 1617-1628.

7. Kot, A.; Zabiegala, B.; Namiesnik, J., Passive sampling for long-term monitoring of organic pollutants in water. *TrAC Trends in Analytical Chemistry* **2000,** *19*, (7), 446-459.

8. Sethajintanin, D.; Anderson, K. A., Temporal bioavailability of organochlorine pesticides and PCBs. *Environmental Science and Technology* **2006,** *40*, (12), 4506-4516.

9. Sower, G. J.; Anderson, K. A., Using passive sampling devices as biological surrogates in ecological and human health risk models. *Environmental Science and Technology* **In progress**.

10. Booij, K.; Sleiderink, H. M.; Smedes, F., Calibrating the uptake kinetics of semipermeable membrane devices using exposure standards. *Environmental Toxicology and Chemistry* **1998,** *17*, (7), 1236-1245.

11. Huckins, J. N.; Petty, J. D.; Lebo, J. A.; Almeida, F. V.; Booij, K.; Alvarez, D. A.; Cranor, W. L.; Clark, R. C.; Mogensen, B. B., Development of the permeability/performance reference compound approach for *in situ* calibration of

semipermeable membrane devices. *Environmental Science and Technology* **2002,** *36*, (1), 85-91.

12. Booij, K.; Hofmans, H. E.; Fischer, C. V.; Van Weerlee, E. M., Temperature-dependent uptake rates of nonpolar organic compounds by semipermeable membrane devices and low-density polyethylene membranes. *Environmental Science and Technology* **2003,** *37*, (2), 361-366.

13. Sower, G. J. Spatial and temporal variations of bioavailable polycyclic aromatic hydrocarbons in the lower Willamette River. Oregon State University, 2008.

14. Neff, J. M.; Stout, S. A.; Gunster, D. G., Ecological risk assessment of polycyclic aromatic hydrocarbons in sediments: Identifying sources and ecological hazard. *Integrated Environmental Assessment and Management* **2005,** *1*, (1), 22-33.

15. McCarty, L. S.; Borgert, C. J., Review of the toxicity of chemical mixtures: Theory, policy, and regulatory practice. *Regulatory Toxicology and Pharmacology* **2006,** *45*, (2), 119-143.

CHAPTER 5 – CONCLUSIONS

Continually elevating environmental quality criteria and the subsequent need to measure concentrations of contaminants at increasingly low levels has caused typical environmental analyses to become more expensive. There is an obvious need for rapid but effective low-cost integrated methods that would enable monitoring of the fate and concentrations of contaminants in the environment directly. Environmental concentrations of contaminants and assessment of the hazards these chemicals pose to the environment and to human health can be evaluated using passive sampling devices; LFT are particularly well suited to this task.

Further development of a simple time-integrative passive sampling device, the LFT, was discussed. A laboratory and analytical method was developed that provides a less wasteful, in terms of labor and solvent, more efficient, and more green alternative to current methods while maintaining comparable limits of quantitation. Successful removal of gel permeation chromatography as a sample clean-up and fractionation step eliminated the need for specialized equipment to processing many PSD samples. LFTs are well suited to PCBs and pesticides for GC/ECD analysis as well as PAHs by HPLC. However, the ability to use GC/MS to analyze complex mixtures sequestered in LFT would be ideal. Experiments are underway in our laboratory to investigate this further. Zebrafish, in a developmental toxicity assay can be exposed to LFT extracts without deleterious biological effects as compared to both fish water and DMSO vehicle controls. As a result, LFT allow for bridging research and knowledge gaps by coupling PSDs with biological analysis of samples collected; potentially providing many insights including the relative toxicological significance of real-world bioavailable aqueous phase contaminant concentrations.

A series of training exchanges between Oregon State University and the CERES/Locustox laboratory in Dakar, Senegal demonstrated that LFT technology as well as associated laboratory and field methods lend themselves to easy

incorporation into a remote monitoring study without the need of extensive resources on hand. The CERES laboratory had neither the prior knowledge or equipment on hand to process other currently used PSD. LFT was fit for the purpose at hand: little to no prior knowledge of passive sampling techniques was needed to ensure successful application by staff to the CERES laboratory. The staff at CERES was mostly amenable to training, but at times showed an over confidence in their abilities which may need to be taken into account when transferring similar sampling methods and technologies to other laboratories similar to CERES. A training manual was developed for a previous training event as part of a portable laboratory and training package to provide instruction specific to transferring methodology for utilizing passive sampling devices for persistent organic pollutant trans-boundary analysis in western Africa. The portable laboratory and training package should assist technology to be transferred to other laboratories easily and successfully in the future with less hands-on training and training exchanges needed.

The principal role of membrane-based passive samplers is to provide easy yet powerful analytical tools for determining dissolved and HOC concentrations in ecosystems. LFTs can also be useful as risk assessment screening tools based on steady state assumptions. LFTs allow for the estimation of exposure of organisms to HOCs compounds in aqueous ecosystems as well as potential tissue concentrations. An assessor of risk could use such information as a standard to evaluate health hazards. LFT can be used as a biological surrogate for fish/shellfish when mass:mass concentrations are used, representing the amount of contaminant an organism would be exposed to through partitioning assuming steady state without metabolism. LFT concentrations are based on the mass of a contaminant collected versus the mass of the sampler.

The research presented here advances science by improving upon a tool useful in environmental monitoring studies and health risk assessments. LFTs offer the same benefits as other current PSDs, but provide a simpler, more efficient, and cost effective alternative. Also, they allow for simple application to remote monitoring studies where many conventional and contemporary methods of sampling prove difficult or impossible.

BIBLIOGRAPHY

Adams, R. G., R. Lohmann, et al. (2007). "Polyethylene Devices: Passive samplers for measuring dissolved hydrophobic organic compounds in aquatic environments." Environmental Science and Technology **41**(4): 1317-1323.

Alexander, M. (2000). "Aging, bioavailability, and overestimation of risk from environmental pollutants." Environmental Science and Technology **34**(20): 4259-4265.

Allan, I. J., B. Vrana, et al. (2006). "A "toolbox" for biological and chemical monitoring requirements for the European Union's Water Framework Directive." **69**(2): 302-322.

Alvarez, D. A., J. D. Petty, et al. (2004). "Development of a passive, *in situ*, integrative sampler for hydrophilic organic contaminants in aquatic environments " Environmental Toxicology and Chemistry **23**(7): 1640-1648.

Anderson, K. A. and W. Hillwalker (2008). Lipid-free tube passive sampling device technology transfer: On-site quality assurance, laboratory and field training, Oregon State University.

Anderson, K. A. and W. E. Hillwalker (in press 2008). Ecotoxicology Bioavailability. Encyclopedia of Ecology. Oxford, Elsevier Publishing Inc.

Anderson, K. A. and E. R. Johnson (2001). "Bioavailable organochlorine pesticides in a semi-arid region of Eastern Oregon, USA, as determined by gas chromatography with electron capture detector." Journal of AOAC International **84**: 1371-1382.

Anderson, K. A., D. Sethajintanin, et al. (2008). "Field trial and modeling of uptake rates of *in situ* lipid-free polyethylene membrane passive sampler." Environmental Science and Technology **42**(12): 4486-4493.

Augustijn-Beckers, P. W. M., A. G. Hornsby, et al. (1994). The SCS/ARS/CES Pesticide properties database for environmental decision-making. II Additional compounds. Reviews of Environmental Contamination and Toxicology. G. W. Ware. New York, Springer-Verlag. **137**.

Booij, K., J. R. Hoedemaker, et al. (2003). "Dissolved PCBs, PAHs, and HCB in pore waters and overlying waters of contaminated harbor sediments." Environmental Science & Technology **37**(18): 4213-4220.

Booij, K., H. E. Hofmans, et al. (2003). "Temperature-dependent uptake rates of nonpolar organic compounds by semipermeable membrane devices and low-density polyethylene membranes." Environmental Science and Technology **37**(2): 361-366.

Booij, K., H. M. Sleiderink, et al. (1998). "Calibrating the uptake kinetics of semipermeable membrane devices using exposure standards." Environmental Toxicology and Chemistry **17**(7): 1236-1245.

Booij, K., F. Smedes, et al. (2002). "Spiking of performance reference compounds in low density polyethylene and silicone passive water samplers." Chemosphere **46**(8): 1157-1161.

Burkhard, L. P., P. M. Cook, et al. (2003). "The relationship of bioaccumulative chemicals in water and sediment to residues in fish: A vizualization approach." Environmental Toxicology and Chemistry **22**(11): 2822-2830.

Carls, M. G., L. G. Holland, et al. (2004). "Monitoring polynuclear aromatic hydrocarbons in aqueous environments with passive low-density polyethylene membrane devices." Environmental Toxicology and Chemistry **23**(6): 1416-1424.

Chimuka, L. and E. Cukrowska (2006). "The role of passive samplers in monitoring of aquatic ecosystems and occupational hygiene pollution." LC/GC North America **June**: 1-11.

Cockerham, L. G. and B. S. Shane, Eds. (1994). Basic Environmental Toxicology. Boca Raton, Florida, CRC Press, Inc.

Comyn, J. (1985). Polymer Permeability. London, England, Elsevier Applied Science LTD.

Cornelissen, G., A. Pettersen, et al. (2008). "Field testing of equilibrium passive samplers to determine freely dissolved native polycyclic aromatic hydrocarbon concentrations." Environmental Toxicology and Chemistry **27**(3): 499-508.

U.S. DHHS. (2006). Public Health Assessment for Portland Harbor. ODHS and ATSDR.

Eisler, R. and A. A. Belisle (1996). Planar PCB hazards to fish, wildlife, and invertebrates: a synoptic review. Washington, D.C., The United States Department of the Interior, National Biological Service.

Escher, B. I. and J. L. M. Hermens (2004). "Internal Exposure: linking bioavailability to effects." Environmental Science and Technology **38**(23): 455A-462A.

Esteve-Turrillas, F. A., A. Pastor, et al. (2007). "Using semi-permeable membrane devices as passive samplers." TrAC Trends in Analytical Chemistry **26**(7): 703-712.

Esteve-Turrillas, F. A., V. Yusà, et al. (2008). "New perspectives in the use of semipermeable membrane devices as passive samplers." Talanta **74**(4): 443-457.

Gale, R. W. (1998). "Three-compartment model for contaminant accumulation by semipermeable membrane devices." Environmental Science and Technology **32**(15): 2292-2300.

Górecki, T. and J. Namiesnik (2002). "Passive sampling." TrAC Trends in Analytical Chemistry **21**(4): 276-291.

Gustavson, K. E., W. M. DeVita, et al. (2000). "Novel use of a dual-zone restricted access sorbent: normal-phase solid-phase extraction separation of methyl oleate from polynuclear aromatic hydrocarbons stemming from semi-permeable membrane devices." Journal of Chromatography A **883**: 143-149.

Hallare, A., K. Nagel, et al. (2006). "Comparative embryotoxicity and proteotoxicity of three carrier solvents to zebrafish (Danio rerio) embryos." Ecotoxicology and Environmental Safety **63**(3): 378-388.

Huckins, J. N., G. K. Manuweera, et al. (1993). "Lipid-containing semipermeable membrane devices for monitoring organic contaminants in water." Environmental Science and Technology **27**(12): 2489-2496.

Huckins, J. N., J. D. Petty, et al. (2006). Monitors of organic chemicals in the enviornment semipermeable membrane devices. New York, Springer.

Huckins, J. N., J. D. Petty, et al. (2002). "Development of the permeability/performance reference compound approach for *in situ* calibration of semipermeable membrane devices." Environmental Science and Technology **36**(1): 85-91.

Huckins, J. N., J. D. Petty, et al. (1999). "Determination of uptake kinetics (sampling rates) by lipid-containing semipermeable membrane devices (SPMDs) for polycyclic aromatic hydrocarbons (PAHs) in water." Environmental Science and Technology **33**(21): 3918-3923.

Huckins, J. N., H. F. Prest, et al. (2004). "Overview and comparison of lipid-containing semipermeable membrane devices and oysters (*Crassostrea gigas*) for assessing organic chemical exposure." Environmental Toxicology and Chemistry **23**(7): 1617-1628.

Huckins, J. N., M. W. Tubergen, et al. (1990). "Semipermeable membrane devices containing model lipid: A new approach to monitoring the bioavailability of lipophilic contaminants and estimate their bioconcentration potential." Chemosphere **20**(5): 533-552.

Kot-Wasik, A., B. Zabiegala, et al. (2007). "Advances in passive sampling in environmental studies." Analytica Chimica Acta **602**(2): 141-163.

Kot, A., B. Zabiegala, et al. (2000). "Passive sampling for long-term monitoring of organic pollutants in water." TrAC Trends in Analytical Chemistry **19**(7): 446-459.

Lebo, J. A., F. V. Almeida, et al. (2004). "Purification of triolein for use in semipermeable membrane devices (SPMDs)." Chemosphere **54**(8): 1217-1224.

Leonard, A. W., H. V. Ross, et al. (2002). "Trimethylpentane-containing passive samplers for predicting time-integrated concentrations of pesticides in water: laboratory and field studies." Environmental Toxicology and Chemistry **21**(12): 2591-2599.

Mackay, D., W.-Y. Shiu, et al. (1992). Illustrated handbook of physical-chemical properties and environmental fate of organic chemicals. Boca Raton, Lewis Publishers.

Mackay, D., W. Y. Shiu, et al. (1999). Physical-chemical properties and environmental fate handbook. Boca Raton, FL, CRC Press.

Mackay, D., W. Y. Shiu, et al. (2006). Handbook of physical-chemical properties and environmental fate for organic chemicals. Boca Raton, CRC Press.

Madrid, Y. and Z. P. Zayas (2007). "Water sampling: Traditional methods an.d new approaches in water sampling strategy." TrAC Trends in Analytical Chemistry **26**(4): 293-299.

Mayer, P., J. Tolls, et al. (2003). "Equilibrium sampling devices." Environmental Science and Technology **37**(9): 185A-191A.

McCarty, L. S. and C. J. Borgert (2006). "Review of the toxicity of chemical mixtures: Theory, policy, and regulatory practice." Regulatory Toxicology and Pharmacology **45**(2): 119-143.

Meadows, J. C., K. R. Echols, et al. (1998). "Estimation of uptake rate constants for PCB congeners accumulated by semipermeable membrane devices and brown trout *(Salmo trutta)*." Environmental Science and Technology **32**(12): 1847-1852.

Namieśnik, J., B. Zabiegała, et al. (2005). "Passive sampling and/or extraction techniques in environmental analysis: a review." Analytical and Bioanalytical Chemistry **381**(2): 279-301.

Neff, J. M., S. A. Stout, et al. (2005). "Ecological risk assessment of polycyclic aromatic hydrocarbons in sediments: Identifying sources and ecological hazard." Integrated Environmental Assessment and Management **1**(1): 22-33.

Opperhuizen, A., E. W. van der Velde, et al. (1985). "Relationship between bioconcentration in fish and steric factors of hydrophobic chemicals." Chemosphere **14**: 1871-1896.

Petty, J. D., J. N. Huckins, et al. (1997). Evaluation of the semipermeable membrane devices (SPMD) as a passive *in situ* concentrator of military organic chemicals in water, Final report, approved for release. Fort Dentrick, MD, US Army Medical Research and Material Command.

Rantalainen, A.-L., W. J. Cretney, et al. (2000). "Uptake rates of semipermeable membrane devices (SPMDs) for PCDDs, PCDFs, and PCBs in water and sediment." Chemosphere **40**: 147-158.

Roberfroid, M. R. S. M. I. A. D. D. M. (1999). "The risks of risk assessment in foods." Food and Chemical Toxicology **37**(2-3): 183-189.

Rusina, T. P., F. Smedes, et al. (2007). "Polymer selection for passive sampling: A comparison of critical properties." Chemosphere **68**(7): 1344-1351.

Sabaliunas, D., J. Ellington, et al. (1999). "Screening of bioavailable hydrophobic toxicants in surface waters with semipermeable membrane devices: Role of inherent oleic acid in toxicity evaluations." Ecotoxicology and Environmental Safety **44**: 160-167.

Sabaliunas, D., J. Lazutka, et al. (2000). "Acute toxicity and genotoxicity of aquatic hydrophobic pollutants sampled with semipermeable membrane devices." Environmental Pollution **109**: 251-265.

Sander, L. C., S. A. Wise, et al. (1997). Polycyclic aromatic hydrocarbon structure index. NIST special publication 922. Gaithersburg, MD, U.S. Dept. of Commerce, Technology Administration, National Institute of Standards and Technology.

Schwarzenbach, R. P., P. M. Gschwend, et al. (2003). Environmental organic chemistry. Hoboken, NJ, John Wiley & Sons, Inc.

Sethajintanin, D. (2003). Bioaccumulation profiles of chemical contaminants in fish from the lower Willamette River, Portland Harbor superfund site and human health risk assessment. Environmental and Molecular Toxicology. Corvallis, Oregon State University**:** 119.

Sethajintanin, D. and K. A. Anderson (2006). "Temporal bioavailability of organochlorine pesticides and PCBs." Environmental Science and Technology **40**(12): 4506-4516.

Setková, L., J. Hajslová, et al. (2005). "Fast isolation of hydrophobic organic environmental contaminants from exposed semipermeable membrane devices (SPMDs) prior to GC analysis." Journal of Chromatography A **1092**(2): 170-181.

Sower, G. J. (2007). Lipid-free tube passive sampling device technology transfer: On-site laboratory and field training, Oregon State University.

Sower, G. J. (2008). Spatial and temporal variations of bioavailable polycyclic aromatic hydrocarbons in the lower Willamette River. Environmental and Molecular Toxicology, Oregon State University. **Ph.D.**

Sower, G. J. and K. A. Anderson (In progress). "Using passive sampling devices as biological surrogates in ecological and human health risk models." Environmental Science and Technology

Stuer-Lauridsen, F. (2005). "Review of passive accumulation devices for monitoring organic micropollutants in the aquatic environment." Environmental Pollution **136**(3): 503-524.

Suffet, I. H., C. T. Jafvert, et al. (1994). Synopsis of discussion session:influences of particulate and dissolved material on the bioavailability of organic compounds. Bioavailability:physical, chemical, and biological interactions. J. L. Hamelink, P. F. Landrum, H. L. Bergman and W. H. Benson. Boca Raton, Lewis Publishers.

Usenko, C. Y., S. L. Harper, et al. (2007). "In vivo evaluation of carbon fullerene toxicity using embryonic zebrafish." Carbon **45**(9): 1891-1898.

Vrana, B., I. J. Allan, et al. (2005). "Passive sampling techniques for monitoring pollutants in water." TrAC Trends in Analytical Chemistry **24**(10): 845-868.

Wells, J. B. and R. P. Lanno (2001). Passive sampling devices (PSDs) as biological surrogates for estimating the bioavailability of organic chemicals in soil. Environmental toxicology and risk assessment: science, policy, and standardization - implication for environmental decisions : Tenth volume, ASTMSTP. B. M. Greenberg, R. N. Hull, M. H. J. Roberts and R. W. Gensemer. West Conshohocken, PA, American Society for Testing and Materials**:** 253-270.

Wennrich, L., B. Vrana, et al. (2003). "Development of an integrative passive sampler for the monitoring of organic water pollutants." Journal of Environmental Monitoring **5**(5): 813-822.

Wenzel, K. D., B. Vrana, et al. (2004). "Dialysis of persistent organic pollutants and polycyclic aromatic hydrocarbons from semipermeable membranes. A procedure using an accelerated solvent extraction device." Analytical Chemistry **76**(18): 5503-5509.

Yusà, V., A. Pastor, et al. (2005). "Microwave-assisted extraction of OCPs, PCBs and PAHs concentrated by semi-permeable membrane devices (SPMDs)." Analytica Chimica Acta **540**(2): 355-366.

APPENDICIES

APPENDIX 1 – FIELD TRIAL AND MODELING OF UPTAKE RATES OF IN SITU LIPID FREE POLYETHYLENE MEMBRANE PASSIVE SAMPLER

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A 1.1 Abstract

Lipid free polyethylene membrane tubing (LFT) has been further developed in response to a growing need for an inexpensive, simple time-integrative sampling device for dissolved hydrophobic contaminants in water. The LFT sampler is based on the diffusion of dissolved hydrophobic target compounds through the aqueous boundary layer and into the polyethylene membrane, mimicking uptake by organisms. We demonstrate through laboratory and field validation studies that LFT provided the same benefits as many other passive sampling devices, without the potential of analytical interference from lipid impurities. A total of 370 LFTs and SPMD (semipermeable membrane devices) were deployed for 21 days in paired studies at highly urbanized, undeveloped, and two Superfund sites, and representing several river conditions. A simple internal surrogate spiking method served as an in situ calibration indicator of the effects of environmental conditions on the uptake rates. A modified extraction method for the LFT increased recoveries while decreasing solvent use and labor compared to other organic extraction procedures. LFT sampling rates were estimated using ratios, in-situ calibration and modeling for over 45 target analytes, including PAHs, PCBs, and pesticides.

A 1.2 Introduction

Passive sampling devices (PSD) are widely used to assess exposure and contamination in water, air, and soils [1-5]. The major advantages of a passive sampling technique are the ability to distinguish between dissolved and bound molecules and the focus on availability and activity, rather than on the mere presence of chemicals [1, 6]. The freely dissolved fraction of the contaminant is related to chemical biological availability, toxicity, mobility, and degradation processes [7, 8]. The passive sampling technique measures the concentration in a reference phase, which can be brought into equilibrium with the medium [6]. The

availability of a chemical is measured based on the chemical potential, which is logarithmically related to its fugacity and linearly related to its dissolved concentration in a particular medium [6].

PSDs have been demonstrated to sequester a variety of non-polar and moderately polar organic contaminant [9, 10]. Many PSDs consist of low density polyethylene tubing containing a small volume of lipid, such as triolein, hexane, and trimethylpentane to name a few [9, 11, 12]. The PSDs are thought to mimic key mechanisms of bioconcentration, including diffusion through biomembranes and partitioning between organism and the surrounding medium [9, 10]. The transient polymer cavity for polyethylene PSDs range to ~10 Å [13] and closely resembles the size of transient cavities in biomembranes estimated at 9.8 Å [14] potentially mimicking -molecular discrimination. Analysis of lipid containing PSDs however, may be hampered by interferences from polyethylene oligomers, and lipid impurities [15, 16]. Size exclusion chromatography may be used to remove polyethylene oligomers [16]. Small amounts of oleic acid and methyl oleate remain and are analytically problematic [16]. Measures to reduce interferences from these lipid impurities are more expensive, laborious, and often sacrifice target analytes. Lipid containing PSDs are also more difficult to model as they represent a 3-compartment model whereas the LFT are a 2-compartment model.

The polyethylene membrane itself, also described as lipid free tubing (LFT), contains a significant portion of the total amount of dissolved target analytes accumulated by PSD as determined in laboratory aquatic studies [17, 18]. For example, Booij *et al* [19] found that the role of triolein in semipermeable membrane devices (SPMDs) in the exchange kinetics was insignificant, for compounds with log K_{ow} > 6 [20, 21]. Thus, by removing the lipid from passive sampling devices, the analysis of a lipid-free polyethylene device could be less laborious and expensive without sacrificing target analytes while improving detection limits. Polyethylene membrane without lipid has been reported to be more susceptible to biofouling and reach equilibrium more quickly than lipid

containing PSDs because of its smaller sorption capacity [19, 22]. We postulated that capacity of LFTs would not differ substantially from lipid containing PSDs, nor be excessively biofouled, using typical deployment times, for ambient Superfund levels of environmental hydrophobic organic contaminants. Information on the kinetic phase of chemical uptake during field deployment can be obtained by using pre-loaded internal standards (IS) also called permeability/ performance reference compounds (PRCs) as an *in situ* calibration approach [19, 23]. PRCs are non-interfering compounds with moderate to relatively high fugacity. These compounds are added to PSDs prior to membrane deployment to assess the effect of environmental variables. Because the uptake and dissipation of organic chemicals are controlled by the same molecular processes, *in situ* dissipation rates of PRCs would allow estimation of PSD uptake rates. For SPMD, triolein served as a convenient carrier for PRC, but a different approach was necessary for a lipid-free polyethylene membrane. Booij *et al* [24] developed an effective, yet laborious, infusion spiking method for triolein- free PSDs, based on equilibration of the membrane in a spiked methanol:water solution.

The feasibility of LFTs as a passive sampler in aquatic systems has been mentioned [19, 21, 22]. However studies [19, 20, 22] involving rigorous field application, demonstration and direct comparison with another types of PSD with co-deployed LFTs are limited [17, 25, 26]. In this study, we evaluate the use of LFTs for assessing dissolved hydrophobic organic contaminants in surface waters. A new method for extracting the LFTs was developed that reduced labor, eliminated chlorinated solvents and reduces overall solvent use while improving recovery of our 45 target analytes, especially low molecular weight analytes, compared with typical extraction methods. An alternative method for spiking PRCs into LFTs was developed and recoveries were tested in both laboratory and field systems and proved effective. LFTs and SPMDs were co-deployed in a series of deployments, at highly urbanized, undeveloped and two Superfund sites. LFT sampling rates (RS) for 33 routinely detected target analytes including PAHs, polychlorinated biphenyls (PCBs), and pesticides were estimated and modeled

using several approaches. Field-derived data demonstrated the effectiveness, reliability and cost effectiveness of LFTs for environmental assessment.

A 1.3 Materials and methods

A 1.3.1 Standards, solvents and certified reference material (CRM)

Pesticide (purities ≥ 98.5%), PAHs (purities ≥ 99%) and PCBs (purities ≥ 99%) standards were from Chem Service, Inc. (West Chester, PA) or AccuStandard (New Haven, CT). CRM of OCs in cod liver oil (1588a) was from the National Institute of Standards and Technology (Gaithersburg, MD). A list of 45 target analytes, abbreviations and selected physicochemical properties are found in supplementary table A S.1. Standard SPMD were from Environmental Sampling Technologies (St. Joseph, MO).

A 1.3.2 Preparation of LFTs and embedding of internal surrogate (PRCs) method

Additive-free low-density polyethylene membrane (Barefoot) was purchased from Brentwood Plastic, Inc (St. Louis, MO, USA). The LFT were 2.7 cm wide, 100-cm long. The polyethylene membrane was pre-cleaned using Optima® grade hexanes from Fisher Chemical, (Fairlawn, NJ). LFTs were loosely rolled, placed in glass jars, covered with hexanes and placed on an orbital shaker with light shaking for 48 h. The hexanes were exchanged twice. The LFTs were dried by vacuum at ambient temperature for up to 48 h or until the solvent was removed. Pre-extracted LFTs were heat-sealed on one end and PRCs were spiked, using < 100uL, into the tube and the end heat-sealed. LFT dimensions are given in supplementary material. PRCs used were chosen based on similarity to our target analytes. PRCs used were not present at the sites, based on previous data [27] and the continued use of field blanks. PRCs used represented a range of log K_{ow} 4.2 to 7.1 compounds see supplementary table A S.2. The micro-volume of PRCs represents ≤ 1% (v/v), whereas, lipids in PSDs typically represent ≥ 25% (v/v). Smaller volumes of PRCs have been subsequently used with equal success. PRCs used and their concentrations are described in supplementary material. The LFTs used in this study are 20% larger in volume than the SPMD, and are estimated to

be about 40% larger than the triolein-free-membranes used in other comparative studies such as Adams *et al.* [17].

A 1.3.3 Laboratory demonstration of LFTs in situ calibration

In a pilot experiment, fortified LFTs, were placed in a darkened glass aquarium tank with tap water at a flow rate 41 ± 7 mL/s and 17 ± 1 °C. After 14 days, unexposed fortified LFTs, fortified LFT procedural blanks, reagent blanks, and fortified reagent blanks accompanied the exposed LFTs and were processed as previously described [3].

A 1.3.4 LFT field deployment

A total of 370 PSDs were co-deployed with SPMDs in the lower Willamette River at Portland, which included the Portland Harbor Superfund site, the McCormick and Baxter Creosoting Co. Superfund site, highly urbanized, and undeveloped sites. Eleven sites and two duplicate sites were established through an 18 mile section of the Willamette River, representing (n=74) samples each with 5 PSDs [4]. Three sampling campaigns in fall 2003 and summer and fall of 2004 were completed. For each campaign the PSDs were deployed for 21 days, temperature, flow conditions, organic and dissolved carbon, suspended and dissolved solids, dissolved oxygen and pH are given in supplementary materials.

A 1.3.5 Alternative extraction methods for LFTs

Four sets of LFTs were prepared and modified extraction methods were performed on each set. Each set consisted of 3 blank LFTs, 3 fortified LFTs and 1 field exposed LFT. A reagent blank and PAH and PCB spikes were also included in each set. Fortification consisted of 20 PAHs and organochlorines at various concentrations. The traditional SPMD extraction method has been described previously [10]. Briefly, this method uses 400 mL hexanes (200 mL x 2), gel permeation chromatography (GPC), and includes several solvent reduction/exchange steps [4]. The second method investigated, utilized the traditional extraction without the GPC step. The third method investigated, also eliminates the GPC step and the LFTs were cut into several pieces and extracted using only 100 mL hexanes (50 mL x 2) for the dialysis step. The fourth method

investigated used an accelerated solvent extraction (ASE) (Dionex ASE 300 (Sunnyvale, CA)) approach. The ASE method consisted of a 5 min heat to 40 °C and pressure to ~1500 psi. Each 34-mL cell contained one LFT and was filled with 100% hexanes and held static for 5 min. A flush consisting of 50% cell volume of hexanes and then a nitrogen purge for 240 seconds.

A 1.3.6 Analysis and quality control and statistical analysis

OC contaminants were determined using GC-μECD (Agilent Technologies 6890N Network GC system) (Palo Alto, CA), with dual capillary columns (db-xlb and db-17, J&W Scientific Inc., Agilent Technologies, Palo Alto, CA) and dual detectors. Injector and detector temperature were at 250 °C and 350 °C, respectively. The GC system was operated with He carrier gas and N2 makeup gas. The oven temperature program was as follows: 100 °C, with a 1-minute hold, and ramped at 1.2 °C min-1 to 265 °C, with a 2-minute hold. PAH contaminants were determined using a 150 x 3.0 mm Phenomenex Luna C18 column (Phenomenex, Torrance, CA) with a Hewlett Packard HPLC series 1100 (Agilent Technologies, Palo Alto, CA) and a flow rate of 1.5 mL/min. The gradient program started at 40:60 acetonitrile:water, with a 10 min hold, ramped first to 70% acetonitrile at 25 min and then to 90% acetonitrile at 36 min, with a 3 min hold. PAH were detected using UV absorbance at 230, 242, and 254 nm and fluorescence with 230 nm excitation and 360, 410, 460 nm emissions.

In addition to the laboratory and field QC described above, PSD trip and field blanks were employed for all field campaigns. Duplicate field samples were setup at two sites. Quality control samples represented 30 to 40% of a sample set. Data interpretation was performed with SPSS® Version 10.0.1 (SPSS Inc., 1989-1999), Sigma Plot 2002 for Windows Version 8.0 (SPSS Inc., 1986-2001), and Microsoft® Office Excel 2003 (Microsoft Corporation, 1985-2003).

A 1.4 Results and discussion

A 1.4.1 Evaluation of alternative extraction methods

Supplementary table A S.3, shows the compound specific recoveries for each of the four extract methods tested. The average recovery for the traditional, without GPC, without GPC and cut PSDs, and ASE methods were 75% (±2), 91% (±3), 93% (±8), and 37% (±10), respectively. Recoveries increased significantly with the elimination of the gel permeation chromatography step, except for the ASE method. Low molecular weight compounds (LMW) using the traditional method had an average recovery of 63% while the method without GPC and cut PSDs had an average recovery of 85%. LMW compounds with higher vapor pressures had the largest percent recovery increases, though high molecular weight compound recoveries also improved slightly. Eliminating several concentration steps likely explains much of the gains in LMW recoveries, while eliminating GPC and associated sample transfers helps explain improved recoveries of all compounds. Non-GPC and non-GPC and cut PSDs methods did not differ significantly demonstrating that the 80% reduction in solvent use of the latter did not negatively affect recoveries. There was small improvement in recoveries of the organochlorine compounds. The ASE method had consistent recoveries, albeit poor, throughout the range of K_{ow}. This suggests the method could be altered to increase the extraction efficiency. The solvent may be modified, the temperature increased, or multiple solvent cycles performed. The temperature was intentionally kept low to prevent decomposition of the low density polyethylene; however, inspection after extraction showed no breakdown of the material. Overall, the non-GPC and cut PSDs method eliminated all chlorinated solvent, and an expensive labor intensive GPC step. In addition, the non-GPC and cut PSDs method reduced solvent transfers, and reduced the hexanes used up to 80% while increasing recovery efficiencies, without compromising chromatography or sacrificing target analytes.

A 1.4.2 Evaluation of the spiking method for internal surrogates (PRCs) in LFT

For purposes of comparison with other passive sampling devices the traditional extraction method was used. Average recovery of LFT internal surrogates was 89% ± 11%, from a 3-week room temperature storage and transport study illustrating that there was no loss of spiked compounds or other stability issues with the approach. These recoveries indicate that dissipation of spiked compounds during sample preparation, storage, handling, and extraction was negligible and that the integrity of the PRCs spiked into the PSD was maintained.

Measuring the PRC loss over an exposure period provides an *in situ* clearance rate constant of PRC ($k_{e\text{-}prc}$) [23].

$$k_{e\text{-}prc} = \ln (N_{PSD\text{-}0} / N_{PSD\text{-}e}) \bullet t^{-1} \qquad \textbf{Eq. A 1.1}$$

$N_{PSD\text{-}0}$ is the initial amount of the PRC at $t = 0$, and $N_{PSD\text{-}e}$ is the amount of PRC remaining in the PSD following exposure, and t is exposure time in days. In flowing-water laboratory exposure study, $k_{e\text{-}prc\text{-}lab}$ for compounds with log K_{ow} > 5.1 were less than 0.008 d^{-1}. These findings substantiate that a LFT has the capacity to retain hydrophobic organic compounds in the polymer [22] and that the PRC spiking method was effective.

A 1.4.3 Demonstration of effectiveness and reliability of LFT for field deployment

Comparison of the $k_{eprc\text{-}field}$, derived from field-exposed PSDs to $k_{eprc\text{-}lab}$, measured in calibration exposure, ($k_{eprc\text{-}field}$ / $k_{eprc\text{-}lab}$) can serve as an *in situ* calibration indicator of differences in the exposure conditions or the effect of environmental variables on PSD sampling [23]. Recoveries and clearance rates ($k_{eprc\text{-}field}$) of PRCs in both LFTs and SPMDs, co-deployed under various field flow conditions are shown in table A 1.1. Retention of PRC in both PSDs indicated linear uptake for all target analytes, as defined [22] by ≥ 40% retention. The similarity in clearance times indicates that, for a 21-day field exposure, LFTs and SPMDs were governed by similar exchange kinetics, previously demonstrated to be first order for SPMDs [10].

The LFT R_S for all compounds studied were found to be insensitive to modest differences in river flow, table A 1.1. Two field events, both at 22 °C, and river flow rates of 235 and 285 m^3/s were found to have less than a 10% difference in LFT R_S. The temperature effect on average for the PAH R_S increases by a factor of 1.7 from 9 °C to 22 °C, resulting in an activation energy of *ca.* 40 kJ/mol. The PCBs on average increased by a factor of 2.4 from 9 °C to 22 °C, resulting in an activation energy of *ca.*70 kJ/mol. The PCBs' activation energy is in good agreement with that reported by Rantalainen *et al.* [28] for PCDD/PCDF and non-ortho substituted PCBs which ranged from 40 to 100 kJ/mol. On average, this means that a temperature increase of 10 °C causes an increase of about a factor of 2 in R_s.

Table A 1.1 PRC recoveries, retention and clearance rates for LFTs and SPMDs under two field conditions. Each sample was a composite of 5 PSDs, avg temperature, and flow rate: A) 22 °C, 235 m^3/s, and B) 22 °C, 285 m^3/s; values are averages and ± 1SD.

A) PRC, 235 m^3/s	**Percent recovery (%) retention**				**PRC clearance rate k_{eprc} ($d^{-1}x10^{-3}$)**		**$k_{eprc\text{-}field}$/ $k_{eprc\text{-}lab}$**
	field sample (n=13)		laboratory and field control (n=7)				
	LFT	**SPMD**	**LFT**	**SPMD**	**LFT**	**SPMD**	**LFT**
PCB 82 (log K_{ow} = 6.1)	62 ± 2.2	50 ± 3.0	81 ± 8.2	80 ± 5.9	10	20	1.25
endrin (log K_{ow} = 5.1)	41 ± 4.9	64 ± 4.9	93 ± 24	80 ± 10	40	10	5
dibenz[ah]anthracene (log K_{ow} = 6.5)	84 ± 6.2	52 ± 13	92 ± 7.2	94 ± 4.2	4	3	2
B) PRC, 285 m^3/s							
PCB 82 (log K_{ow} = 6.1)	65 ± 7.8	54 ± 6.0	73 ± 3.4	85 ± 8.9	6	20	0.75
endrin (log K_{ow} = 5.1)	33 ± 7.7	89 ± 11	87 ± 4.7	106 ± 15	40	8	5
dibenz[ah]anthracene (log K_{ow} = 6.5)	85 ± 4.4	65 ± 7.6	92 ± 8.5	100 ± 4.8	4	2	2

LFT sorption capacity estimate: Another concern in using the lipid-free polyethylene membrane as a PSD is its potentially smaller sorption capacity due to lack of lipid [21, 22]. Lack of lipid did not appear to affect the capacity of LFTs to accumulate hydrophobic organic contaminants under realistic exposure conditions, figure A 1.1. An estimate of sorption capacity is partly based on device volume (equation A 1.1), and the device used here is nominally 20% larger in volume than the SPMDs.

Comparison of PSDs: The ability to compare results of PSDs with datasets from other PSD studies is desirable. Concentrations of contaminants accumulated in LFTs and SPMDs at each sampling site for each deployment were statistically compared (a paired *t*-test, two-sided *p*-value = 0.05 was considered significantly different). The mean concentrations for p,p'-DDT, p,p'-DDD, p,p'-DDE, and Σ_{25}PCBs (sum of 25 individual PCB congeners) in LFTs were slightly higher than in SPMDs under all 3 conditions. No significant difference was observed for the mean concentration for ΣPAH. However, the mean concentrations of three-ring PAH with log $K_{ow} < 4.5$ were higher in the SPMD. The mean concentrations of PAH with log $K_{ow} \geq 6.0$ were higher in LFT. No differences were observed between the concentrations in LFT and in SPMD for PAH with log K_{ow} between 4.5 and 5.5. For the 45 contaminants tested, 23 were taken up by the LFTs faster than by SPMDs, and 10 compounds were taken more slowly (supplementary figure A S.1). All analytes that were detected in the SPMDs were detected in the LFTs and in some instances, additional PCB congeners were above detection limit in the LFTs but below detection limits in the SPMDs due to cleaner background in the LFTs. Our field studies corroborate the laboratory exposure results by Booij *et al* [19], demonstrating the overall effectiveness and reproducibility of LFTs for sampling hydrophobic contaminants. The congener distribution of PAHs and PCBs accumulated by LFT and SPMD from side by side field deployments were also very similar, figure A 1.2. Overall, the amount sequestered and the PCBs and PAHs congener distribution were similar or when they were statistically different the differences were small (< 20%).

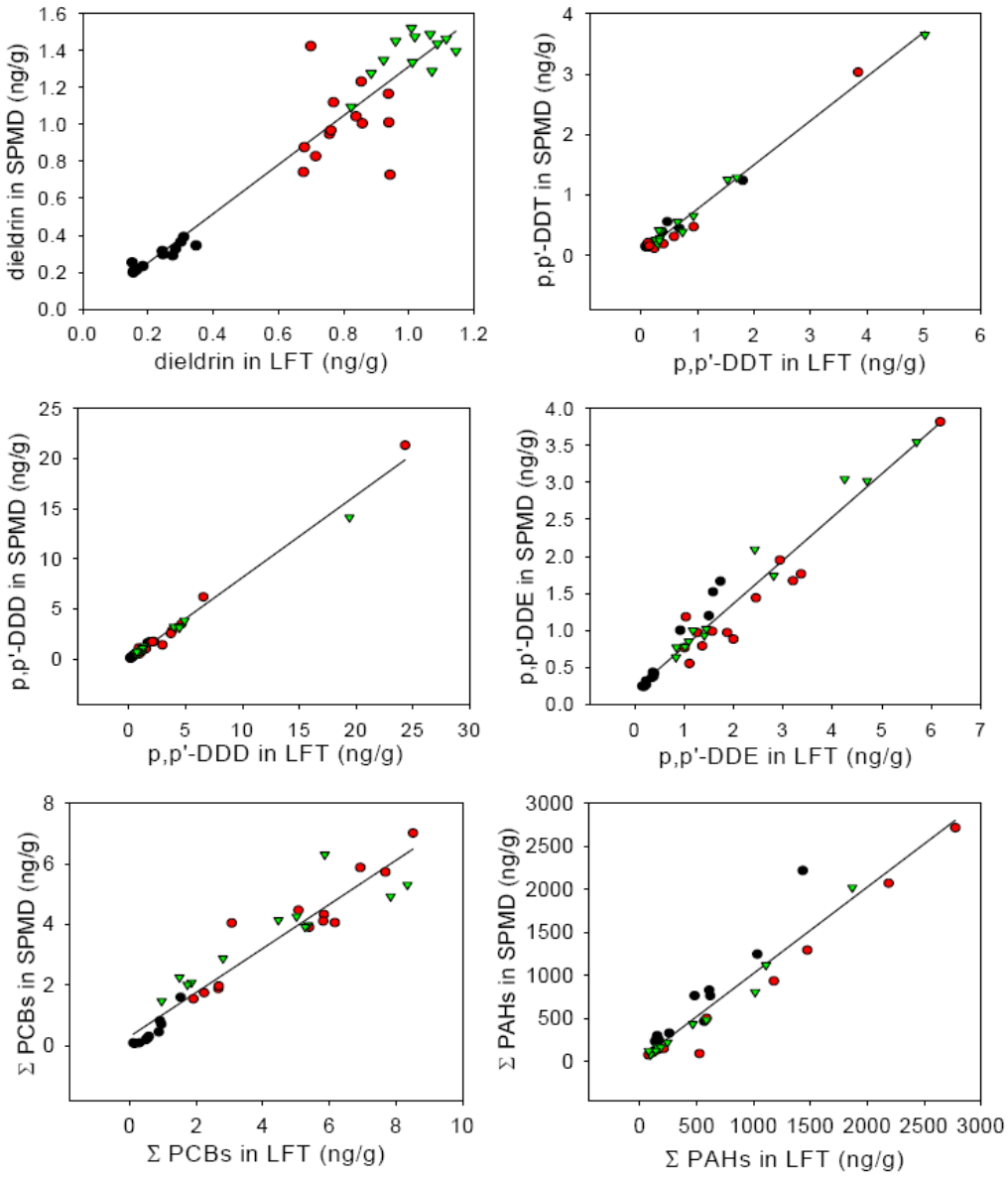

Figure A 1.1 Relationship of contaminant concentrations in the lipid-free polyethylene lay flat tubing (LFT) and in semipermeable membrane devices (SPMD) from paired LFT and SMPD field exposure. Correlation (amount of contaminant accumulated per mass of PSD ng/g) suggests both devices are governed by the same uptake process. Water temperature and river flow: ● 9 °C, 395 m^3/s; ● 22 °C, 235 m^3/s; and ▼ 22 °C, 285 m^3/s.

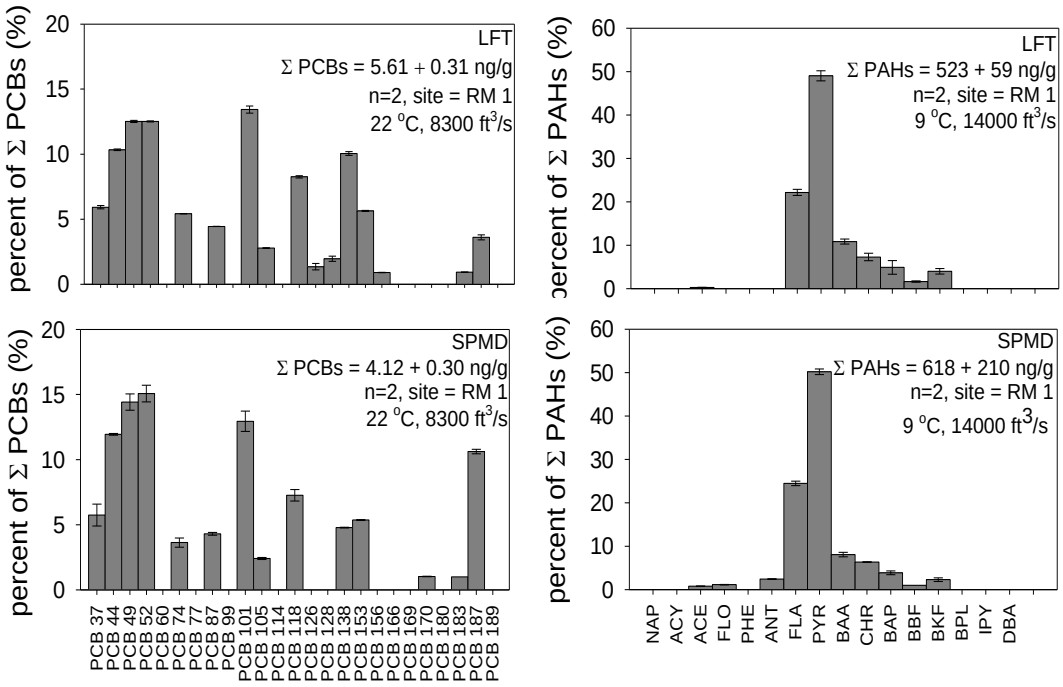

Figure A 1.2 Percent composition of PCBs and PAHs (average ± 1SD) in the lipid-free polyethylene tubing (LFT) and semipermeable membrane device (SPMD) which were co-deployed total concentrations and field condition are noted in each panel. See supplementary table A S.1 for abbreviations.

Evaluation of LFT R_s and target analyte chemical physical properties:

When considering all chemical classes together, sampling rates initially increase with increasing log K_{ow}, then they level off, supplementary figure A S.1 and A S.2. However, LFT R_s for PAHs are relatively insensitive to log K_{ow}, above log K_{ow} 4, figure A 1.3. There is a small initial increase of PAH Rs with increasing log K_{ow}, molecular weight, and molar volume indicative of membrane-controlled uptake. A lack of change of sampling rates with these properties is more likely indicative of ABL-controlled (aqueous boundary layer) uptake.

The polyethylene membrane itself may become rate limiting for large compounds. The selectivity of the polymer cavities (9 to 10 Å) [10] impedes the diffusion of large and particle-bound contaminants. All chemicals tested (PAHs, PCBs and pesticides) did demonstrate a decrease in R_s with increasing molar volume, once the volume was > 250 cm^3/mol, figure A 1.2. These differences may

contribute to the large variation of LFT sampling rates for large K_{ow} compounds. We observed that for large K_{ow} at higher temperatures the R_s were not as widely distributed as for low temperature, supplementary figure A S.2. Elevated temperatures may decrease the variation of sampling rates for high K_{ow} compounds by increasing polymer chain movement within the membrane, which can facilitate chemical transport through the membrane. Additional studies will be required to test this hypothesis.

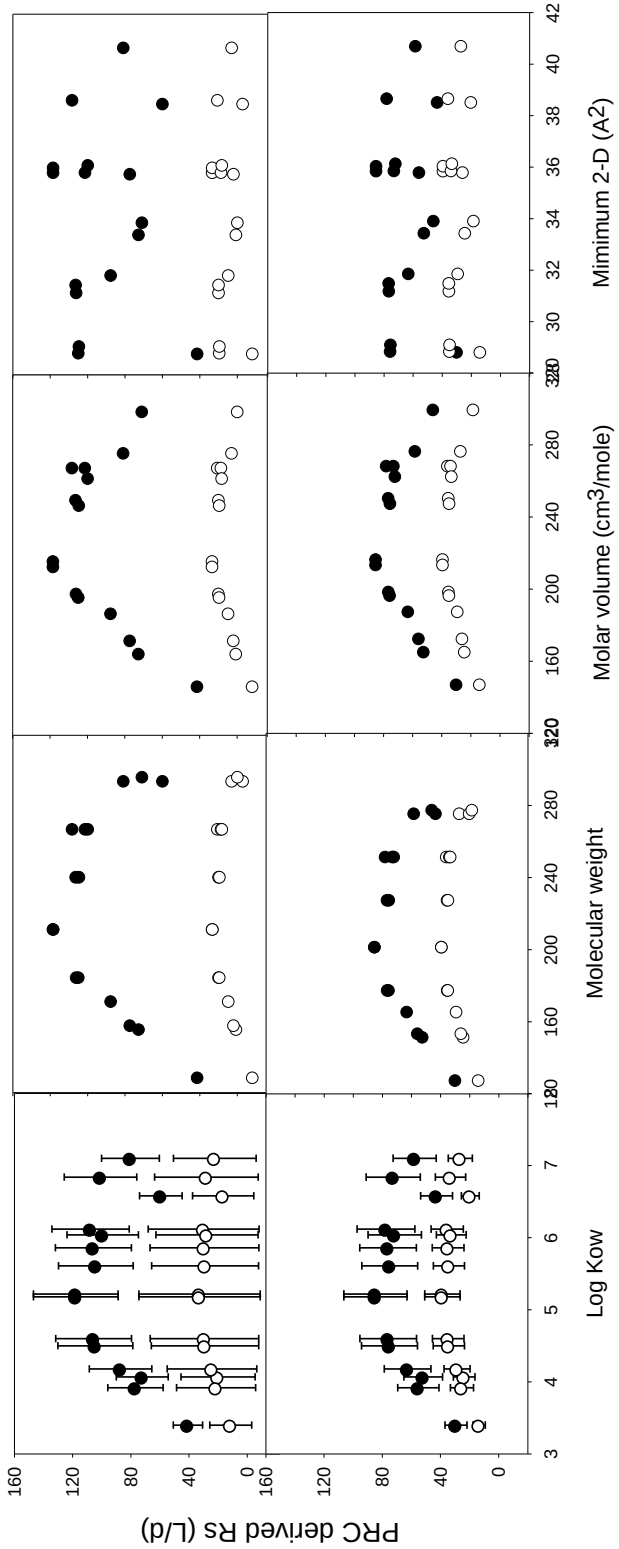

Figure A 1.3 Sampling rates versus physical chemical parameters are shown for two field events and two passive sampling devices. PRC derived sampling rates from equation 5 in text, for LFT (O) and SPMD (●) PAHs, LFT (□) and SPMD (■) PCBs, and LFT (△) and SPMD (▲) organochlorine pesticides. The average is from 11 field and two duplicate field sites (n=13) the first graph in each panel also includes one standard deviation, both events were at 22°C, panel A 235 m^3/s, and panel B was 285 m^3/s river flow.

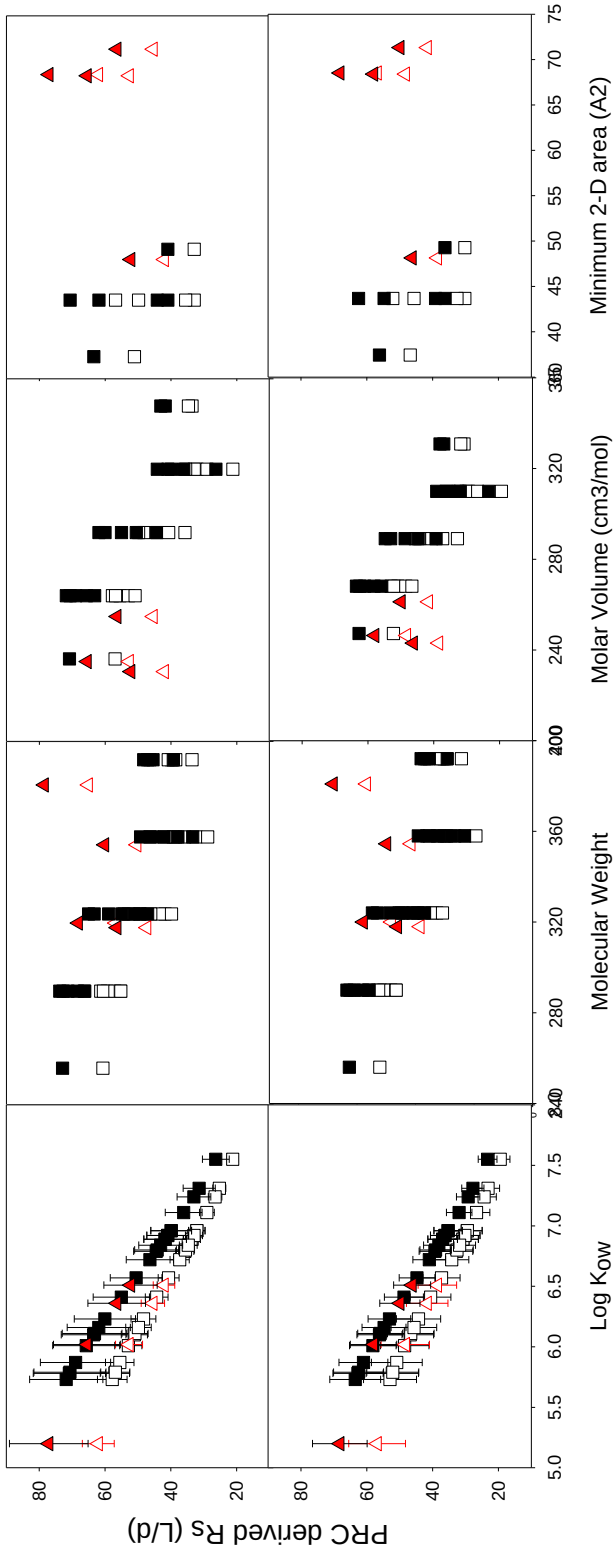

Figure A 1.3 (Continued)

A 1.4.4 Modeling, evaluation and comparison of sampling rates for LFT and SPMD

Several approaches were employed to estimate contaminant sampling rates for PSDs. The first approach was to co-deploy the LFTs with a passive sampling device (i.e., SPMD) with established sample rates. The second approach was to determine sample rates based on PRCs coupled with empirically developed models. Finally, a third approach was based on theoretical principles of aqueous diffusivity and membrane partitioning.

Estimation of LFT contaminant sampling rates based on co-deployments with established devices: Estimated water concentration (C_w) by PSDs that are co-deployed with devices with known sampling rates (R_s) may be obtained from the following equation:

$$R_{s\text{-}LFT} = C_{LFT} \,.\, M_{LFT} \,.\, R_{s\text{-}SPMD} \,.\, C_{SPMD}^{-1} \,.\, M_{SPMD}^{-1} \qquad \textbf{Eq. A 1.2}$$

where C_{PSD} is the concentration of the contaminant in the PSDs (i.e., SPMD or LFT), M_{PSD} is the mass of PSD in grams, R_s is the sampling rate in liters per day of PSD. R_s for the SPMDs [10] are from Huckins *et al* [23, 29]. The field exposure R_s at 9 and 22^{o}C ranged from 1 to 10 and 1 to 20 L/day respectively, table A 1.2**,** similar to the rates reported by Huckins; however, this approach is not as accurate as using PRC [2, 10].

Table A 1.2 Sampling rates for one LFT estimated from co-deployed PSDs, PRC Empirical and Aqueous Boundary Layer Models, using equations from text

Compounds	Co-deployed Avg R_s 9°C Eq. A.2 n = 4-12	SD	Co-deployed Avg R_s 22°C Eq. A.2 n = 7-25	SD	PRC Empirically Modeled Avg Rs 22°C Eq. A.5 n = 13	SD	Aqueous Boundary Layer Modeled Avg Rs 22°C Eq. A.9 n = 13	SD
PAHs								
Naphthalene (NAP)	1.3	±0.8	[a]	[a]	13.9	±4.3	28.6	±12.9
Acenaphthylene (ACY)	2.2	±0.8	[a]	[a]	24.5	±7.7	27.3	±12.4
Acenaphthene (ACE)	[a]	[a]	1.5	±0.5	26.1	±8.2	26.8	±12.2
Fluorene (FLO)	3.4	±1	2.3	±0.6	29.5	±9.3	26.0	±11.8
Phenanthrene (PHE)	4.3	±2	3.5	±0.8	35.8	±11.2	25.4	±11.5
Anthracene (ANT)	3.5	±0.5	4.2	±0.9	35.4	±11.1	25.5	±11.6
Fluoranthene (FLA)	3.7	±0.4	6.9	±1	40.0	±12.5	24.6	±11.1
Pyrene (PYR)	4.5	±0.5	8.3	±0.9	40.0	±12.5	24.7	±11.2
Chrysene (CHR)	[a]	[a]	12.6	±5	35.9	±11.2	23.2	±10.5
benz[a]anthracene (BAA)	1.9	±0.3	6.4	±1	35.3	±11.1	23.3	±10.6
benzo[b]fluoranthene (BBF)	[a]	[a]	4.6	±1	36.5	±11.4	22.6	±10.2
benzo[k]fluoranthene (BKF)	2.4	±0.6	7.7	±4	34.2	±10.7	22.6	±10.2
benzo[a]pyrene (BAP)	[a]	[a]	12.9	±6	33.7	±10.6	22.8	±10.3
benzo[ghi]perylene (BPL)	NA	NA	NA	NA	27.3	±8.5	22.3	±10.1
indeno[123-cd]pyrene (IPY)	NA	NA	NA	NA	20.2	±6.3	22.1	±10.0
Pesticides								
Dieldrin	1.1	±0.1	4.5	±0.8	56.8	±8.6	43.2	±6.6
p,p'-DDE	1.6	±0.3	13.6	±3	38.5	±5.9	43.9	±6.7
p,p'-DDD	2.4	±0.3	8.7	±2	48.3	±7.3	43.5	±6.6
p,p'-DDT	3.5	±1	7.9	±3	41.6	±6.3	42.4	±6.4
PCBs								
PCB 37	[a]	[a]	7.1	±5	52.2	±7.9	47.7	±7.2
PCB 44	9.7	±1	13.7	±2	52.9	±8.0	46.2	±7.0
PCB 49	6.0	±1	9.5	±3	50.9	±7.7	46.2	±7.0
PCB 52	5.9	±1	11.5	±4	52.1	±7.9	46.2	±7.0
PCB 60	NA	NA	NA	NA	48.5	±7.4	46.2	±7.0
PCB 74	6.3	±0.9	17.1	±3	46.8	±7.1	46.2	±7.0
PCB 77	NA	NA	NA	NA	46.6	±7.1	46.2	±7.0
PCB 87	5.8		16.8	±3	44.3	±6.7	44.9	±6.8
PCB 99	NA	NA	NA	NA	40.6	±6.2	44.9	±6.8
PCB 101	7.4	±1	17.3	±4	45.6	±6.9	44.9	±6.8
PCB 105	[a]	[a]	11.5	±2	32.8	±5.0	44.9	±6.8
PCB 114	NA	NA	NA	NA	34.2	±5.2	44.9	±6.8
PCB 118	4.1	±0.5	19.7	±3	37.3	±5.7	44.9	±6.8
PCB 126	NA	NA	NA	NA	30.8	±4.7	44.9	±6.8
PCB 128	NA	NA	NA	NA	29.4	±4.5	43.7	±6.6
PCB 138	7.3	±1	16.6	±9	30.2	±4.6	43.7	±6.6
PCB 153	3.5	±1	15.0	±4	32.6	±4.9	43.7	±6.6
PCB 156	[a]	[a]	12.4	±4	26.6	±4.0	43.7	±6.6
PCB 166	NA	NA	NA	NA	23.1	±3.5	43.7	±6.6
PCB 169	NA	NA	NA	NA	19.4	±2.9	43.7	±6.6
PCB 170	[a]	[a]	10.0	±3	29.4	±4.5	42.6	±6.5
PCB 180	[a]	[a]	16.5	±2	30.8	±4.7	42.6	±6.5
PCB 183	[a]	[a]	13.9	±5	30.2	±4.6	42.6	±6.5
PCB 187	[a]	[a]	7.4	±2	31.8	±4.8	42.6	±6.5
PCB 189	NA	NA	NA	NA	24.3	±3.7	42.6	±6.5

[a] concentration below detection limits.

LFT sampling rates determined by in situ PRC and Empirical Model: The use of *in situ* calibration to determine site-specific sampling rate constants is based on the hypothesis that the same mechanisms k_e (exposure rate constant) occur as k_u (uptake rate constant) [19, 23]. *In situ* R_s may be determined that account for the physico-chemical factors as well as the environmental site-specific factors including temperature and fouling. The exposure and site specific PRC R_s may be determined from:

$$R_{s,\,PRC} = (K_{mw\text{-}prc} \cdot k_e \cdot V_s) \qquad \textbf{Eq. A 1.3}$$

Where k_e is calculated from equation 1, and K_{mw} is determined from an empirical model reported by Booij *et al* [21] and described in supplementary material. Examples of LFT R_s from equation 3 based on 11 sites from one field exposure event are presented in table A 1.2. At 22 °C the PAHs, PCBs and pesticides LFT R_s range from 15 to 40, 25 to 52, and 40 to 57 L/day, respectively. The LFT sampling rates are larger than those typically reported for SPMDs by Huckins *et al* [2] without PRC, however they are consistent with the results found by Booij *et al* [21] and Meadows *et al* [30] when using SPMDs with PRC or other modeling approaches.

Employing equation 3 for both the SPMDs and LFTs results in SPMD R_s that are a factor of 2-6 times larger for PAHs and about 0.5 times smaller for PCBs and organochlorines pesticides than LFT R_s, figure A 1.3. The SPMD PAH R_s were quite different for the two field events, on average a 200% difference, while the LFT R_s were typically ≤ 10% different.

Estimation of sampling rates from Theoretical Uptake Model: R_s that are controlled by the aqueous boundary layer, ABL, may be modeled by:

$$R_s = k_w A \qquad \textbf{Eq. A 1.4}$$

Where A is the surface area of the PSD and k_w the mass transfer coefficient, is estimated from the Hayduk-Laudie diffusion equation described in supplementary

material [10]. Generally, the PAH R_s from the ABL modeling are only 10-35% smaller than the PRC Empirically modeled R_s, with the exception of NAP, ACY and ACE. The pesticide ABL R_s are 0-25% different than the PRC Empirical models, dieldrin ABL modeled R_s was smaller while the other pesticides R_s were the same or slightly larger. The PCB congeners 37-77 ABL R_s are generally smaller than those calculated from the PRC Empirical model, while PCBs 105-189 were generally larger. This results from the large effect of K_{ow} in the PRC Empirical model calculations versus the molecular volume effect in the ABL calculations. The latter two modeling approaches for LFT R_s, under all exposure conditions studied resulted in ≤ 50% difference and often only 10% difference between field exposures.

It has been hypothesized that the transition from membrane controlled to ABL is driven by the K_{ow} of the analyte [21]. Booji *et al* [21] empirically expressed both membrane- and ABL- models as a function of K_{ow} and combined them into a single uptake model. This K_{ow}-only model did not readily predict our data sets. We evaluated an approximation based on combining diffusion, K_{ow} and minimal molecular 2-dimensionns and found it was statistically significant ($p < 0.05$)

PSDs that contain triolein lipid required more solvent and cost intensive extractions, and had higher detection limits than LFT. In both laboratory and field studies the LFT was proved to be both reliable and cost effective, and uptake rates were effectively modeled.

A 1.5 Acknowledgments

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Supplementary material for Appendix 1

A 1.6 Materials and methods

Table A S.1 Selected physicochemical properties of target analytes

Chemical	MW	Log K_{ow}	minimal box dimension (Å)			molecular volume ($Å^3$)	molar volume (cm^3/mol)
			length	breadth	depth		
PAHs [29, 31-34]							
naphthalene (NAP)	128	3.4	8.9	7.2	3.1	126.9	147.6
acenaphthylene (ACY)	152	4.1	8.8	8.4	3.1		165.7
acenaphthene (ACE)	154	3.9	8.8	8.1	3.2	148.8	173
fluorene (FLO)	166	4.2	11.1	7.2	3.1	160.4	188
phenanthrene (PHE)	178	4.6	11.5	7.7	3.1	169.5	199
anthracene (ANT)	178	4.5	11.7	7.2	3.1	170.3	197
fluoranthene (FLA)	202	5.2	10.7	9.0	3.1	187.7	217
pyrene (PYR)	202	5.2	11.4	9.5	3.1	186	214
benz[a]anthracene (BAA)	228	5.6	13.7	9.4	3.1	212.9	248
chrysene (CHR)	228	5.9	13.6	7.7	4.4	212.2	251
Benzo[a]pyrene (BAP)	252	6.5	13.6	8.9	3.1	228.6	263
Benzo[b]fluoranthene (BBF)	252	6.1	13.6	9.3	4.5	230.3	268.9
benzo[k]fluoranthene (BKF)	252	6.8	13.3	9.1	3.1	231.1	268.9
benzo[ghi]perylene (BPL)	276	7.1	11.5	10.2	3.1	244.3	277
indeno[123-cd]pyrene (IPY)	276	6.6	13.2	10.0	3.1		NA
dibenz[ah]anthracene[a] (DBA)	278	6.5	15.6	9.3	3.1	255.4	300
OC pesticides [33, 35, 36]							
p, p'-DDT	355	5.7	13.1	8.7	8.2		261.3
p ,p'-DDD	320	6.1	13.0	9.5	7.2		246.4
p, p'-DDE	318	6.0	13.5	8.6	5.6		243.1
Dieldrin	381	3.5	10.3	8.9	7.7		
methoxychlor[a]	346	4.2	13.4	11.0	6.6		
endrin[a]	381	5.5	10.2	8.8	8.8		
PCBs [31, 33, 37]							
- di-CBs PCB 8[a]	223	5.1				234.9	226.4
- tri-CBs PCB 37	256	5.9					247.3
- tetra-CBs PCB 44	290	6.0				259.1	268.2
PCB 49	290	6.1				262.8	268.2
PCB 52	290	6.1	11.4	7.8	5.6	262.8	268.2
PCB 60	290	6.3				265.2	268.2
PCB 74	290	6.7	13.9	7.8	4.8		
PCB 77	290	6.5				267.6	268.2
- penta-CBsPCB 82[a]	324	6.1					
PCB 87	324	6.5				272.4	289.1
PCB 99	324	6.6					289.1
PCB 101	324	6.4	12.6	7.8	5.6	276.1	289.1
PCB 105	324	6.0					289.1
PCB 114	324	6.7					
PCB 118	324	7.1					289.1
PCB 126	324	6.9					
- hexa-CBs PCB 128	358	7.0				282.4	310
PCB 138	358	6.7	13.9	7.8	5.6		310
PCB 153	358	6.9	13.9	7.8	5.6	289.4	310
PCB 156	358	7.2					310
PCB 166	358	7.0					
PCB 169	358	7.6				265.2	310
- hepta-CBs PCB 170	392	7.1					
PCB 180	392	7.2					330.9
PCB 183	392	7.2	13.9	8.8	5.6		
PCB 187	392	7.2					330.9
PCB 189	392	7.7					

[a] used as permeability/performance reference compounds (PRCs) in passive sampling devices

A 1.6.1 PSD sample extraction and chemical analysis

The 5 PSD were dialyzed together in hexanes for 18 h, followed by a second dialysis with hexanes for 6 h (2x400mL). The combined dialysates were concentrated by rotary evaporation and a TurboVap® LV evaporator (Zymark®, Hopkinton, MA). The samples were cleaned and fractionated using gel permeation chromatography (GPC) (Waters, Milford, MA) with dichloromethane at a flow rate of 5 mL/min. The appropriate fraction was collected and reduced to 2 mL using a TurboVap® LV evaporator and then split into two 1 mL fractions for organochlorine contaminants (OC) and for PAHs. The OC fraction (e.g. PCBs and pesticides) was exchanged into iso-octane and the PAH fraction was exchanged into acetonitrile.

The LFT used in the laboratory experiment were fortified with 50-100 μL containing 40 ng of PCB 8, PCB 82, PCB 170 and endrin, 80 ng of methoxychlor and 2 μg of dibenz[ah]anthracene in 40:60 hexane. The LFT used in the field exposures were fortified with 50-100 μL containing PCB 82 and endrin (each at 20 ng) and dibenz[ah]anthracene (2 μg) in 40:60 toluene:iso-octane.

Three sampling campaigns in fall 2003 and summer and fall of 2004 were completed, for each campaign the PSD were deployed for 21 days, temperature, flow conditions, organic carbon, suspended solids and pH are given in supplementary materials. (the average water temperature and flow rate were: 9 ± 1 °C , 400 ± 130 m^3/s; 22 ± 1 °C, 235 ± 11 m^3/s; and 22 ± 0.3 °C, 285 ± 48 mt^3/s respectively). The total organic carbon average were 1.8 ± 0.2 mg/L, the dissolved organic carbon average was 1.8 ± 0.2 mg/L, the total suspended solids was 7.3 ± 1.8 mg/L, and total dissolved solids were 80 ± 10 mg/L, the pH average was 7.2, and the dissolved oxygen average was 9.0 ± 1 mg/L.

Table A S.2 Laboratory exposure study percent recoveries of permeability/performance reference compounds (PRC) using a simple spiking method

Permeability/performance reference compound (PRC)	Percent recovery (%)		PRC clearance rate k_{eprc} (d^{-1})
	exposure under flowing water (n=3)	unexposed laboratory control (n=9)	
PCB 8 ($\log K_{ow}$ = 5.1)	26 ± 8.1	62 ± 11	0.060
PCB 82 ($\log K_{ow}$ = 6.1)	67 ± 2.5	75 ± 9.1	0.008
PCB 170 ($\log K_{ow}$ = 7.1)	86 ± 4.0	87 ± 10	0.0008
endrin ($\log K_{ow}$ = 5.5)	98 ± 7.1	110 ± 20	0.008
methoxychlor ($\log K_{ow}$ = 4.2)	53 ± 16	114 ± 15[a]	0.050
dibenz [ah] anthracene ($\log K_{ow}$ = 6.5)	91 ± 4.2	93 ± 13[a]	0.002

[a] n = 2

A 1.7 Results and discussion

Table A S.3 PAH compound specific percent recoveries for the four methods tested, OC recoveries showed similar results (data not shown)

Compound	Traditional Extraction Method	Extraction Method without GPC	Extraction Method without GPC and cut PSD	Accelerated Solvent Extraction
NAP	42 ± 5	73 ± 1	65 ± 9	28 ± 7
ACY	54 ± 4	75 ± 3	70 ± 8	31 ± 8
ACE	56 ± 4	75 ± 3	71 ± 8	29 ± 8
FLO	66 ± 5	88 ± 3	86 ± 8	33 ± 10
PHE	66 ± 4	86 ± 3	92 ± 7	38 ± 11
ANT	79 ± 4	98 ± 3	101 ± 5	43 ± 14
FLA	71 ± 4	89 ± 1	97 ± 7	40 ± 11
PYR	68 ± 4	90 ± 3	97 ± 6	33 ± 12
CHR	87 ± 3	98 ± 3	103 ± 6	42 ± 12
BAA	80 ± 4	94 ± 3	98 ± 5	38 ± 11
BBF	84 ± 4	96 ± 3	99 ± 5	37 ± 9
BKF	85 ± 4	96 ± 3	99 ± 4	38 ± 10
BAP	84 ± 4	99 ± 2	101 ± 11	38 ± 11
DBA	91 ± 4	104 ± 4	111 ± 14	46 ± 8
BPL	92 ± 4	110 ± 11	124 ± 23	58 ± 9
IPY	97 ± 14	82 ± 6	73 ± 17	20 ± 12
LMW Avg	**63 ± 4**	**84 ± 2**	**85 ± 7**	**34 ± 10**
HMW Avg	**87 ± 1**	**97 ± 4**	**101 ± 10**	**40 ± 10**
AVG all	**75 ± 2**	**91 ± 3**	**93 ± 8**	**37 ± 10**

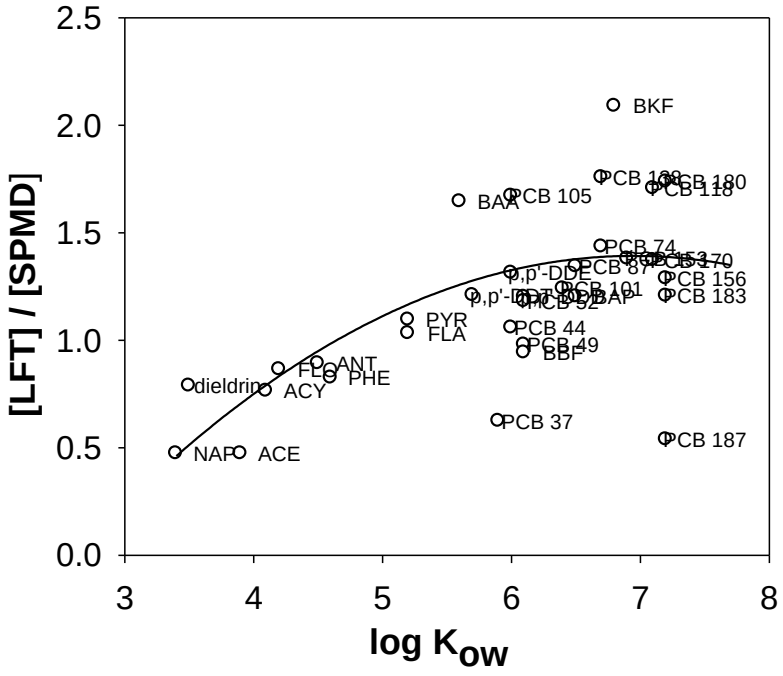

Figure A S.1 Average ratios of the concentration of contaminants accumulated in lipid-free polyethylene tubing (LFT) and in semipermeable membrane device (SPMD) as a function of log octanol-water partitioning coefficient (log K_{ow}). See supplementary table A S.1 for abbreviations.

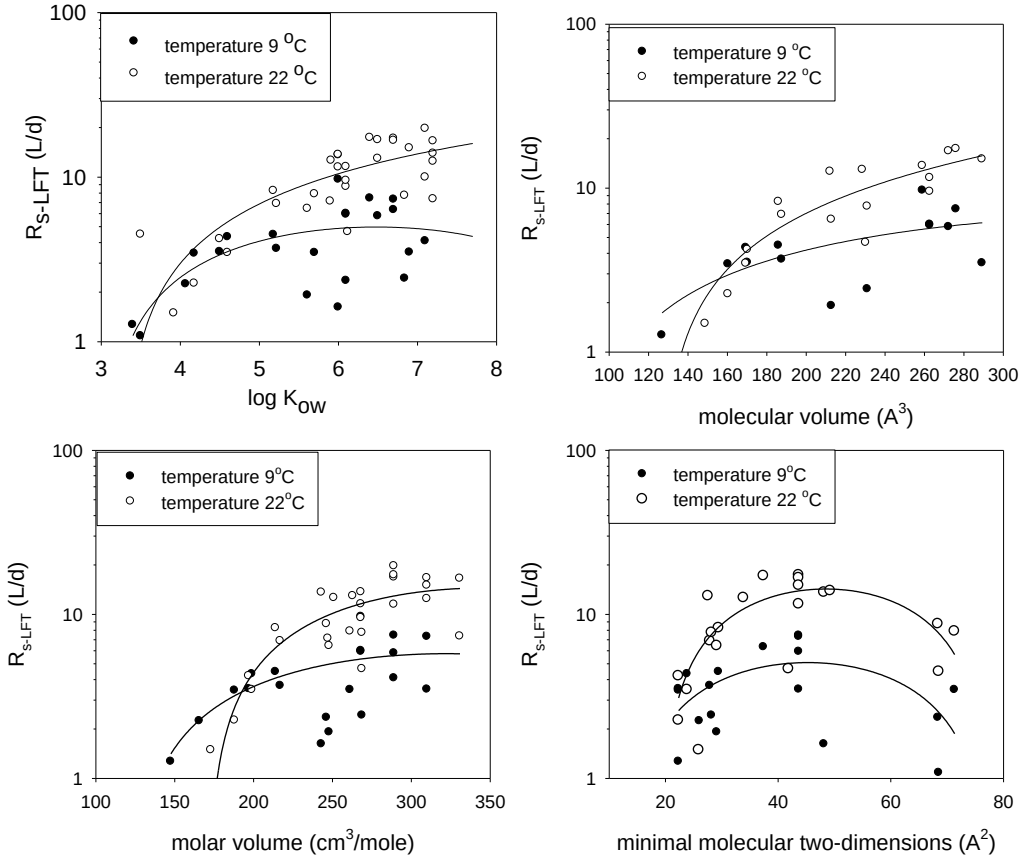

Figure A S.2 Sampling rate estimates ($R_{s\text{-}LFT}$) of lipid-free polyethylene lay flat tubing (LFT) as a function of log K_{ow}, molecular volume, molar volume, and minimal molecular two-dimension. R_s as calculated from equation A.2 from text, includes all chemical classes (pesticides, PCBs and PAHs).

A 1.8 Modeling:

Measuring the PRC loss over an exposure period provides an *in situ* clearance rate constant of PRC ($k_{e\text{-}prc}$) [23].

$$k_{e\text{-}prc} = \ln (N_{PSD\text{-}0} / N_{PSD\text{-}e}) \bullet t^{-1} \qquad \textbf{Eq. A S.1}$$

The exposure and site specific PRC R_s may be determined from:

$$R_{s,\,PRC} = (K_{mw\text{-}prc} \cdot k_e \cdot V_s)/1000 \qquad \textbf{Eq.A S.2}$$

Where k_e is calculated from equation 1, Booij *et al* [21] reported an empirical model to determine the polyethylene membrane-water partition coefficients, K_{mw}, as:

$$\log K_{mw} = a_0 + 2.321(\log K_{ow}) - 0.1618(\log K_{ow})^2 \qquad \textbf{Eq. A S.3}$$

Where a_0 = -2.61 for PCBs, PAHs, 4,4'-DDE and -3.2 for polar pesticides [10] and V_s is 6.1 cm^3 for LFT, R_s is then reported in L/day. The sampling rates (R_s) for specific target analytes (*i*) in different exposure conditions (*j*) may be estimated from:

$$R_{s,\,i,j} = R_{s,\,PRC} \cdot (\alpha_{i,j} / \alpha_{PRC\text{-}j}) \qquad \textbf{Eq. A S.4}$$

If isotope labeled surrogates are employed equation A S.3 is used directly; however if labeled surrogates are unavailable an estimate from chemically similar compounds can be made from the following empirical relationship developed by Huckins *et al* [10] :

$$\log \alpha = 0.0130 \log K^3_{ow} - 0.3173 \log K^2_{ow} + 2.244 \log K_{ow} \qquad \textbf{Eq. A S.5}$$

Estimation of sampling rates from Theoretical Uptake Model: R_s that are controlled by the aqueous boundary layer, ABL, may be modeled by:

$$R_s = k_w A \qquad \textbf{Eq. A S.6}$$

where k_w is the mass transfer coefficient, and A is the surface area of the PSD. Flow through the PSD cage is considered tortuous; and hydrodynamic correlations are established [10]. One example is where $k_w \cong D_w^{2/3}$, and the water diffusion coefficient may be used as an approximation [10]. Employing the Hayduk-Laudie diffusion equation to estimate the diffusion coefficient, $D_w \cong V_m^{-0.589}$, where V_m is the molar volume, then PSD sampling may be estimated from:

$$R_s \cong V_m^{-0.39} \qquad \textbf{Eq. A S.7}$$

Lost in this simplification is the temperature dependence of diffusion; which is discussed later. One may both experimentally test the model and apply a correction factor by utilizing the PRC. The R_s for specific target analytes (*i*) in different exposure conditions (*j*) may be estimated from:

$$R_{s,i,j} = R_{s\text{-}prc,j} \left(V_{prc,j}/V_{i,j}\right)^{0.39} \qquad \textbf{Eq. A S.8}$$

Input data and calculations

SPMD Vs =	4.95	cm^3	LFT Vs =	6.10	cm^3
exposure duration in days (E) =			21	days	
PRC amount in PSD,					
at t_0 =	5000	ng	from 5 spiked tubes		
at t_E =	2710	ng	from PRC Recovery Calc worksheet		

$\text{Log } K_{sw} = a_0 + 2.321(\text{log}K_{ow}) - 0.1618(\text{log}K_{ow})^2$

a_0 = -2.61 for PCBs, PAHs, 4,4'-DDE

a_0 = -3.2 for polar pesticides

(from Huckins)

Step 1. Calculation of PRC R_s

$k_e = -\ln (t_E / t_0) / E =$ 0.0292

$\log K_{sw, PRC} =$ 5.68

$R_{s, PRC} = (V_s \cdot 10^{\log Ksw, PRC} \cdot k_{e)/1000} =$ 69.9 L/day

Step 2a. Calculation of the relative Rs of analyte and PRC

$\log \alpha = 0.0130 \log K^3_{ow} - 0.3173 \log K^2_{ow} + 2.244 \log K_{ow}$

(from Huckins)

Step 2b. Calculation of the Rs of analyte for ABL

$R_{s, PRC} \cdot (\text{LeBas vol}_{PRC} / \text{LeBas vol x1})^{0.39} = R_{s, x1}$

Step 3. Calculate the Rs of the analyte

$R_{s, PRC} \cdot (\alpha_{x1} / \alpha_{PRC}) = R_{s, x1}$

Step 4. Calculate the aqueous concentrations

$C_{w, x1}$ = x1 accumulation/ (Vs · Ksw (1 - exp (-(Rs (mL/d) · E)/ (Vs · Ksw)))*1000 = ng/L

Figure A S.3 Example of calculations for PRC from empirical models [10] and first principles

A 1.9 Literature Cited

1. Allan, I. J.; Vrana, B.; Greenwood, R.; Mills, G. A.; Roig, B.; Gonzalez, C., A "toolbox" for biological and chemical monitoring requirements for the European Union's Water Framework Directive. **2006,** *69*, (2), 302-322.

2. Alvarez, D. A.; Petty, J. D.; Huckins, J. N.; Jones-Lepp, T. L.; Getting, D. T.; Goddard, J. P.; Manahan, S. E., Development of a passive, *in situ*, integrative sampler for hydrophilic organic contaminants in aquatic environments *Environmental Toxicology and Chemistry* **2004,** *23*, (7), 1640-1648.

3. Anderson, K. A.; Johnson, E. R., Bioavailable organochlorine pesticides in a semi-arid region of Eastern Oregon, USA, as determined by gas chromatography with electron capture detector. *Journal of AOAC International* **2001,** *84*, 1371-1382.

4. Sethajintanin, D.; Anderson, K. A., Temporal bioavailability of organochlorine pesticides and PCBs. *Environmental Science and Technology* **2006,** *40*, (12), 4506-4516.

5. Wells, J. B.; Lanno, R. P., Passive sampling devices (PSDs) as biological surrogates for estimating the bioavailability of organic chemicals in soil. In *Environmental toxicology and risk assessment: science, policy, and standardization - implication for environmental decisions : Tenth volume, ASTMSTP*, Greenberg, B. M.; Hull, R. N.; Roberts, M. H. J.; Gensemer, R. W., Eds. American Society for Testing and Materials: West Conshohocken, PA, 2001; pp 253-270.

6. Mayer, P.; Tolls, J.; Hermens, J. L. M.; Mackay, D., Equilibrium sampling devices. *Environmental Science and Technology* **2003,** *37*, (9), 185A-191A.

7. Alexander, M., Aging, bioavailability, and overestimation of risk from environmental pollutants. *Environmental Science and Technology* **2000,** *34*, (20), 4259-4265.

8. Escher, B. I.; Hermens, J. L. M., Internal exposure: Linking bioavailability to effects. *Environmental Science and Technology* **2004,** *38*, (23), 455A-462A.

9. Namieśnik, J.; Zabiegała, B.; Kot-Wasik, A.; Partyka, M.; Wasik, A., Passive sampling and/or extraction techniques in environmental analysis: a review. *Analytical and Bioanalytical Chemistry* **2005,** *381*, (2), 279-301.

10. Huckins, J. N.; Petty, J. D.; Booij, K., *Monitors of organic chemicals in the enviornment semipermeable membrane devices*. Springer: New York, 2006; p 223.

11. Chimuka, L.; Cukrowska, E., The role of passive samplers in monitoring of aquatic ecosystems and occupational hygiene pollution. *LC/GC North America* **2006,** *June*, 1-11.

12. Leonard, A. W.; Ross, H. V.; Pablo, F., Trimethylpentane-containing passive samplers for predicting time-integrated concentrations of pesticides in water: laboratory and field studies. *Environmental Toxicology and Chemistry* **2002,** *21*, (12), 2591-2599.

13. Comyn, J., *Polymer Permeability*. Elsevier Applied Science LTD: London, England, 1985.

14. Opperhuizen, A.; van der Velde, E. W.; Gobas, F. A. P. C.; Liem, A. K. D.; van der Steen, J. M. D.; Hutzinger, O., Relationship between bioconcentration in fish and steric factors of hydrophobic chemicals. *Chemosphere* **1985,** *14*, 1871-1896.

15. Gustavson, K. E.; DeVita, W. M.; Revis, A.; Harkin, J. M., Novel use of a dual-zone restricted access sorbent: normal-phase solid-phase extraction separation of methyl oleate from polynuclear aromatic hydrocarbons stemming from semi-permeable membrane devices. *Journal of Chromatography A* **2000,** *883*, 143-149.

16. Lebo, J. A.; Almeida, F. V.; Cranor, W. L.; Petty, J. D.; Huckins, J. N.; Rastall, A.; Alvarez, D. A.; Mogensen, B. B.; Johnson, B. T., Purification of triolein for use in semipermeable membrane devices (SPMDs). *Chemosphere* **2004,** *54*, (8), 1217-1224.

17. Adams, R. G.; Lohmann, R.; Fernandez, L. A.; MacFarlane, J. K., Polyethylene Devices: Passive samplers for measuring dissolved hydrophobic organic compounds in aquatic environments. *Environmental Science and Technology* **2007,** *41*, (4), 1317-1323.

18. Huckins, J. N.; Tubergen, M. W.; Manuweera, G. K., Semipermeable membrane devices containing model lipid: A new approach to monitoring the bioavailability of lipophilic contaminants and estimate their bioconcentration potential. *Chemosphere* **1990,** *20*, (5), 533-552.

19. Booij, K.; Sleiderink, H. M.; Smedes, F., Calibrating the uptake kinetics of semipermeable membrane devices using exposure standards. *Environmental Toxicology and Chemistry* **1998,** *17*, (7), 1236-1245.

20. Booij, K.; Hoedemaker, J. R.; Bakker, J. F., Dissolved PCBs, PAHs, and HCB in pore waters and overlying waters of contaminated harbor sediments. *Environmental Science & Technology* **2003,** *37*, (18), 4213-4220.

21. Booij, K.; Hofmans, H. E.; Fischer, C. V.; Van Weerlee, E. M., Temperature-dependent uptake rates of nonpolar organic compounds by semipermeable membrane devices and low-density polyethylene membranes. *Environmental Science and Technology* **2003,** *37*, (2), 361-366.

22. Gale, R. W., Three-compartment model for contaminant accumulation by semipermeable membrane devices. *Environmental Science and Technology* **1998,** *32*, (15), 2292-2300.

23. Huckins, J. N.; Petty, J. D.; Lebo, J. A.; Almeida, F. V.; Booij, K.; Alvarez, D. A.; Cranor, W. L.; Clark, R. C.; Mogensen, B. B., Development of the permeability/performance reference compound approach for *in situ* calibration of semipermeable membrane devices. *Environmental Science and Technology* **2002,** *36*, (1), 85-91.

24. Booij, K.; Smedes, F.; van Weerlee, E. M., Spiking of performance reference compounds in low density polyethylene and silicone passive water samplers. *Chemosphere* **2002,** *46*, (8), 1157-1161.

25. Carls, M. G.; Holland, L. G.; Short, J. W.; Heintz, R. A.; Rice, S. D., Monitoring polynuclear aromatic hydrocarbons in aqueous environments with passive low-density polyethylene membrane devices. *Environmental Toxicology and Chemistry* **2004,** *23*, (6), 1416-1424.

26. Cornelissen, G.; Pettersen, A.; Broman, D.; Mayer, P.; Breedveld, G. D., Field testing of equilibrium passive samplers to determine freely dissolved native polycyclic aromatic hydrocarbon concentrations. *Environmental Toxicology and Chemistry* **2008,** *27*, (3), 499-508.

27. Sethajintanin, D. Bioaccumulation profiles of chemical contaminants in fish from the lower Willamette River, Portland Harbor superfund site and human health risk assessment. Oregon State University, Corvallis, 2003.

28. Rantalainen, A.-L.; Cretney, W. J.; Ikonomou, M. G., Uptake rates of semipermeable membrane devices (SPMDs) for PCDDs, PCDFs, and PCBs in water and sediment. *Chemosphere* **2000,** *40*, 147-158.

29. Huckins, J. N.; Petty, J. D.; Orazio, C. E.; Lebo, J. A.; Clark, R. C.; Gibson, V. L.; Gala, W. R.; Echols, K. R., Determination of uptake kinetics (sampling rates) by lipid-containing semipermeable membrane devices (SPMDs) for polycyclic aromatic hydrocarbons (PAHs) in water. *Environmental Science and Technology* **1999,** *33*, (21), 3918-3923.

30. Meadows, J. C.; Echols, K. R.; Huckins, J. N.; Borsuk, F. A.; Carline, R. F.; Tillitt, D. E., Estimation of uptake rate constants for PCB congeners

accumulated by semipermeable membrane devices and brown trout *(Salmo trutta)*. *Environmental Science and Technology* **1998,** *32*, (12), 1847-1852.

31. Mackay, D.; Shiu, W.-Y.; Ma, K.-C., *Illustrated handbook of physical-chemical properties and environmental fate of organic chemicals*. Lewis Publishers: Boca Raton, 1992; Vol. 1: Monoaromatic hydrocarbons, chlorobenzenes, and PCBs.

32. Mackay, D.; Shiu, W. Y.; Ma, K.-C., *Physical-chemical properties and environmental fate handbook*. CRC Press: Boca Raton, FL, 1999.

33. Mackay, D.; Shiu, W. Y.; Ma, K.-C.; Lee, S. C., *Handbook of physical-chemical properties and environmental fate for organic chemicals*. 2nd ed.; CRC Press: Boca Raton, 2006.

34. Sander, L. C.; Wise, S. A.; NIST, Polycyclic aromatic hydrocarbon structure index. In *NIST special publication 922*, U.S. Dept. of Commerce, Technology Administration, National Institute of Standards and Technology: Gaithersburg, MD, 1997.

35. Augustijn-Beckers, P. W. M.; Hornsby, A. G.; Wauchope, R. D., The SCS/ARS/CES Pesticide properties database for environmental decision-making. II Additional compounds. In *Reviews of Environmental Contamination and Toxicology*, Ware, G. W., Ed. Springer-Verlag: New York, 1994; Vol. 137.

36. Petty, J. D.; Huckins, J. N.; Orazio, C. E.; Lebo, J. A.; Clark, R. C.; Gibson, V. L. *Evaluation of the semipermeable membrane devices (SPMD) as a passive in situ concentrator of military organic chemicals in water, Final report, approved for release*; US Army Medical Research and Material Command: Fort Dentrick, MD, September 11th, 1997, 1997.

37. Eisler, R.; Belisle, A. A. *Planar PCB hazards to fish, wildlife, and invertebrates: a synoptic review*; Biological report no. 31 Contaminant hazard reviews report no. 31; The United States Department of the Interior, National Biological Service: Washington, D.C., 1996.

APPENDIX 2 – SUPPLEMENTARY INFORMATION FOR CHAPTER 3

A 2.1 Edited weekly training log

The contents of the following section were adapted from an online training blog that was updated daily.

Week 1

January 27, 2009

The objectives were to begin GC troubleshooting and maintenance and to perform an inventory of supplies shipped from OSU to the CERES laboratory during 2008.

GC/ECD instrument trouble-shooting

- It was brought to my attention that the front column or DB-17ms was broken. At first glance, it appeared to be broken in half, but I later found that only a 1-m section from the end was broken. It is great news that a column should not need to be located or shipped from OSU.
- We took apart the GC inlet, which was found dirty, especially the gold seals.
- To meet our method requirement the GC should have high purity helium (He) for the inlet and nitrogen (N2) for the make-up gas, but both were plumbed with the He gas line. We fixed this problem but will not install new gas traps until we are ready to make GC runs.
- The exhaust outlet for the GC was venting into the laboratory onto the lab bench. It was discussed how to fix this by running exhaust tubing to the outside for contamination and health reasons.

GC gases

- A red light on the nitrogen generator may indicate that the filter is bad. This is a guess because a manual for the nitrogen (N2) generator was not located. The CERES staff indicated that the filter has never been changed. I recommend that the filter be changed regularly. Without it reliable chromatography is unlikely.
- There is one full tank of class 2 helium.

Inventory of supplies shipped from OSU in 2008

- 2 Rainin pipets that were shipped over in the summer of 2008 were missing, but we did find 3 pipets that may work just as well. We will test them tomorrow.
- Most of the glassware was present, but some beakers and flasks were missing. Ground glass stoppers of appropriate size for volumetric flasks are needed for standards.

I was told by Adama that the PSD deployment cages that will house the LFT during field sampling would be finished by Saturday. I hope to view them tomorrow. Also tomorrow we will be doing mostly GC maintenance and pipet verification.

January 28, 2009

I was able to view the cages and the building operation. There are 32 cages being built. They appear to be smaller in diameter than the cages purchased from EST used by OSU. The cages are ~ 6 to 7 inches in diameter and all need more finishing and welding. The spiders were being made while we were there. The spiders also seem a bit small. The spider is a device that the LFT is wound upon before being place in the deployment cage to allow water to access the membrane freely during deployment. I will post pictures later.

We performed inlet maintenance on the GC today. The gold seals and inserts were dirty. We attempted to clean the gold seals with some success, but we will not really know until we reconnect the columns and put the inlets back together. I would recommend new gold seals be ordered.

There is another nitrogen generator that has much less apparent use time on the filter; we will try to use this one for the GC. An electrician connected it to the power conditioner to keep it running when power is poor.

I met with Hama Garba (UN-FAO) and Baba Gagji (CERES interim director) today to discuss the project and the discrepancies found with the inventory of supplies from OSU I did yesterday.

Tomorrow, I hope to put the inlets back together on the GC and re-install

the columns. Also, we should be re-plumb some of the gas tubing and insert traps for the N2 and He gases. After that, we should have a better idea of contamination on the GC and the condition of the columns and/or detectors.

January 29, 2009

Today was another day of GC maintenance. We put the inlets back together, trimmed and re-installed the columns. Tomorrow we will re-plumb the gas lines and practice LFT construction.

Also, we got the balance in working order and verified a few pipets. The pipets we found a few days earlier are new and seem to be accurate. I saw the stock room today with Adama's help and we found several more pipets. The pipet volumes ranges include 2-20 µl, 100-1000 µl, and 1000-5000 µl. We only have tips for the 2-20 and 100-1000.

The cages should be finished on Monday. The cages are smaller than the standard cages from Environmental Sampling Technologies (EST) used by OSU.

Figure A 2.1 Production facility for PSD cages being built for the CERES laboratory

Figure A 2.2 Cage and spider shown: This was the prototype

Comment by KAA January 29th, 2009

> "Lucas, I suspect that you will have trouble loading the PSD onto the spiders given that the cages and the spiders are smaller than the standard size. I preferred the tube PSD cages because they do not require that the complicated spider also be manufactured. The tube design also is more forgiving with tube length and size compared with the spider."

January 30, 2009

Today we were met with many new challenges. Before we could re-plumb the GC helium and nitrogen gas lines and insert working traps, we observed that the pressure regulator on the helium was not functioning correctly and leaked. I removed it and took it apart revealing that the regulator was badly corroded (rusted shut). I pieced together and cleaned another regulator from spare regulators and it seems to be working properly and holding pressure well. We relocated the helium tank, from the outside to inside the lab in order to reduce wear and tear on the regulator. We were successful in re-plumbing both gas systems and the nitrogen generator is holding pressure and cycling correctly.

The goals for next week are to bring the GC online and start troubleshooting contamination issues. OSU shipped a GC maintenance kit, including GC parts and repair items, to the CERES laboratory.

Week 2

February 2, 2009

Our goals were to bring the GC online as well as load the new method for the current list of analytes. Before that could happen, we noticed that the nitrogen generator we were going to use would not hold pressure. We went back to the first generator with the red light and large number of use hours recorded and found that the indicated use hours were falsely represented on the generator. We are attempting to use this nitrogen generator now.

We brought the inlet, oven and detectors up to typical operating temperatures and they will remain at those temperatures overnight. I will assess the baseline tomorrow. When I left for the day, the detectors were reading ~2,000 to 1,500 Hz and dropping slowly.

Adama, Anna, Marie, and Vieux (a trainee) and I were present for the first bit, but once I ramped up the temperatures, I mostly was alone, but was showing Anna, Marie, and Adama what I was doing and explained why.

Once again, we experienced problems with the He pressure regulator but have worked to fix it. Adama, Anna, Marie, Vieux, Baba, Makhfousse, and I were present.

Baba, Adama, and I paid a visit to the PSD cage production facility. I guess there was some miscommunication somewhere because there was only one spider per cage made, not the two as I was expecting. It does not look like we will be going out to the field for a deployment until Tuesday and Wednesday of week 3.

The cages and spiders that were produced were of nice quality but both the cages and spiders were smaller than the dimensions typically used by OSU. The cage dimensions are as follows; diameter = 15.5 cm, height = 15 cm. Spider dimensions; diameter = 15 cm, height = 4 to 3.5 cm.

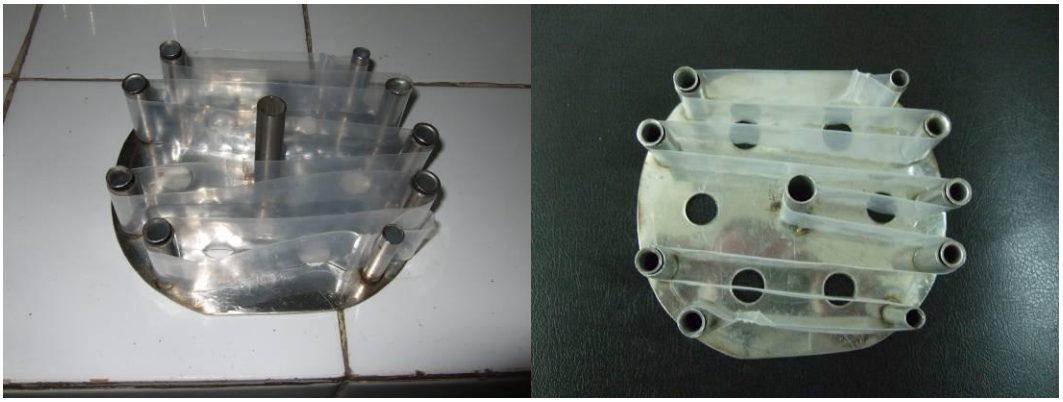

Figure A 2.3 Pictures showing that a standard size 1-m long LFT does not fit the newly constructed spider (left picture), but would fit on the newly constructed spiders if the LFT were wound around the center pin (right picture)

Figure A 2.4 LFT construction: Adama in the foreground, Vieux sitting down and Anna on the right

Adama, Marie, Anna, Vieux, and I practiced LFT construction today. All seem to be proficient after some practice. Now we need to work on efficiency. Tomorrow my goals are to continue GC trouble-shooting and maintenance, begin documenting standards preparation and organize supplies for the upcoming field deployment.

February 3, 2009

The bake-out oven, necessary for trace pesticide analysis glassware cleaning, still has not arrived. The understanding was that the oven would be here before the training would begin. I have not heard back from Makhfousse on the status yet.

The GC ran overnight to allow the baseline to level off. In the morning, the baseline at 300 ºC (maximum temperature of the GC pesticide method) was steady at 500 Hz for the front detector and 750 Hz for the back detector. At 110 ºC, the starting temperature of the method, the baseline was around 250 Hz for each, which is much better than the previous day.

Anna observed as I replaced the injector needles, rinsed the auto sampler rinse vials and added new solvent. We ran several injections of hexanes (all solvents were newly opened bottles). There are 2 or 3 stubborn peaks that show up in both front and back chromatograms starting at 24 minutes and some high grassy baseline towards the end of the run. Each successive injection showed the peaks at the same height.

The CERES staff worked to bring up a Varian GC for pesticide verification work. Baba was present and involved in that.

We searched for the 1,000-10,000 µl pipet that was in the lab the previous week and it could not be located. It would be nice to have had it along with pipet tips that fit that the automated pipet, but this was overlooked until after the shipment from OSU. Other items that would be nice to have would be GC vial inserts to limit wasting standards and samples, but analysis can still occur without these items.

For making standards, Marie, Adama, Vieux and I washed some ground glass stoppers and will bake them out using an oven that is in the biology lab here that can reach a temperature beyond 400 ºC as designated by the OSU FSES SOP 1120 for glassware cleaning. The problem with this oven is that it is very small and not practical for the needs of the GEF/PRM project.

I could not complete my GC trouble-shooting goals for today. At ~3:00 pm electricians shut down the power to replace a part in the power house.

February 4, 2009

Still no oven; the manufacturer could not be reached. Baba, Marie, and I visited the blacksmith working on the cages. I asked that he make the spiders a priority as they are most crucial to the project at this time. I let him know that we needed the finished cages and spiders by Monday afternoon at the latest.

After the electrical shut down yesterday, it took a long time to bring the GC back online and achieve a stable low baseline. We ran some hexanes solvent blanks, which showed extraneous and large peaks seen in figure A 2.5. For

preparing analytical standards we need a 100 µl pipet, but have not found one that works well.

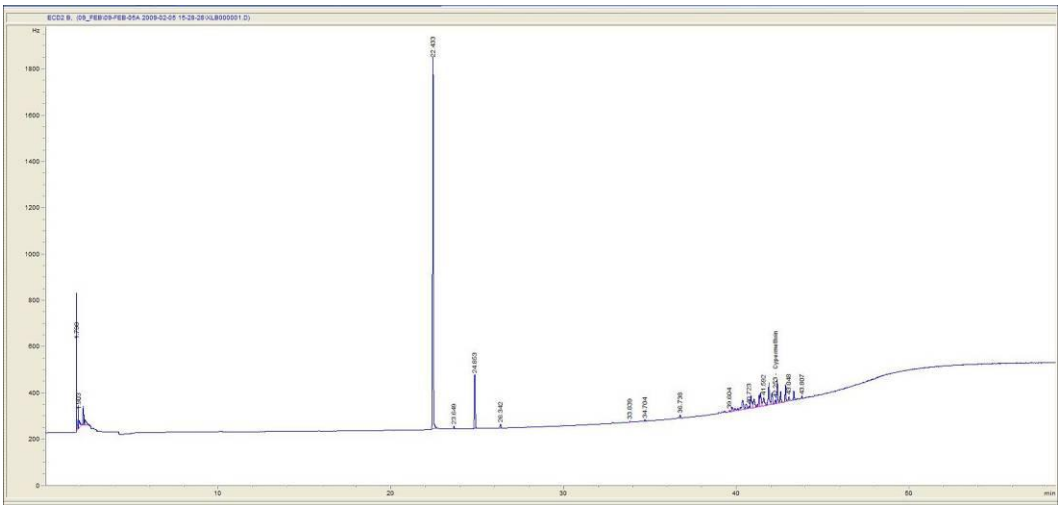

Figure A 2.5 Chromatogram showing baseline for hexanes solvent blank (VWR brand). The large peak is usually around 2,000 Hz.

February 5, 2009

Still no oven. Baba told me that the manufacturer forgot to send it over here. Baba and I paid a visit to Air Liquid, the helium supplier for the CERES lab, to confirm the purity of the helium: It is grade 4.5 or 99.995% pure.

My goal, to make the performance reference compound (PRC) spike for LFT construction, could not happen due to a continued need to trouble-shoot GC contamination. After performing an air injection on the GC which turned out clean, shown below in figure A 2.6, I suspect the contamination from yesterday came from the hexanes (VWR brand new bottle) not the GC itself. This was confirmed by injecting a different brand of hexanes (Panreac) shown below in figure A 2.7. It shows much smaller peaks (same scale on left) if you compare it to the chromatogram from the previous day (see figure A 2.5). We also injected another new bottle of hexanes (VWR) of the same lot and got similar results as yesterdays runs. Anna and I worked on most of this together.

There was not enough time in the day to make the standard and we will proceed tomorrow and if it checks out on the GC we will begin making LFT. If

need be, the CERES staff will be happy to work on Saturday to ensure the success of this project. We found a 10-100 µl pipet of decent quality; brand name Hightech lab series LM. This should aid us in making standards. We will try to pick up the GC "care package" that Glenn sent to CERES on Friday morning.

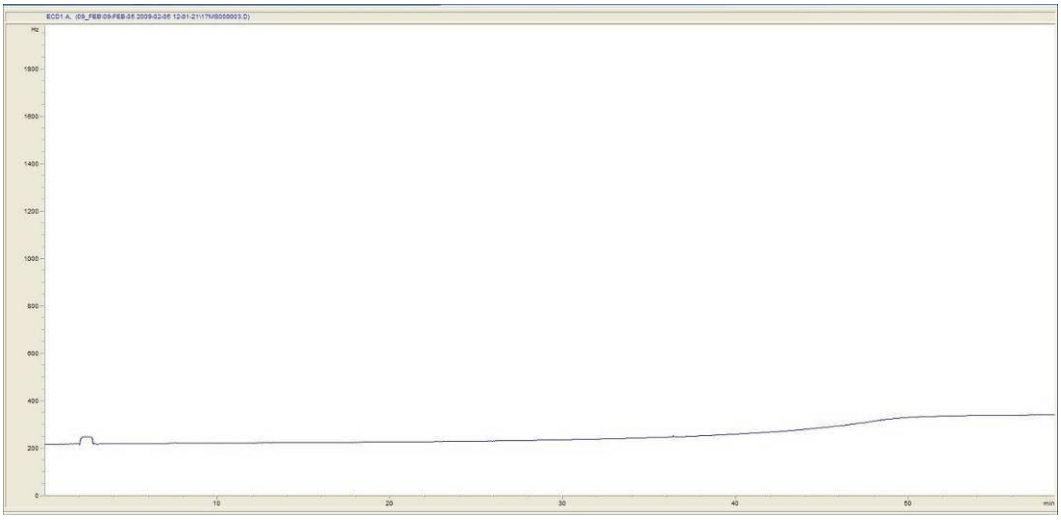

Figure A 2.6 Chromatogram showing baseline for air injection blank

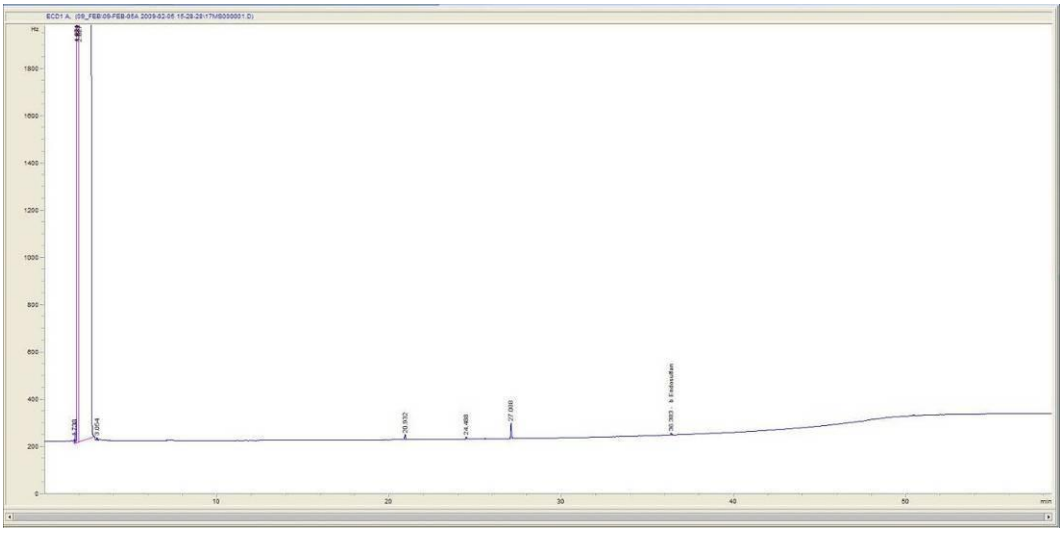

Figure A 2.7 Chromatogram showing baseline for hexanes solvent blank (Panreac brand)

February 6, 2009

The GC care package did not arrive at CERES today. Adama made the PRC standard with the Panreac hexanes; Anna, Marie, and Vieux were present, I observed and stepped in and provided instruction and feedback when needed.

We were experiencing power outages all day, but mostly for only a minute or two at a time. We were going to check the standard on the GC to verify it but during the run, the power went out for the remainder of the day. The battery back-up (UPS) and its capacitors only held up for about 35 minutes around 1:45 PM during the middle of a GC run. The PRCs will need to be confirmed on the GC before the LFT construction can be completed. The CERES staff and I will come in Saturday to construct the LFT, though we have until Tuesday afternoon to finish before we travel to the field site.

February 7, 2009

Anna, Adama, Vieux, and I constructed open-ended LFT today for the field deployment scheduled for Week 3. Open-ended means the LFT is constructed with one looped end and with one open end so that it can be easily fortified with the PRC solution later. We spent the rest of the afternoon organizing for the deployment next week.

I spent much of the morning bringing the GC back online after the power failure on Friday. It had lost power just after an injection so it took a while for the compounds to come off the column. By the time we left for the day, the baseline was still not at an ideal level. I will wait to run and verify the PRC solution on the GC Monday.

Week *3*

February 9, 2009

Jeff Jenkins (OSU), Paul Jepson (OSU), Bill Settle (FAO), Hama Garba (FAO), and Makhfousse Sarr (FAO) visited the CERES lab during the morning hours.

The retrieval has to be pushed back to Wednesday/Thursday of Week 3 due to delays in the cage production and power outages. The 32 complete cages arrived today and needed to be cleaned with Alconox (detergent) and tap water. 3 spiders were made too small and had to be retrofitted by the blacksmith. Today we finished cleaning the cages; tomorrow we will finish the spiders. The cleaning procedure, set forth in OSU FSES SOP 2110, requires that the cages and spiders be baked-out. The oven has still not arrived, so we will rinse at least the spiders with solvent (acetone and hexanes).

The GC injection of the PRC mix revealed a problem with the compound dibutylchlorendate. After some discussion with Jeff Jenkins, we think it may be degrading in the inlet. We are seeing consistently two peaks even when we inject dibutylchlorendate singly. This other peak does not show up in any of our solvent or air injections. Trouble-shooting will continue tomorrow.

February 10, 2009

Today Adama, Marie, Anna, Vieux, and I spiked LFT with the PRC mix and finished their construction for the first field deployment on Thursday, February 11.

We sanded down sharp edges on a few of the spiders in order to minimize cutting the LFT; it was also a safety hazard for anyone handling them. The spiders were cleaned; soaked in detergent and scrubbed with brushes, thoroughly rinsed with tap water, then solvent rinsed (submersion and agitation in acetone then hexanes) and air-dried. Once dry, the spiders were packaged in clean plastic bags. Organization for the trip to the field continued.

GC trouble-shooting: Anna and Adama were present while I installed new liners for the GC inlet, removing some of the glass wool prior to installation. I also changed out the gold seals and tightened all the column connections. The new liners appeared to make the multiple peak problem worse, especially for the front detector. At the end of the day, I cooled and shut down the GC. The recent power

failures led to this decision. I did not want the GC turning off hot while we were in the field.

February 11 and 12, 2009

Adama and I reviewed the deployment bench sheets. Anna, Adama, the driver and I left mid-day for St. Louis, where we stayed for the night, and picked up Marie along the way. We did not have enough time on Wednesday to visit the sampling sites so we did everything on Thursday.

In the morning, we traveled to the field site, Pont Gendarme, NE of St. Louis and picked up Abdoulaye Djiop (our field guide). A spatial layout with sampling sites indicated is shown below in figure A 2.8. We were only able to find three sites to deploy at, we were hoping for four. Two sites were the same as when Greg Sower visited in 2007 [1] (the outlet and inlet, sites 1 and 2 shown in figure A 2.8). The site located on the irrigation canal that Greg sampled was dry due to one of the pumps being broken. The third site we sampled was at one of the only remaining canals containing water. It was very stagnant water though. It also appeared to be very close to where Greg deployed for site 3 during the previous training event. Pictures of the three sites are shown below. I also included a picture of the dry land adjacent to the dry irrigation canal.

Pont-Gendarme: *Spatial Layout*
Rainy season (hivernage): June –October 2007

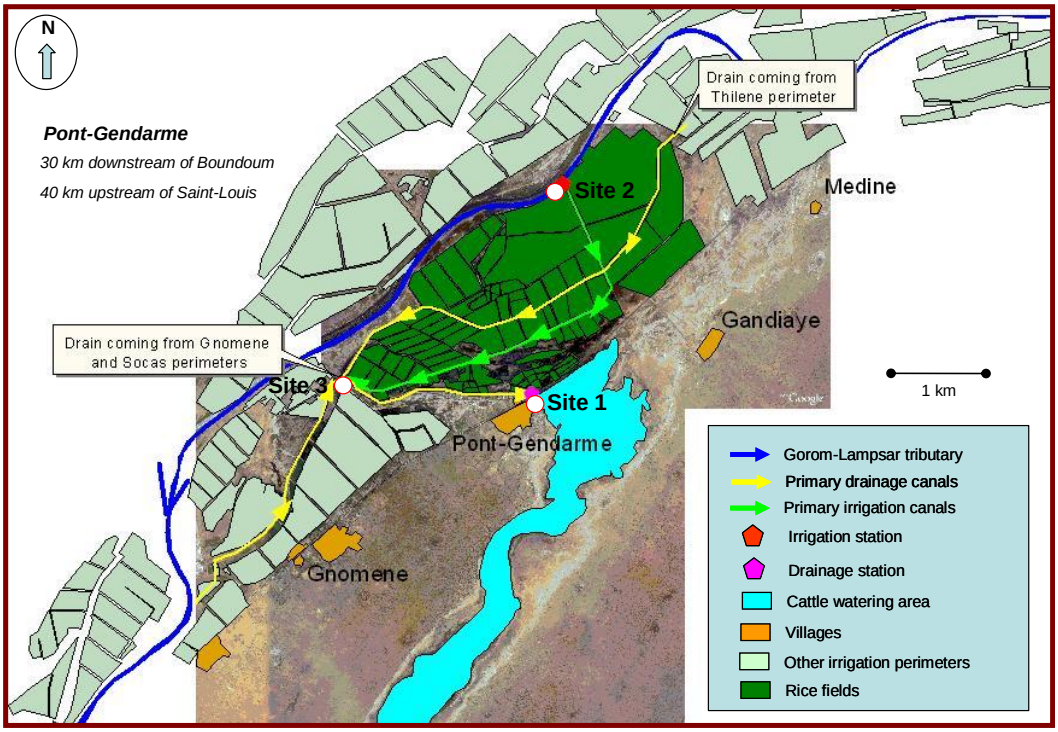

Figure A 2.8 Pont Gendarme field site representation with the PSD training sites as white circles (sampling sites 1 through 3)

Each site received eight cages with two LFT in each cage. A trip blank was kept in a small amber jar in a cooler that we transported the LFT in. The CERES staff approximated and collected water depth and cage depth measurements at each site. Mr. Djiop assisted greatly in the deployment at all sites. He ventured into the water, helped positioned the cages, and helped take water temperature readings. I took photos with a digital camera and used a GPS to record coordinates for each site as well as instructed upon the correct deployment techniques.

The first site sampled was the main drainage pumping station at the south of the fields. I presented a brief demonstration of proper handling and mounting of an LFT onto a spider then into a cage, then the staff and I prepared the remaining cages. The LFT were deployed in the outflow of the pumping station, as was the case with the previous training event. Behind the station, the area was secured by a

fence and gate though there was no lock. There was not any sediment due to the basin's concrete lining. Many fish were observed feeding and living inside the basin. There is strong evidence that fishing practices are on going here (dead fish, nets, and traps) and some residents were seen fetching water.

Figure A 2.9 Field sampling site 1: Loading and transporting PSD cages. From left to right: Lucas, Adama, Marie, Anna.

Figure A 2.10 Field sampling site 1: The drainage pumping station

The second sampling site was adjacent to the primary irrigation inlet pumping station (north). Many residents were observed fetching water and washing clothing in the canal just north or behind the pumping station. Several donkeys were seen grazing and drinking the water as well.

Figure A 2.11 Residents fetching water near the inlet pumping house adjacent to field sampling site 2

The PSDs were located slightly (~3-4 m) northwest of the canal that leads to the pumping station and near the shore of the tributary as was done in the previous training event. They were attached by rope to tree limbs in order to decrease potential contact with sediment and to deter theft.

Figure A 2.12 Field sampling site 2: PSD located near shore on a tributary adjacent to the inlet pumping station

Figure A 2.13 Field sampling site 3: A stagnant canal

The third site was located in a stagnant canal right next to a bridge/culvert. A field exposure blank was exposed to the ambient air at the site for the duration of the deployment. Mr. Djiop helped us very much at this site as he offered to wade in to deploy the cages and hide the ropes.

Figure A 2.14 Fields adjacent to the second site Greg Sower sampled in 2007 [1]. During this sampling event, the canal was dry.

February 13, 2009

Today was a short day. It took the morning to bring the GC back online. Adama, Anna, and I were the only ones that came in today. We checked the rotary evaporators (rotovaps) to make sure they were in working condition. They worked just fine, but some replacement parts will probably be needed soon. We also tested the nitrogen blow-down module. This took a long time to do since we had to find another gas regulator that worked. So far, we have not located GC-grade hexanes in Senegal. They are expecting some soon at one location but it seems as if they really do not know any details beyond that. I ran out of time today and will pursue

this more in depth on Monday. Baba knows of our need for high purity hexanes and it being essential for the success of this project.

Week 4

February 16, 2009

Baba, Anna and I worked on tracking down pesticide grade hexanes in Senegal since the only grade in the lab is standard reagent grade. It looks as if there is none in Senegal. It would take at least 2 weeks to ship it, probably more since most of the companies we have contacted have not responded to our inquiries in a timely manner. In addition, the French websites for some of the solvent distributors are not set-up the same as they are in the U.S. For the same company, they do not use the same product numbers, so it took a very long time to research solvents. We did find some pesticide grade ethyl acetate in the laboratory, which will work for preparing standards.

We discovered that the round bottom flasks sent from OSU had a smaller neck size than the rotovaps used in the CERES lab. Adama found an adapter in a storage room but the problem is that only one was found. Therefore, only one rotovap is operational at this time. This will increase blow down time, limiting the number of samples that can be processed as a batch. We will continue to search for another fitting in the lab. In addition, we have been steadily baking what glassware we can in the biology oven until the new bake-out oven purchased for the CERES lab arrives.

Trouble-shooting the degradation of dibutylchlorendate on the GC inlet continues. I replaced the liner for the front but not the back inlet. The degradation does not seem as extensive as before for all the compounds in the PRC mix, but dibutylchlorendate is still a problem. One suggestion is to remove it from the PRC mix. When I make and run the standard mix of 14 analytes, it will help with trouble-shooting the degradation problem.

February 17, 2009

At about 5:15 pm the oven arrived. I was told they will install it on Wednesday. From correspondence between OSU and FAO, we determined that the oven is in fact a muffle furnace and presents the following problems as a glassware bake-out oven: 1) The internal dimensions are smaller than expected and will be able to bake-out less glassware at a time, and 2) As the furnace does not have an external exhaust it will take longer to cool glassware.

Figure A 2.15 Glassware muffle furnace ordered for CERES.

We are still trying to find a pesticide grade hexanes distributor. I am not sure we can get high quality hexanes before I leave. This has not only created problems during my short time here, but it also falls to the responsibility of the CERES director and staff to find a distributor when I leave.

We made the stock solution for the 14 analytes with the high purity ethyl acetate we found yesterday. I ran several injections of the ethyl acetate on the GC to make sure it did not show extraneous peaks. An unknown peak shows up in both detectors very consistently, which I have observed before. It seems the

unknown peak is taller and more spread out during the first injection of the day if the GC remains idle overnight.

We made up the PRC and analyte mix (14 analyte standard mix) spiked LFTs to practice PSD processing and analysis. We have no alternative but to use the reagent grade hexanes for the dialysis.

Wednesday we will practice dialysis of the spiked laboratory LFT samples then leave for the St. Louis. We will retrieve the field samples from Pont Gendarme on Thursday then head back to Dakar in the afternoon.

February 18, 2009

Anna, Marie, Adama, Vieux, and I started the dialysis of our spiked laboratory LFT samples today at 9:00 am. As it takes at least 4 hours to travel to St. Louis, we left Vieux to finish the second half of the dialysis. Anna, Marie, Adama, and I left for St. Louis Wednesday around 3:00 pm.

February 19, 2009

Today we have completed our first field retrieval. About 10 miles outside of the first site (outlet pumping station), the pickup broke down. We had to hitch a ride with what I believe was one of the driver's friends to the first field site. For the rest of the retrieval we used a horse cart to travel from site to site. The horse cart was driven by Mr. Djiop's brother. The retrieval was successful and all cages were accounted for, though some rust was observed on the cages. Most of the rust occurred along the welds. The cages will have to be repaired before deploying again. During the retrieval event, there was a minor oversight: Due to the nature of the teflon bags used to store the LFT upon retrieval, we could not write vital sample information on the bags (the ink would not stick to the bags). We wrote the site and sample information on nitrile gloves then placed the Teflon bags inside the gloves and tied them off as a solution to the problem. This seemed to work out quite well. The pickup was towed back to St. Louis and fixed relatively fast, but we did have to wait several hours after the retrieval before we were picked up.

Figure A 2.16 Photo 1: First retrieval, site 1, Abdoulaye Djiop (standing), Marie, Anna, Adama (from left to right). Staff cleaning LFT from retrieved cages. Photo 2: Horse-drawn cart.

Figure A 2.17 An example of the rust observed on cages after deployment

February 20, 2009

Today was a short day, I left at 2:00-ish, so did the rest of the staff. The plan for today was to blow down the spiked laboratory LFT samples in preparation to run them on the GC. It became apparent that only certain parts of the Techne N2 blow-down module worked properly, which is similar in function to the OSU FSES laboratory's TurboVap LV evaporator but different in design. The CERES staff admitted that they had experienced problems with the module in the past and had previously attempted to fix it. Even with functioning parts, I do not think the N2 flow through the 1/8-in copper supply tubing would be sufficient to blow-down several simultaneous samples. I took apart the blow-down module and ascertained that new parts need to be ordered from the manufacturer if CERES was to continue using the equipment. It might be in CERES' best interest to buy a new and better-suited evaporator. The CERES staff and I discussed an idea to construct our own blow-down apparatus and we will test it on Monday. Adama, Marie, and Vieux performed the rotovap blow-down and sample transfer. Anna was present, but did not practice the transfer.

Week 5

February 23, 2009

Week 5 Expected outline of activities:

Monday - Create a blow-down module, do final blow down of spiked laboratory LFT samples, bake out glassware.

Tuesday - Make serial dilutions of PRC and mix of analytes for calibration curve, make internal standard composite, log in samples, do solvent cleaning of field samples from the first retrieval, do dialysis of field samples and QC, run spiked laboratory LFT samples on GC, check calibration standards on GC

Wednesday - Blow down field retrieval samples, run calibration standards on GC, travel to St. Louis

Thursday - 2nd and final retrieval, travel back to Dakar

Friday - Log in samples, solvent cleaning of 2nd retrieval samples, run 1st retrieval samples on GC, clean glassware.

Today Adama and I modified the current solvent blow-down system (the result is seen in pictures below) to meet the needs of the GEF/PRM project. It should suffice until a suitable replacement can be purchased, such as a TurboVap.

Figure A 2.18 Modified nitrogen blow down module

We tested the oven today and found that there is a long cool down period due to the absence of an exhaust fan. This will increase the amount of time needed to clean glassware because of the increased oven cool-down time. We can crack the door and get a decent rate of cool-down, but unfortunately, when the oven shuts the heating coils off in the middle of the night it does not cool much before we arrive in the morning.

We started working on the paperwork for the standards to make tomorrow go faster. Normally the paperwork should be done on the same day you create the standards, but with the several setbacks that have occurred and the time constraints

put on the project we are trying to do the paperwork when we have down time in the laboratory to save time later. In general, the staff needs to pay more attention to detail when filling out paperwork. Often the paperwork is filled out after the laboratory work is finished if at all.

On Friday I talked to Baba about getting the fume hood motors fixed since only one worked and it was underpowered. This creates a health hazard for the CERES staff. A technician came by today to check it out and told Baba he would need to upgrade all the motors and replace them for the hoods to work effectively. In addition, we had a person visit concerning the stainless steel counter-top and hood. He made measurements and can probably start this week. I think Baba said it would take about 2 to 3 days to complete when he starts. I believe it will be made with 1.5 mm stainless.

February 24, 2009

Tasks and status:

1. Make PRC and mix of analytes serial dilutions for calibration curve DONE
2. Make internal standard composite DONE
3. Solvent cleaning of field samples from the first retrieval DONE
4. Dialysis of first retrieval samples and QC DONE
5. Run practice samples on GC PENDING
6. Check calibration standards on GC PENDING

Tuesday was a long day. The staff arrived at 8:00 am and left at 7:00 pm. The decision was made to have Anna and I not go out to the field on Wednesday. We felt that we could use that time to train Vieux in the field and Anna and I could spend more needed time on the GC. We got a lot done, but still have a lot of work ahead of us.

February 25, 2009

In the morning, Adama, Anna, Marie, Vieux, and I performed rotary evaporation and N2 final blow down of the first retrieval samples. The blow down took a very long time as only one rotovap could be used at a time due to the

glassware compatibility problem I posted before. Some calibration standards and the internal standards were verified on the GC today. Additionally, we washed dishes today in preparation of the field samples from retrieval 2. Adama, Marie and Vieux left for St. Louis this afternoon for the second retrieval.

February 26, 2009

Today, the CERES staff minus Anna and I performed the second retrieval. Last night Anna and I ran the internal standards and mix of PRC and 14 analytes so that we could update the calibration table retention times in Chemstation (GC software). At first glance, the chromatograms look great, but we are starting to lose a little resolution between some compounds and gain some with others. The elution order and peak identification of chlorpyrifos, fipronil, PCB-100, and fenitrothion were presenting a problem on the back column (db-xlb) only (retention times near the middle of the run). Their peak height ratios did not resolve the retention time issue. We found some standards in the freezer that had been previously used for other projects at CERES, performed some dilutions, and ran them for retention time. We are still sorting out two compounds. The earlier problem with breakdown of the PRC compound (potentially at the inlet) was not seen during these runs.

At the end of the day, we realized that we were going to have a problem with pipeting 1 ml into or out of GC auto sampler vials due to the pipet tip being too wide. After several email exchanges with Glenn Wilson (OSU) and Skype conversation with Paul Jepson (OSU) we decided that we could just use the 1 and 2-ml graduations on the centrifuge tubes as volumes.

Anna and I ran two hexane blanks overspiked with our internal standards on the GC to determine the volume accuracy of the centrifuge vs. the automated pipet method. This is an important step because it is the final volume of the sample blow-down step and is used for calculating compound concentration. The samples were 1 mL of hexanes spiked with 100 ppb internal standard. The final volume step was either:

1) 1 mL of hexanes transferred using a 1 mL automated pipet, or

2) 1 mL hexanes transferred using graduations on a centrifuge tube

Adama, Marie, and Vieux arrived from the Pont Gendarme field site (retrieval 2) and performed the solvent cleaning of the second retrieval samples. Tomorrow we plan to extract the samples and run the calibration standards on the GC.

February 27, 2009

The results from the overnight GC run revealed similar peak areas for the 100-ppb internal standard spiked hexane blanks when using either centrifuge tubes marked at 1 ml or a 1-mL automated pipet for determining the final volume of the sample after blow-down. If the final volume is determined by marking the blow-down centrifuge tube then it is extremely important that the CERES staff verify pipettes and pay close attention during 1-mL transfer with the pipet. This final volume step is crucial for accurate calculation of concentration.

Given this information, we prepared standards for the calibration table and included the internal standards (tetrachloro-m-xylene and dibromobiphenyl). The use of internal standards are common in chromatography to account for per batch shifts in retention times and for potential changes in volume and concentration (such as evaporation). Adama pipetted the standard mix and I pipetted the internal standard. These will run overnight.

Adama, Anna, Vieux, and Marie performed the dialysis of the second retrieval samples and some of the field QC samples. I will be taking some QC samples (1 trip blank, 1 field exposure blank, and 1 lab cleaning blank) back with me to OSU as part of the QC sample split objective from the LOA. I was present to observe the dialysis and corrected the CERES staff's technique where needed, but for the most part did not assist. The staff's LFT processing technique has improved from the 1st retrieval sample processing.

February 28, 2009

Anna, Adama, and I spent most of the day working with the GC Chemstation software updating the calibration table and training on the GC. We also now have all compound retention times in the calibration table correctly. While updating, it was clear that a few compounds do not show up on the GC here as well as they do at OSU, especially fipronil, dimethoate, and propanil. These compounds may need to be dropped from the method or the inlet liner type changed. Much time was lost today because of a software glitch with Chemstation. The calibration table would not communicate correctly with its own software and had to be rebuilt several times. It was a good opportunity to troubleshoot software problems.

We prepared the first retrieval samples to be run on the GC over Saturday night and Sunday. Adama brought the samples up to the 2-mL volume in the centrifuge tubes with hexanes, I transferred them to the GC vials, and Anna spiked the internal standard. One sample was compromised because of a bad spike, but the samples were analyzed in duplicate.

Week 6

March 2, 2009

The first retrieval samples ran over the weekend on the GC. GC training was done with Anna mostly (Adama, Marie, and Vieux were watching, but also in and out). We processed the front column (db-17ms) chromatograms using the Chemstation software and looked at the standards we ran with the samples.

While viewing and processing the QC samples it is obvious that there is possible cross contamination with most of the analytes in our method. The most contaminated sample was the laboratory reagent blank. This QC sample is processed with the field sample batch and indicates if cross-contamination occurs during sample processing among other issues. This was also the last sample transferred and processed.

Part of the day was spent troubleshooting the contamination. A centrifuge tube that had hexanes in it that was in the blow-down manifold that had been exposed to the air in the lab for approx. one week was run on the GC. We found no peaks that were identifiable, so the contamination is probably not coming from the ambient air. I had not previously seen contamination from solvent blown down on the rotovap, so that is likely not the source either.

We made four blank LFT and set them up in the hood, bench top, freezer, and LFT construction rig to represent a laboratory cross-contamination worse case scenario. We plan to set them out for several days then extract and look for contamination.

Tuesday I hope to prepare and run samples from the second retrieval. We will also finish updating the calibration curve for the back column and, if there is time, finish analyzing chromatograms from the first retrieval samples so we can start working with the calculation spreadsheet.

March 3, 2009

We deduced that the contamination issue with the reagent blank from yesterday may be the result of a mislabeled sample or a lack in attention to detail by the staff. The reagent blank and a field sample had similar sample numbers assigned to them and were likely switched during sample transfer. This was confirmed through duplicate analysis, as the reagent blank looked identical to a field site. We still do not know where the other lower level contamination came from in the other QC samples. Tomorrow when we inject our second retrieval samples, we will have a better idea.

If we are unable to find the source of the apparent contamination in the other QC samples by the time I leave, the CERES staff is very motivated to find out where it came from; they want to succeed. Anna has been a fast learner throughout the GC training.

The internal dimensions of the oven are: DxWxH with usable dimensions located in parentheses; 55cm(53), 33cm(29, shelf braces), 31cm(31). We can fit a

maximum of seven cages inside (more practically six). I do not think it is possible to put an exhaust fan or port on it to decrease the cool-down period.

Anna and I, Marie, Adama, and Vieux were present while the GC calibration table was updated again for the front and back detectors. We keep running into problems with propanil, dimethoate, and fipronil continually shifting retention times. We ran into other problems with the back column (db-xlb). I would recommend purchasing new and backup columns before future GEF/PRM work is pursued. This would minimize the time spent troubleshooting column problems during critical sample throughput periods and would provided a fall back if accidental damage or breakage occurred. We made new standards and are running those tonight. Tomorrow we will run the second retrieval samples on the GC and start working on the calculation spreadsheet. We extracted the blank LFT placed in the lab to investigate external sources of contamination. I am not sure if we can run these on the GC before I leave. If not, I will follow up with the CERES staff on analyzing these samples.

March 4, 2009

We ran into problems with the GC today. It looks as if some of our analytes are being degraded in the inlet with consecutive injections as before. From the Friday of week 5 to today I can see a definite progression of lost or smeared peaks for several analytes. It is most noticeable for propanil, fipronil, dimethoate, and DDT.

Most of my time was spent working with Anna on interpreting chromatograms, manual integrations, compiling data into a raw data master spreadsheet for data work-up, and general troubleshooting of the inlet problems. The other staff, especially Adama, was present and attentive.

Adama and I transferred fresh standards for the second retrieval samples and set up a run on the GC overnight. Anna is scheduled to review the GC batch run tomorrow and update the calibration table.

March 5, 2009

Today I had a meeting with Hama (FAO), Makhfousse (FAO), Baba (CERES director), Amadou (CERES), and Papa Sam Gueye (CERES). During this meeting, I discussed in length my summary of the training. This meeting lasted much of the afternoon. An outline of the summary is shown below with respect to what had been accomplished, challenges, and some suggestions I had for the laboratory.

What had to be done upon arrival to create a functioning set-up?

1. The GC was not operational: In general, it was ill-maintained and the following problems were rectified.
    a. There was a broken column
    b. Dust everywhere, in general
    c. Very dirty inlet
    d. Gas lines were incorrectly installed
    e. Gas traps were incorrectly installed
    f. Gas regulators were not in good operational condition
    g. All GC syringe wash vials missing diffuser insert
    h. GC exhaust and purge lines were not plumbed
    i. There was no GC maintenance kit
    j. Some consumables were not available
2. Nitrogen blow down module was not in good operating order
    a. A module was made with tubing until the original can be satisfactorily fixed or a suitable replacement can be bought (Turbovap)
3. Paint was peeling off of the ceiling and walls in the laboratory and falling on to bench tops and the analytical balance
    a. We had the ceiling in the balance room scrapped as a temporary solution
    b. We moved sensitive materials away from areas where the peeling was occurring
4. Upon an inventory inspection on what items were sent over from OSU several glassware pieces were missing, but most notable was two expensive Rainin automated pipets
    a. Several other automated pipets were found in a storage room and were used for the remainder of my stay
5. Some general glassware was not available (most notably; ground glass stoppers)
    a. I had some needed glassware items sent from OSU.

6. GC grade/pesticide residue analysis grade solvents were not present
    a. A local supplier for the correct grade was difficult find
7. The stainless steel cages and spiders were not constructed as previously indicated and were smaller than what was specified
    a. The finished cages did not arrive until week 3 of the training
8. The exhaust hoods were not is good or operational condition.
    a. Most of the motors were broken and those that did work were too weak to be efficient
    b. This could be a health concern
9. Most of the freezers and refrigerators in my opinion do not work as they should
    a. They do not hold temperature
    b. This results in the need to use the same space for standards and samples and LFT; which is not ideal
10. One oven was capable or reaching 400-450 ºC, but it was prohibitively small for effective glassware cleaning
    a. The ordered oven did not arrive until week 4, but was not operational until week 5

What lab work was accomplished?

1. Staff prepared what looks to be very good standards
2. Set up a fully functioning GC; still working out some problems though
3. Put in place a protocol for washing glassware for the project
    a. SOP available on the GC computer
4. Set aside areas to be used for the project in the lab
5. Made a nitrogen blow down module
6. Analyzed the samples from the 1st retrieval on the GC
7. Staff learned basic GC maintenance, which can also be found in the manual previously given to the lab by OSU in French
    a. Trained Anna on making sequences, running the GC, updating calibration tables, and processing data
8. Staff learned how to correctly use and verify automated pipets
9. Staff learned the correct procedure to make analytical standards and dilutions of analytical standards
10. Trained the staff on constructing, deploying, and processing LFT in the lab
    a. Made spiked LFT and QC for two paired deployments
    b. Deployed LFT samples at 3 separate sites in Pont Gendarme
    c. Performed 2 retrievals; 1 week and 2 week deployments from Pont Gendarme
    d. Performed sample cleaning and processing (dialysis and blow-down) on those samples and the QC

Suggestions for the lab

1. Implement SOPs, checklists, routine maintenance logs for commonly used equipment (GC, balance, freezer/refrigerator, ovens, pipets)
2. Implement a gas log for the helium used on the Agilent GC
3. Develop a hierarchy in the lab – it is unclear at the moment
    a. Who is the chemist in charge of the GC?
    b. Who is in charge of the lab?
4. Repair the fume hoods
    a. Replace the motors and upgrade the current motors
    b. Place stainless steel in one hood
    c. Add another level stop
5. Make and install a stainless steel countertop
    a. Where other countertops need to be clean use the Teflon OSU sent
6. Buy a new sample concentrator such as a Turbovap or repair the Techne sample concentrator correctly
7. Buy a stainless steel tub or preferable a sink for washing "organics" glassware. Plastic is not the best material to use
8. Designate trace analysis work such as pesticide residue work for the Agilent GC only
9. Designate other analyses (such as pesticide formulations) for the Varian GC or other equipment
    a. Do routine maintenance for the Varian GC
    b. Install moisture and oxygen trap for the nitrogen going into the Varian GC
10. Either choose to do formulation type work or trace level analysis or set up a new lab room for formulation only work.
    a. You cannot do both in the same area
11. Remove rust from cages and have several of them re-welded
    a. It looks as if some of the cages were welded with iron and not stainless steel rods like they should have been
    b. Buy welding paste to remove rust
12. Find a local supplier for pesticide residue analysis grade solvents or find a reliable over-seas supplier
13. Find a local or over-seas supplier of general laboratory consumables
    a. Kimwipes
    b. Nitrile gloves
    c. Alconox or similar detergent specific to scientific laboratories
    d. General glassware; beakers, volumetric flasks, etc
    e. Eye protection, and implement their mandatory usage
14. Find a local or over-seas supplier of GC consumables
    a. Columns, inlets, septa, syringes, ferrules, etc…

In the morning, we prepared the samples from retrieval 2 and started a run on the GC that will not be over until the morning on Friday. The computer froze after a few injections so we had to start the run over in the afternoon. We are still seeing degradation of some analytes in the inlet, but chose to run the samples anyway to better facilitate training.

The problem in the inlet seems most likely to be the glass wool. If I had more time I would have removed the glass wool from the liners and evaluated the degradation and compared the two inlet set-ups.

We also encountered a problem at the final step of the sample blow-down and transfer (a critical step for compound concentration calculations). During the preparation of the samples that included bringing the final volume up to 2 ml, drawing off 1 mL, and inserting an internal standard spike, Adama noticed that the graduations on the centrifuge tubes were not accurately done. I suspect that this was caused by incorrect or sloppy pipeting technique. To ensure it was done correctly and to retrain the staff I marked new centrifuge tubes and we quantitatively transferred all the samples to these new tubes before proceeding. This added about two hours to the whole process. It also highlights the need for each staff member to pay attention to details, especially during critical steps.

Anna was busy most of the day updating the calibration table on the GC Chemstation software with the standards that were run last night. We saved this new calibration table under a new processing method name specific to retrieval 2. I was with Anna helping her off and on during the day. Adama prepared the samples. I consider Adama a good worker, familiar with the LFT technology and processing and usually pays close attention to detail.

Much of the staff's time today was shared between working on this project and another project that had come in a few days earlier looking at pesticides in what I believe was peanut oil.

March 6, 2009

Today not much work was done in the laboratory. The GC run of the retrieval two samples at first glance showed the same type of contamination as retrieval one. This is only a provisional statement until I can look at the chromatograms more in depth. It looks as if the reagent blank is clean. This leads me to believe that the contamination either was introduced during the storage of the LFT prior to use or during LFT construction. I think we can come to a better conclusion when the data is actually worked up and the blank LFT from the lab is analyzed. I feel that determining where this contamination came from is an absolute must before any other LFT can be constructed, deployed, or processed at the CERES lab for actual data acquisition.

I performed a thorough review of the bench sheets filled out during this project and found that more attention to detail is needed when filling these out. Consistently the staff did not record critical information on bench sheets in a timely manner and there was reluctance to go back and finish or fix errors in bench sheets or other documentation. I had Anna, Marie, and Adama go over the bench sheets to verify that they were filled out correctly and fix errors that had occurred. It is obvious that some sort of error correction code system or protocol needs to be put into action such as OSU's SOP 1200. Documentation is a critical part of a QA process. Also, the staff will complete training surveys designed to collect their input on the training performance at a later date and send them to OSU via email.

A 2.2 Proposed training schedule for training event

Table A 2.1 Proposed training schedule for the CERES/Locustox personnel training. Dates are September 26 to March 6, 2009.

Week	1st half of week	2nd half of week
1st week Jan 26-30	Orientation, introductions Performance evaluations GC/ECD training - contamination trouble shooting and maintenance GC/ECD training - transfer of new methods Inventory and location – of needed lab supplies Inspect new cages	GC/ECD training - general PSD - basics; testing knowledge and retention of training, make sample LFT Confirm supply of gases Clean and bake (if available or week 2) glassware
2nd week Feb 2-6	Confirmation (GC dependent) of glassware and cleanliness Make calibration standard Make PRC solution Make internal standards Confirm standards (especially PRC on GC/ECD)	Set-up clean spaces (stainless steel or Teflon) PSD - construction of and spiking LFT for deployment Prep for field deployment (includes generating bench sheets) Field deployment PSD - training, and make spiked practice LFT
3rd week Feb 9-13	PSD - processing of spiked practice LFT to be used for GC and dialysis training Prepare for field retrievals GC/ECD training - general * potentially moving deployment to Monday Feb 9th	GC run of practice samples Review and data analysis of GC run of practice samples Calculation spreadsheet introduction and initial implementation
4th week Feb 16-20	1st field retrieval 1st field retrieval sample cleaning 1st field retrieval dialysis	GC run of 1st retrieval Review and data analysis of GC run of 1st retrieval Calculation spreadsheet QC summary
5th week Feb 23-27	2nd field retrieval 2nd field retrieval sample cleaning 2nd dialysis	GC run of 2nd retrieval Review and data analysis of GC run of 2nd retrieval Calculation spreadsheet with QC summary
6th week Mar 2-6	More GC training Calculation spreadsheet with QC summary Performance evaluations Follow up on inadequate training shown from performance evaluations Discuss method validation packet	Follow up on inadequate training shown from performance evaluations Discuss method validation packet * potentially deploy Feb 9th, retrieve the 16th and 23rd

A 2.3 Literature Cited

1. Sower, G. J. *Lipid-free tube passive sampling device technology transfer: On-site laboratory and field training*; Oregon State University: 2007.

APPENDIX 3 – SUPPLEMENTARY INFORMATION FOR CHAPTER 4

Table A 3.1 Parameters used in the ATSDR Portland Harbor PHA

Parameter	Value	Units	Description
Concentration (C)	N/A	µg/g	PSD accumulation (mass:mass)
Conversion Factor (CF)	0.001	kg/g	
Ingestion Rate (IR)	17.5	g/day	Average consumption (90th percentile)
Ingestion Rate (IR)	142.4	g/day	High consumption (99th percentile)
Body Weight (BW)	70	Kg	Adult
Exposure Frequency (EF)	365	days/year	Daily consumption
Exposure Duration (ED)	30	Years	Area residence time
Averaging Time (AT)	10950	Days	30 years (non-cancer)
Averaging Time (AT)	25550	Days	70 years (cancer)