AN ABSTRACT OF THE DISSERTATION OF

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Title: New Applications and Emerging Contaminants: Developing Silicone Passive Sampling Devices for Environmental and Novel Monitoring Applications.

Abstract approved:_____

Kim A. Anderson

Passive sampling is a popular technology for environmental monitoring, and silicone is an ideal choice for a variety of passive sampling applications. The silicone work described here encompasses laboratory and field studies that demonstrate the use of this polymer in novel environments, for new applications, and for emerging compounds. Unique attributes of silicone polymers make them advantageous for targeting semi-polar contaminants not typically targeted in environmental research. Oxygenated polycyclic hydrocarbons (OPAHs) represent an emerging class of contaminants with chemical properties well suited to silicone passive sampling. The first challenge was to create a robust OPAH analytical method to examine these compounds in silicone, and two independent methods (liquid as well as gas chromatography) were optimized and demonstrated for 24 ketone-containing aromatic hydrocarbons, more than other methods published at that time. An isotopically labeled OPAH was used as an internal standard in contrast to previous methods which used only labeled polycyclic aromatic hydrocarbons (PAHs). The efficacy of each method was further demonstrated by comparing standard addition to internal standard quantitation. Next, OPAHs, PAHs and pesticides were used to compare several silicone materials with low density polyethylene (LDPE) at Portland Harbor Superfund field sites. Target analyte detection, precision, and practical considerations in the field and laboratory were used to evaluate

silicone materials. Individual differences between LDPE and the most optimal silicone polymer for OPAHs highlighted the importance of using optimized methods or polymer choice for a particular analyte class. Biggest differences were found for 9-fluorenone, benzanthrone, and 5,12-naphtacenequinone. After this successful polymer comparison, the next study involved a novel application of silicone wristbands as personal passive samplers. Commercially available silicone was modified to serve as personal samplers and tested in both an ambient and occupational settings. Silicone wristbands provided a valuable tool to monitor individual exposures that were time weighted averages of personalized exposure. The ambient study captured 49 individual compounds including PAHs, personal and consumer products, pesticides, phthalates, and as well as other industrial compounds. In the occupational study, roofers working with hot asphalt wore silicone samplers and evidence of both temporal (day versus week deployment, $p < 0.05$), and spatial (between two roofing sites $p < 0.05$), sensitivity was found for PAHs, and two OPAHs were detected in some samplers as well (9-fluorenone and benzofluorenone). Finally, another novel application was developed for silicone as an *in vivo* monitor of persistent organic pollutants. Human silicone breast explants were found to contain chlorinated pesticides, and p,p-DDE and PCB 118 were used in murine models as an *in vivo* sampler to explore silicone as a biomonitor and sink of organic contaminant exposure. Silicone was found to sequester both compounds *in vivo*, and partition values from mouse data were used to predict human adipose tissue concentrations that were within literature values. This work presents silicone as a complimentary and useful material in traditional and novel environmental monitoring applications in order to promote a better understanding of exposures, chemical mixtures and environmental contamination.

New Applications and Emerging Contaminants: Developing Silicone Passive Sampling Devices for Environmental and Novel Monitoring Applications

by
Steven G. O'Connell

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APPROVED:

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Dean of the Graduate School

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

Steven G. O'Connell, Author

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CONTRIBUTION OF AUTHORS

In all chapters, Dr. Kim Anderson contributed to intellectual formulation, writing, and where appropriate, study design and method development. In Chapter 2, Theodore Haigh and Glenn Wilson aided in instrument optimization, method creation, and editing of the final manuscript. In Chapter 3, Melissa McCartney and L. Blair Paulik contributed to sample analysis and editing, Dr. Sarah Allan aided in manuscript preparation and study design, while Lane Tidwell and Glenn Wilson contributed to study design, method development and final editing. For Chapter 4, Dr. Laurel Kincl contributed to study design and worked with roofing professionals in order to be able to add that portion of the study to the final manuscript. Finally, for Chapter 5, Jamie Pennington and Dr. Nancy Kerkvliet provided *in vivo* expertise during study design, housing, and surgery of mice, Dr. Diana Rohlman aided in manuscript preparation, study design, autopsy, and additional *in vivo* expertise, while Dr. Susan Carozza contributed to concept design

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Chapter 1 - Dissertation Introduction

"*If we are going to live so intimately with these chemicals eating and drinking them, taking them into the very marrow of our bones - we had better know something about their nature and their power*."

- Rachel Carson, *Silent Spring (1)*

As environmental concerns and awareness grow, there is an ever increasing pressure to answer two core questions in toxicology: what chemicals are present, and whether they are harmful. The focus of this dissertation is of the first concern; specifically, what toxicants might be present in a variety of environments, and in what quantity. In that regard, some pollution may be obvious like the Cuyahoga River catching fire on several occasions from 1936-1969 *(2)*, or during an oil spill like the Deepwater Horizon tragedy in 2010 *(3)*. But even in obvious cases of pollution or chemical releases, it may be difficult to identify the specific chemical, or mixture of chemicals that are of toxicological interest. Adding to that challenge are degradation products that may or may not result in additional insults to the environmental system being studied. The field of environmental chemistry is constantly challenging itself to target an ever-growing list of compounds in the environment. In fact, one of the top priorities of the National Institute of Environmental Health Sciences (NIEHS) is characterizing mixtures of chemicals and supporting methods to assess health effects associated with complex exposures *(4)*. Current trends in environmental chemistry are in response to these priorities and this dissertation will describe how silicone has been used in three avenues of research: 1) new analytical methods for emerging compounds; 2) new technologies to address the complexity of sampling mixtures; and 3) novel applications in which these technologies may be applied.

1.1 An Example of Emerging Contaminants: Oxygenated-PAHs

As of 2012, more than 80,000 chemicals are currently registered in the United States *(5)*, and of those, at least 2,200 are high volume production chemicals (*i.e.* at least one million pounds produced or imported per year) *(6)*. Despite intricate knowledge about the application of these chemicals, information concerning fate, distribution, and occurrence for most compounds is often limited, or more information is sought *a priori*, or after health effects are seen in wildlife or human populations. On the other hand, there could be a plethora of information about a particular compound class, but nearly nothing is known about the abiotic or biotic products from parent compounds. One recent example includes oxygenated polycyclic aromatic hydrocarbons (OPAHs), which are current compounds of interest despite homologous parent PAHs having been studied for carcinogenic and other toxicity since the 1930s *(7)*. OPAHs consist of one or more oxygen atoms attached to an aromatic ring *(8)* (**Figure 1.1**), and have been increasingly of interest due to environmental presence and potential toxicity *(9)*. Some of the earliest work on this compound class occurred in 1975 when it was noticed that chromatographic peaks associated with a neutral polar fraction of carbon black contained oxygenated aromatic ringed compounds identified as cyclopenta[def]phenanthrenone and benzo[cd]pyrenone *(10)*. Formations of these compounds are derived from petrogenic and pyrogenic sources, and through abiotic or biotic degradation of unsubstituted PAHs *(9)*. Also, there is increasing evidence that some OPAH compounds are more toxic than the unsubstituted PAH analogue *(9,11-15)*. OPAHs represent an emerging compound class that requires new analytical methods.

Quantifying OPAHs is challenging. Because of the diverse physiochemical properties of these compounds, adequate solvation and stability are critical for laboratory and analytical use. OPAHs have been identified using both gas (GC) *(16-22)* and liquid (LC) *(23-28)* chromatographic systems, but few papers target more than 10 OPAHs. Another avenue of research is adequate internal and surrogate standards over

previous methods using only PAHs that might vary greatly in behavior through an analytical system *(29)*. Deuterated and labeled OPAHs have become increasingly available, and current practices would benefit from closely matched laboratory surrogates. In addition, multiple ionization methods might be necessary to quantify environmentally relevant OPAHs since ionization of a particular compound may or may not be optimal for all OPAHs. If better analytical methods can be developed for OPAHs, then field samples could be collected to examine these and other emerging chemicals in the environment.

1.2 New technologies: passive sampling using silicone rubber

One technique to address analytical challenges in the field is passive sampling. As far back as 1853, the idea of using inert material to sample for target compounds has been evolving *(30)*. Over 900 papers have been published on passive sampling since 1979 from a recent search on Web of Science™ *(31)*, but much of the growth has come very recently within the last decade (**Figure 1.2**). Uptake of target compounds occurs through passive diffusion into the membrane or receiving phase of choice. Models of this uptake predictably follow first-order kinetics, and involve a linear uptake phase followed by equilibrium with the surrounding environment *(32)*. Many advantages of passive sampling lie in the practical aspects of the device. Single grab samples of water, sediment pore water, or air only represent concentrations of analytes within the brief time period in which the sample was taken. At first this may seem desirable, but considering the non-static nature of the environment, it is apparent that a single grab sample may not represent the average contamination to a particular site or system, especially considering changes due to seasonal shifts *(33)*. In addition, a single time point may not have enough material to be detected on even the most sensitive equipment. Passive sampling on the other hand, represents time-weighted averages of chemical mixtures, and continually sample throughout the deployment period *(30,34-37)*. The resulting extracts are concentrated over the sampling duration, and may

provide increased analytical sensitivity over a grab sample. Another advantage to passive sampling is that the freely dissolved concentrations are captured *(38)*. This distinction is important to toxicological work since the freely dissolved fraction in sample media is bioavailable to organisms *(39)*.

Passive sampling is a technology that has an incredible range of potential materials that are able to sample freely dissolved compounds. For example, some popular passive sampling devices (PSDs) include: the POCIS (polar organic chemical integrated sampler) consisting of polar and semi-polar sorbents, polyoxymethylene (POM), solid phase micro-extraction (SPME) devices using various coatings around small fibers, SPMDs (semi-permeable membrane devices) consisting of triolein inserted into a polyethylene tube, or simply just thin films of polymers like low-density polyethylene and silicone rubbers *(30,34,35,37)*. PSDs are used in a variety of applications such as measuring endocrine disrupting compounds in waste water treatment plants *(40)*, monitoring contamination during oil spills *(41)*, having open ocean estimates of pesticides *(42)*, riverine concentrations of chlorinated biphenyls *(43,44)*, or atmospheric and sediment concentrations of PAHs *(45)*. In addition to all of the physical factors of the environment that influence the bioavailability of the target compound, there are the inherent intermolecular forces that control uptake between the compound and the receiving phase. The choice of material then, depends largely on the compounds being targeted.

Silicones are used in diverse applications from electronic device covers to gasket linings, bath tub sealant and even medical implants. Silicone was used in passive sampling devices as early as 1984, when it was reported as an outside housing around activated carbon *(46)*. Over time, silicone became the receiving phase for PSDs as coated fibers (SPME) and stir bars, and as solid rods, tubing, and thin films beginning in the 1990s *(35)*. Silicone might be advantageous to other polymers because of the

physical properties that drive passive uptake. Namely, in addition to Van der Waals forces, silicone might be able to take advantage of dipole-dipole interactions between the material itself and the compounds of interest. Silicone consists of a silicon-oxygen backbone *(35)*, so the overall nature of compound specificity is more polar compared to the hydrocarbon structure of another popular thin film, low-density polyethylene (LDPE) (**Figure 1.3**). If additional intermolecular forces are influencing passive uptake in silicone, then this polymer is well suited to sequester a broad range of contaminants. The wide range of potential targets has been alluded to in works by Allan *et al*., who found that silicone could sequester greater amounts of compounds of a lower octanol-water coefficient (K_{ow}) than other materials *(47,48)*. If silicone is a good choice of PSD material for a wide range of compounds, then it might be advantageous for emerging compounds such as OPAHs.

1.3 Novel applications of passive sampling

Most studies use passive samplers to sample legacy environmental pollutants such as PAHs and polychlorinated biphenyls (PCBs), but recent research is expanding usage of passive samplers both in chemical acquisition and environmental application. For example, applications of PSDs used as personal monitoring devices, or for *in vivo* sampling represent rich areas of ongoing research.

Beginning with water vapor and SO_2 measurements in the early 1970s *(49)*, PSDs have been used as personal monitoring devices. In contrast to early PSDs that monitored for only one or a few specific compounds, current research targets classes of organic contaminants and chemical mixtures *(50)*. A recent example is polyurethane foam used as personalized passive samplers for PAHs *(51)*. However, it is unclear if polyurethane foam devices will be able to exclude non-biologically relevant particulate sizes with modified deployment *(51)*, or sample more polar contaminants like OPAHs. Since silicone has sequestered a wide range of contaminants such as volatiles (ex:

benzene, toluene, and xylene) and semi-volatiles containing hydroxyl, ketone, or carboxyl groups *(35,48)* in addition to hydrophobic compounds *(44,47,48,52,53)*, it is likely that silicone is more suitable for sampling a wide range of organic pollutants for personal monitoring. An additional benefit of using silicone is that the material will limit sampling to the vapor phase, which may include up to 86% of the toxicological dose in industrial exposures for PAHs *(54)*.

In addition to personal monitoring, some research has employed passive sampling techniques *in vivo*, which is useful for monitoring or bioaccumulation studies *(55-58)*. Since tissue samples may be difficult to process, and some tissues like blood and plasma represent relatively short-term exposures to contaminants, there is a need for sampling tissues that represent long-term exposures with a greater degree of precision than traditional tissue samples. Additionally, organic interferences are largely excluded from passive sampling methodology, so analytical data may be easier to interpret *(59)*. Silicone is a popular choice for *in vivo* sampling in this application as well *(55-58)*, and diffusion properties between the tissue and silicone may be used to characterize exposure to contaminants. In one example, human silicone implants from surrounding adipose tissue were used to identify several classes of compounds including PCBs, brominated dioxins, and several other chlorinated pesticides including DDT and p,p-DDE *(60)*. Human implants are made with similar silicone as used in the more common PSD sampling applications, so passive uptake kinetics should be able to be used to characterize *in vivo* exposure similarly to environmental studies. Furthermore, if silicone is absorbing contaminants within an organism, those passive samplers may even reduce the concentration of contaminants from surrounding tissues and help explain decreased incidence of breast cancer observed in those individuals receiving silicone implants *(61,62)*.

1.4 Objectives and Hypotheses

Silicone may be used in a variety of applications as a passive sampler to sequester a wide range of contaminants in order to better understand what toxicants might be present, and in what quantity. To address this central theme of this dissertation, four projects were undertaken:

- <u>OPAH Method Development</u>
    - Create analytical methods for the emerging compound class of OPAHs so that they may be used in future research with silicone PSDs.
        - *H_a: Utilizing deuterated and labeled compounds of similar physicochemistry to OPAHs as well as solvation and surface chemistry improvements in instrumental methods will make quantitative analysis more accurate and more precise than previous methods.*

- <u>Environmental Silicone Passive Sampling</u>
    - Develop silicone passive samplers that are able to sequester a wide range of organic contaminants including PAHs, pesticides, and OPAHs from environmental deployments.
        - *H_a: If silicone has a more polar structure than polyethylene, then contaminants like OPAHs will be absorbed in greater frequency and abundance in silicone over LDPE.*

- Silicone Personal Samplers
    - Utilize silicone passive sampling methodology to develop a passive sampler that represents personalized exposures to environmental contaminants.
        - *H_a: Silicone will be able to sequester a wide range of contaminants from an atmospheric deployment that will be spatially and temporally sensitive to personalized exposure.*

- *In vivo* Silicone Sampling
    - Use previous passive sampling techniques to examine human silicone implants for toxicants, and dose a model organism with identified compounds to examine changes in body burden due to silicone *in vivo* sampling.
        - *H_a: If silicone is able to absorb compounds within living tissues and interstitial fluid, then silicone implants will be able to be used as passive sampling devices.*

        - *H_a: If silicone implants are in vivo PSDs, then surrounding tissues will have lower concentrations of contaminants.*

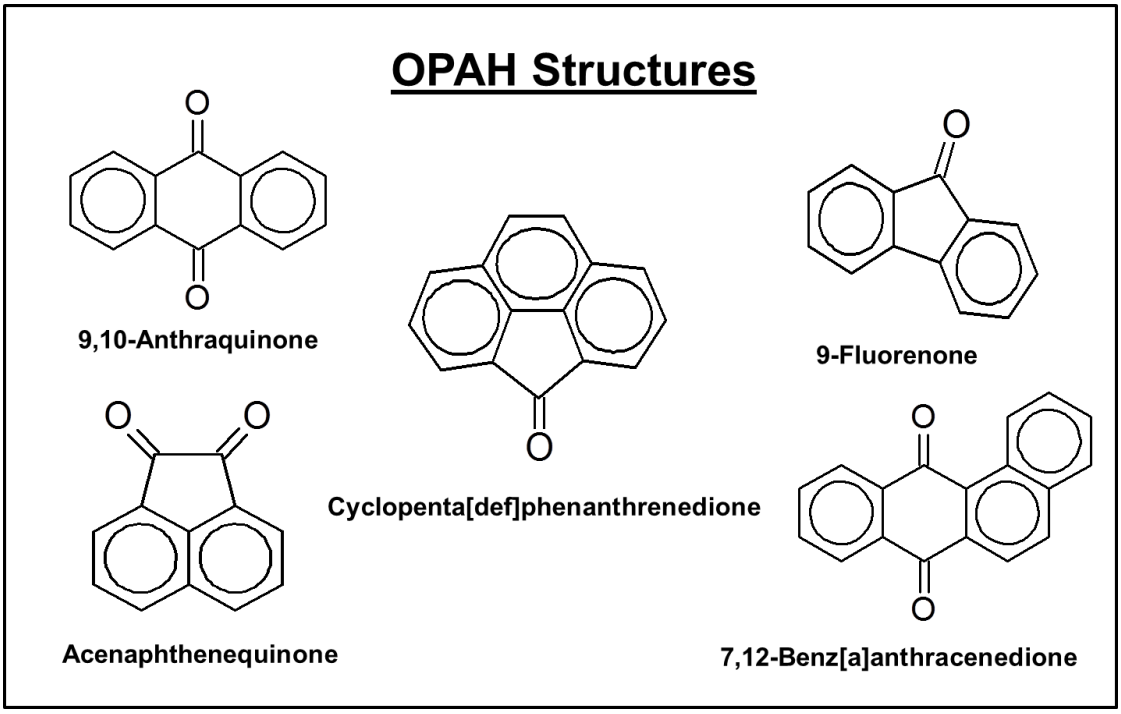

Figure 1.1 Example structures of several OPAHs

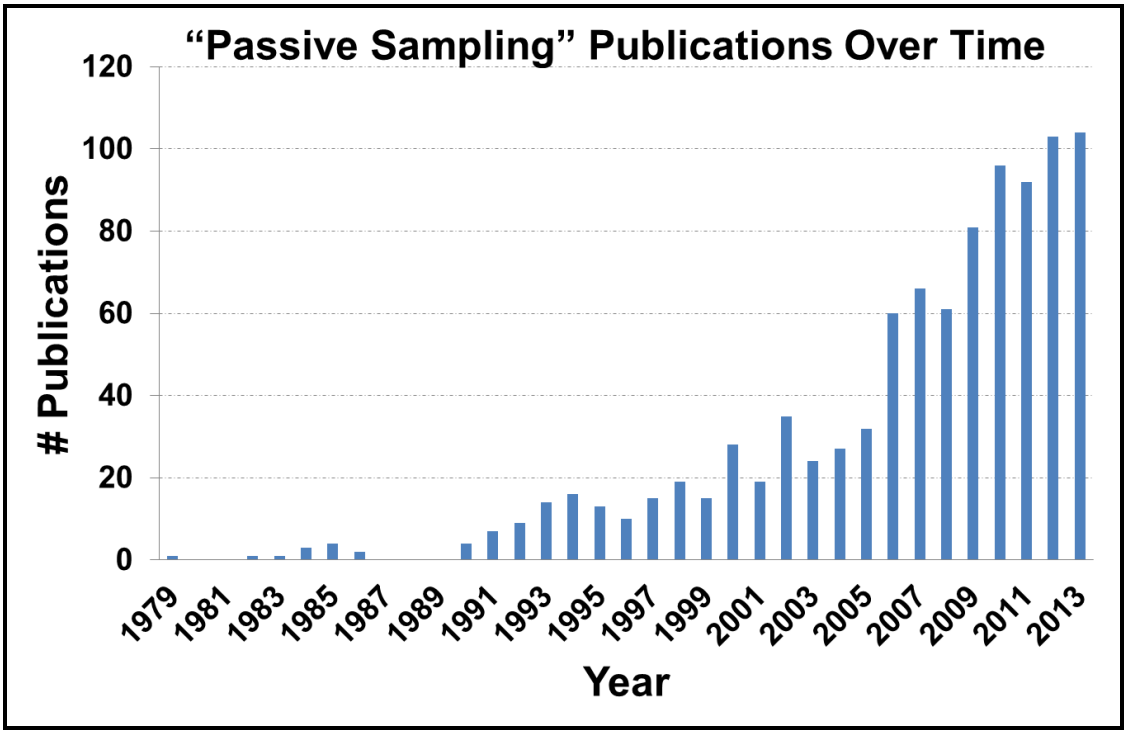

Figure 1.2 Growth of passive sampling research over time. Data was obtained from a search on Web of Science™ using "passive sampling" as search criteria on 10/1/2013 and again on 4/1/2014 for 2013 data.

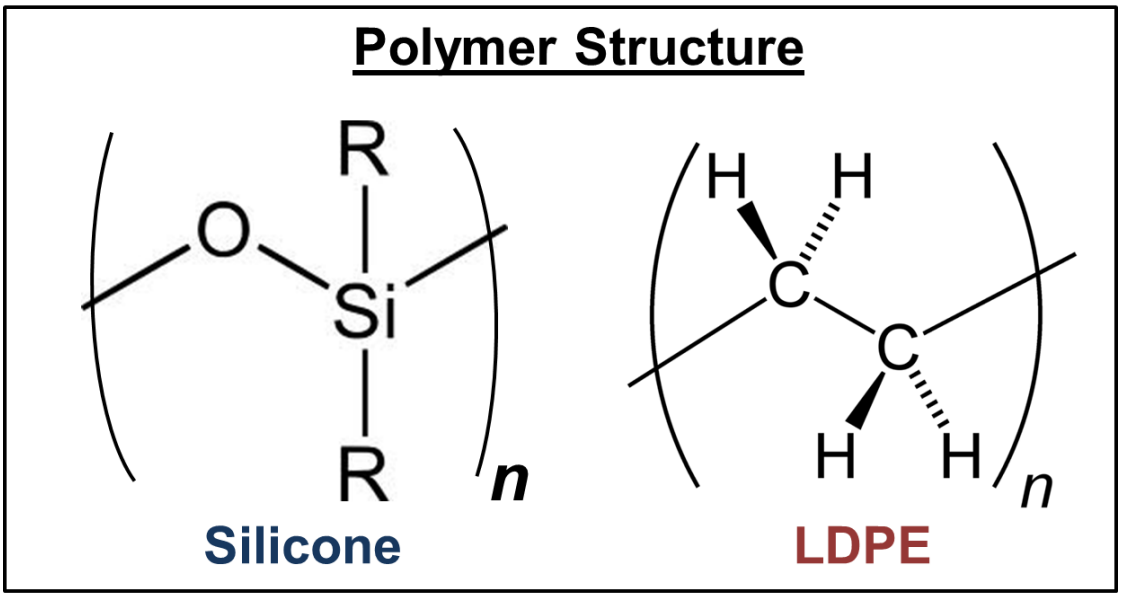

Figure 1.3 Differences in polymer structure might influence absorption of different analytes into each material. Silicone and polyethylene are both common PSDs used as coatings or thin films.

Chapter 2 - An Analytical Investigation of 24 Oxygenated-PAHs (OPAHs) using Liquid and Gas Chromatography-Mass Spectrometry

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2.1 Abstract

We developed two independent approaches for separation and quantitation of 24 oxygenated polycyclic aromatic hydrocarbons (OPAHs) using both liquid chromatography-atmospheric pressure chemical ionization/mass spectrometry (LC-APCI/MS) and gas chromatography-electron impact/mass spectrometry (GC-EI/MS). Building on previous OPAH research, we examined laboratory stability of OPAHs, improved existing method parameters, and compared quantification strategies using standard addition and an internal standard on an environmental sample. Of 24 OPAHs targeted in this research, 19 compounds are shared between methods, with 3 uniquely quantitated by GC-EI/MS and 2 by LC-APCI/MS. Using calibration standards, all GC-EI/MS OPAHs were within 15% of the true value, and had less than 15% relative standard deviations (RSDs) for inter-day variability. Similarly, all LC-APCI/MS OPAHs were within 20% of the true value, and also had less than 15% RSDs for inter-day variability. Instrument limits of detection ranged from 0.18-36 ng mL^{-1} on the GC-EI/MS, and 2.6-26 ng mL^{-1} on the LC-APCI/MS. Four standard reference materials were analyzed with each method, and we report some compounds not previously published in these materials, such as perinaphthenone, and xanthone. Finally, an environmental passive sampling extract from Portland Harbor Superfund, OR was analyzed by each method using both internal standard and standard addition to compensate for potential matrix effects. Internal standard quantitation resulted in increased precision with similar accuracy to standard addition for most OPAHs using 2-fluoro-fluorenone-^{13}C as an internal standard. Overall, this work improves upon OPAH analytical methods and provides some considerations and strategies for OPAHs as focus continues to expand on this emerging chemical class.

2.2 Introduction

Scientific interest for oxygenated polycyclic aromatic hydrocarbons (OPAHs), also known as oxy-PAHs, has increased in the last decade due to environmental presence and concern over potential toxicity *(9)*. OPAHs consist of one or more oxygen atoms attached to an aromatic ring structure that may also contain other chemical groups *(8)*. Formations of these compounds can derive from petrogenic and pyrogenic sources, specifically through chemical oxidation, photo-oxidation, or biological transformation of the unsubstituted PAHs *(9)*. Ongoing research has described several processes of incomplete biodegradation leading to production of substituted PAHs, including OPAHs *(63-66)*. Determinations of individual and mixed OPAH toxicities are active areas of research, but there is increasing evidence that some OPAH compounds are more toxic than the unsubstituted PAH analogue *(9,11-15)*. Additionally, it has been observed that OPAHs are persistent in the environment as opposed to other transient organic compounds, which contributes to increased attention of this chemical class *(9,64,66)*. Other concerns are that OPAHs are not routinely monitored, accumulate at PAH contaminated sites *(9,63)*, or are potentially formed from bio-remediation strategies *(13)*.

Although some analytical methods include OPAHs that contain hydroxylated or carboxylated compounds *(10,16,17,23,24)*, our focus is on OPAHs that have at least one ketone group. Ketone-containing OPAHs include those previously reported in environmental samples such as 9,10-anthraquinone, and 9-fluorenone *(17,18)*, or those that have shown potential for mutagenicity such as perinaphthenone, benzanthrone, and benzo[cd]pyrenone *(67)*. Quantitative analysis is challenging for these OPAHs due to wide ranges in solubility. Because of diverse physiochemical properties, adequate solvation and stability become key factors in successful analytical method development. Both gas (GC) *(16-22)* and liquid (LC) *(23-28)* chromatographic systems have been effectively used for OPAH analyses, but only 4 of the above papers target more than 10

ketone-containing OPAHs. Of those papers, only one has quantitated and separated more OPAHs than this work using GC mass spectrometry (MS) *(19)*, but it utilizes only a single response factor with a deuterated PAH of similar retention time to quantitate several OPAHs. Response factors ranged from 0.09 to 0.74 *(19)*, indicating that PAHs and OPAHs might differ dramatically in analytical response regardless of retention time. Although this strategy would be useful at the time of publication, deuterated OPAHs have become increasingly available in recent years, and the following work highlights OPAHs that could benefit from closely matched laboratory surrogates or internal standards. Liquid chromatographic methods using atmospheric pressure chemical ionization (APCI), or LC-APCI-MS, have also been used successfully to quantitate 15-17 individual OPAHs *(24,25)*, but both papers use both positive and negative mode which can lead to losses in sensitivity, or requires running each sample twice to quantify all target compounds. Because of the large variability in physicochemistry of OPAHs (log K_{ow} 0.2-5.31), ionization of a target compound may or may not be optimal for a single chromatography system; it may be necessary to use multiple ionization methods to quantify larger sets of environmentally relevant OPAHs.

To develop methods for quantifying as many OPAHs as possible, 24 target OPAHs were analyzed on both GC and LC systems. To facilitate analysis on either GC or LC, we developed a final extraction preparation that employed a single solvent (ethyl acetate) that is both LC and GC compatible. We also discovered significant response differences with solvents, inlet temperatures, inlet liners and OPAH stability not previously reported for this compound class. Our initial objective was to optimize and validate each method for increased sensitivity, accuracy and precision for as many OPAHs as each system could quantify. The second objective was to demonstrate each method using real environmental matrices, including National Institute of Standards and Technology (NIST) Standard Reference Materials (SRMs). Our third objective was to illustrate differences between instruments due to any potential matrix effects, and compare the effectiveness

of standard addition over our choice of an internal standard quantitation using an aqueous sample from a National Priority List Superfund site. In this research, we detail the identification and quantification of 24 ketone-containing OPAHs on two independent methods, providing a foundation of analytical investigation as new OPAHs are identified, become commercially available, or targeted through ongoing environmental research.

2.3 Experimental Section

Chemicals and Reagents

Analytical grade standards (purity ≥ 97%) were obtained from several vendors including: 9,10-anthraquinone-D8 (9,10-ANTQ-D8), 9-fluorenone-D8 (9-FLUO-D8), 1,4-naphthoquinone-D6 (1,4-NQ-D6), 2-methyl-1,4-naphthalenequinone-D8 (2me-1,4-NQ-D8), and 2-fluoro-fluorenone-^{13}C (2F-FLUO-13C) from CDN Isotopes (Pointe-Claire, Quebec, Canada); 6H-benzo[cd]pyrenone (B[cd]PYRO), 1,4-phenanthrenedione (1,4-PHED), and 1,4-benzo[c]phenanthrenequinone (1,4-B[c]PHEQ) from Chiron (Trondheim, Norway); 9-fluorenone (9-FLUO), 9,10-anthraquinone (9,10-ANTQ), and 1,9-benzanthrone (BANO) from Fluka (part of Sigma-Aldrich, St. Louis, MO); benzo[a]pyrene-1,6-dione (1,6-B[a]PYRD) and benzo[a]pyrene-7,8-dione (7,8-B[a]PYRD) from NCI (Bethesda, MD); 1,4-benzoquinone (1,4-BQ), chromone (CHRO), 9,10-phenanthrenequinone (9,10-PHEQ), 5,12-naphthacenequinone (5,12-NAPQ), benz[a]anthracene-7,12-dione (7,12-B[a]ANCQ), xanthone (XAN), 1,2-napthoquinone (1,2-NQ), 1,4-naphthoquinone (1,4-NQ), perinaphthenone (PNAPO), pyrene-4,5-dione (4,5-PYRD), aceanthrenequinone (AANEQ), benzo[a]fluorenone (B[a]FLUO), 1,2-acenaphthenequinone (1,2-ANAPQ), 2-ethyl-9,10-anthraquinone (2-ethANTQ), and cyclopenta[def]phenanthrenedione (CP[def]PHED) from Sigma-Aldrich. In total, 29 compounds were acquired including 24 non-labeled OPAHs, and 5 labeled OPAHs (**Figure 2.1**).

All solvents used were at least Optima-grade (Fisher Scientific, Pittsburg, PA) or equivalent. Whenever possible, non-chlorinated solvents were chosen preferentially in order to reduce chlorinated waste. However, attempts at using only non-chlorinated solvents for initial stock solutions resulted in incomplete dissolution for some OPAHs even after sonication. Therefore, all OPAH stock solutions were prepared from neat at approximately 130-1100 µg mL^{-1} in a mixture of ethyl acetate: dichloromethane, 95:5 (v:v), to ensure solvation. Further dilutions and mixtures used only ethyl acetate. Due to solubility constraints at low temperatures, all solutions were brought to room temperature and sonicated for at least 15 minutes prior to any dilution or further use. This step is crucial since some initial stock compounds recrystallized at 4°C. No additional peaks were identified as co-eluters with other compounds of similar ion mass/charge ratios, so any impurities in OPAH standards were deemed negligible. Finally, 2-fluoro-fluorenone-^{13}C was chosen as an instrumental internal standard for target OPAHs due to similar physicochemistry, excellent response on each method, and certified stability. Laboratory surrogates (deuterated OPAHs other than 2F-FLUO-^{13}C) were used only in environmental samples, and data reported in this paper is not-corrected for any potential laboratory losses unless otherwise stated.

Method Parameters and Optimization

***LC-APCI/MS**:* For LC analysis, we used an Agilent (Agilent, Santa Clara, CA) 1100 liquid chromatography binary pump stack coupled to a single quadrupole MS (Agilent/1956B) with an APCI source (Agilent/G1947A). Each OPAH was optimized individually for MS acquisition parameters by performing flow injection analysis for fragmentor voltages ranging from 50 V to 200 V with a step increase between injections of 10 V. The largest response was used for each respective compound. Target ions and fragmentor voltages used for extracted ion chromatograms are listed in **Table 2.1**. Other final MS parameters used in this study include: gas temperature at 350 °C, vaporizer temperature at 400 °C,

drying gas flow at 5 L min^{-1}, nebulizer pressure at 50 psi, capillary voltage at 4500 V and the corona current at 10 µA (negative mode).

The non-aqueous mobile phase (B) consisted of dichloromethane: methanol (1:99, v/v), and the aqueous phase (A) was filtered through a Barnstead D7389 (Dubuque, IA). Mobile phases passed through the system at a flow rate of 0.5 mL min^{-1}, for a total of 55 minutes. Initially, the percentage of A:B was 95:5, and increased to 70:30 in the first 7 minutes, ramped to 60:40 in the next 8 minutes, then to 25:75 in the next 10 minutes, and finally increased to 10:90 over 15 minutes. The final percentage of the non-aqueous phase was then held for 10 minutes, and ramped back down to 95:5 over 5 minutes. Similar to a previous method*(24)*, a phenyl column (150 x 3 mm length and diameter, particle size 3 µm, Agilent) was chosen to achieve separation for most of the OPAHs on the LC (**Table 2.1**). Column temperature was kept above ambient at 42 °C, and 20 µL was used for each sample injection.

GC-EI/MS: Experiments to improve upon previous GC-EI/MS OPAH methods were performed on an Agilent 7890A gas chromatograph coupled to a 5975C mass spectrometer (Agilent) under electron ionization (70eV). Oven temperatures were evaluated and modified for a wider range of OPAHs from a previous study *(18)*. Briefly, temperature was ramped from 60 to 180 °C at 10 °C min^{-1}, to 290 °C at 5 °C min^{-1}, to 310 °C at 25 °C min^{-1} and held at maximum temperature for 2 minutes for a total run time of 37.8 minutes. A DB5-MS column (30 m length, 0.25 mm inner diameter, 0.25 µm film thickness, Agilent) was used to separate target OPAHs, with a 1 mL min^{-1} flow rate using helium (>99.99 %) as a carrier gas. MS temperatures included the thermal auxiliary control set at 280 °C, the MS source at 230 °C, and the MS quadrupole at 150 °C. Quantitation ions are listed in **Table 2.2**, as well as qualification ions used to help identify OPAHs in complex matrices.

Inlet parameters were optimized to 3 mL min^{-1} septum purge, a purge to split flow of 20 mL min^{-1} at 0.75 min, an injection pulse pressure of 35 psi until 0.05 min, and an injection temperature of 300 °C. Sample volume was 1 µL. Initially, deactivated glass wool was used in 4mm injection liners (Restek, Bellefonte, PA) to reduce non-volatile components from environmental samples getting on the column. Over several consecutive runs with composite calibration solutions, it was observed that 14 compounds had relative standard deviations (RSDs) over 15 %, and 1,2-NQ was no longer identifiable. Further experiments using other inlet liners, glass wool, and glass liners with no filter in place were evaluated.

Method Calibration and Validation

Each method was calibrated using extracted ion chromatograms for each OPAH. The calibration curve ranged from 5–5000 ng mL^{-1}, and included 9 calibration points. To determine instrument detection limits (IDLs) and the limit of quantitation (LOQ), the lowest calibration standard that resulted in a signal to noise ratio greater than 3:1 was found for each compound and for each method. The lowest calibration standard was repeatedly analyzed (n≥4), and a standard deviation was calculated for each compound. IDLs were determined by multiplying the resulting standard deviation estimates with the student *t*-value corresponding to the appropriate degree of freedom and 99 % confidence *(68)*. **Table 2.1** and **2.2** report the resulting IDLs for each compound and instrument method. LOQs were calculated by multiplying the IDL by five which was considered as a conservative estimate for reasonable quantitation.

Validation for each method utilized two test series using 500 ng mL^{-1} concentrations. First, several ethyl acetate aliquots spiked only with the internal standard 2F-FLUO-^{13}C, served as blank controls. A second series of composite solutions with all target OPAHs including deuterated compounds was used to explore accuracy

and repeatability as shown in **Table 2.1** and **2.2**. To examine inter-day accuracy and precision, the set of calibration standards were run on at least two days.

OPAH Stability

All deuterated compounds included in this study were certified as viable for a three year period, but due to limited commercial availability and novelty of OPAHs, not all target compounds had known expiration dates when purchased. To examine OPAH stability, a set of 15 aliquots at 500 ng mL^{-1} in ethyl acetate was kept at approximately 4 °C (± 2 °C) with all native OPAHs listed in **Tables 2.1** and **2.2**. At the beginning of the experiment, 2F-FLUO-^{13}C was added into each sample as an internal standard. A set of 3 aliquots were analyzed on days 0, 14, 32, 67, and 111 (LC-APCI/MS) or 116 (GC-EI/MS). Area counts were corrected for the internal standard, but analytes were not quantitated since calibration curves over the course of the study potentially changed with the aliquots, and if degradation had occurred, quantitating responses would have masked any temporal changes.

Method Demonstration using Environmental Matrices

Environmental extracts were spiked with 2F-FLUO-^{13}C at 500 ng mL^{-1} before instrumental analyses. Calibration check standards were run before and after each set of samples, and were considered successful if native OPAHs quantitated at ±30% the true value for 90% of compounds in each method. Qualitative analytes as noted **Tables 2.1** and **2.2** were not included in the 90% criteria. At a minimum, all extracts were run in triplicate on both methods.

NIST Standard Reference Material*:* Four different environmental extracts were examined on each method as verification that OPAHs could be quantitated successfully in environmental matrices. Multiple matrices were chosen to exemplify a range of complexity including: urban dust, river sediment, diesel extract, and diesel particulate

matter (NIST SRMs: 1649b *(69)*, 1944 *(70)*, 1975 *(71)*, and 1650b *(72)*, respectively). Extracts were analyzed from SRM material extracted and reported elsewhere *(18)*. In the original extraction, 9-FLUO-D8 and ANTQ-D8 were spiked as surrogates in each SRM extract and were solvent exchanged to ethyl acetate from hexane and stored until this analysis at 4 °C. Accelerated solvent extraction (ASE) and solid phase extraction cleanup of these SRMs were described in the original work *(18)*. The purpose of analyzing this set of extracts was not to characterize or compare previously reported values of NIST SRM material as previously described *(18)*, but to demonstrate the applicability of our two independent instrumental methods for environmental samples, and to compare values between the LC and GC generated data.

Standard Addition on Portland Harbor Superfund Passive Sampler Extract: To reduce uncertainty about matrix effects between instruments and to examine our choice for internal standard, we quantified an environmental extract from Portland Harbor Superfund, OR using both internal standard and standard addition quantitation. A silicone strip was cut to approximately 3.2 x 99 cm from purchased commercial material, (Stockwell Elastomerics Inc., Philadelphia, PA) and was exposed for 27 days in the Willamette River, mile 3.5 West, within Portland Harbor Superfund, OR from September 3-30th, 2010. Once recovered, the silicone was rinsed two times with ultra-pure water, and then with isopropyl alcohol to remove excess water and stored at -20 °C until extraction. To track potential losses in the laboratory, the sample was spiked with 2me-1,4-NQ-D8 and 9-FLUO-D8 at 500 ng mL^{-1}, extracted twice with ethyl acetate, and finally reduced under filtered nitrogen to 1 mL. Extractions were performed on an S-500 orbital shaker (VWR, Radnor, PA) for a total period of approximately 14 hours, and solvent reduction was performed by using closed cell reduction devices (Zymark, Hopkinton, MA). Each sample was stored at 4 °C until analysis.

Standard addition samples consisted of four dilutions. In each dilution, 100 µl of Portland Harbor extract was placed into a 250 µl chromatography vial. For the initial dilution, an additional 100 µl of ethyl acetate was added to make a total volume of 200 µl. In each subsequent addition, 10, 20, or 30 µl of a 1,000 ng mL^{-1} stock of target OPAHs was added, corresponding to an equivalent of 50, 100, or 150 ng mL^{-1}, respectively. The addition scheme was no more than 10 times the average response of OPAHs in either method, with most responses within a factor of 3. Estimates of variability for standard addition values incorporated the standard deviation of both the slope and intercept of each regression model as described in Bader, 1980 *(73)*.

2.4 Results and Discussion

Method Optimization

LC-APCI/MS: APCI was found to be sensitive for both ketone and diketone OPAHs, while some compounds produced no ions with electron ionization similar to a detailed investigation of HPLC-MS ionization sources *(23)*. Once initial MS parameters were set, methanol and water were used as mobile phases similar to other papers *(23-25)*, but some OPAHs had lower than expected responses. Therefore, several dopants were assessed including formic acid and ammonium formate. However, dramatic improvement was only observed with dichloromethane. Most notable was the effect on 5,12-NAPQ (**Figure 2.2a**), where peak shape improved and the peak response increased about 5-fold. Enhanced responses of dichloromethane dopant in the mobile phase has been found for other structurally similar aromatic compounds as well *(74)*. Improvements from dichloromethane may be due to increases in solvation of some OPAHs in the LC system, or from enhancement of ionization efficiency by stabilizing the charge. Enhancement of ionization efficiency has been shown to be greatly affected by different dopants or solvents for APCI ionization *(15,74)*. While positive mode was more sensitive for a subset of the compound list, negative mode provided better responses over the entire target compound list. One benefit of running solely in negative mode is

reduced analysis time compared with running samples twice in negative and positive mode *(25)*. Additionally, if an LC method is run with dual positive/negative mode it might suffer from reduced sensitivity depending on the number of analytes in the quantitation window. The sensitivity of our LC-APCI/MS method (2.6-26 ng mL^{-1}) is comparable or better than OPAHs of another method with similar ionization parameters (0.10-250 ng mL^{-1}) *(25)*.

***GC-EI/MS**:* Even with the addition of 8 more target compounds compared to a similar GC-EI/MS method *(18)*, we were able to increase resolution and sensitivity, which resulted in lower detection limits (0.18-36 compared to 0.5-50 ng mL^{-1}). Specifically, a lower starting temperature of 60 °C (versus 70 °C) increased the response by over 400-fold for 1,4-BQ, (**Figure 2.2b)** while still being able to acquire slower eluters like 1,4-B[c]PHEQ, 5,12-NAPQ, 7,12-B[a]ANCQ, and B[cd]PYRO in under 40 minutes (**Figure 2.3)**. In addition, better separation was achieved by slowing the rate of oven temperature increase between 1,4-PHED and 9,10-ANTQ *(18)*. Improvements are also likely due to pulsed splitless injection over previous non-pulsed splitless injection *(16-18)*, by getting compounds on-column more efficiently. Pulsed splitless injection has been found to improve recoveries in organophosphorus pesticides with physiochemical properties similar to the OPAHs studied here *(75)*.

Glass wool was thought to be a source of variability for OPAHs due to surface chemistry between the ketone groups of our target compounds and active sites in the wool created in preparing the liner for analysis. **Figure 2.2c** shows the RSDs from 5 or more consecutive runs of calibration aliquots. Using no inlet packing decreased the average RSD for all OPAHs dramatically (8.5 ± 1.4 % vs 21 ± 2.5 % on 95 % confidence intervals) compared with glass wool filters. CarboFrit™ liners improved repeatability over using no inlet packing (6.6 ± 0.7 % on 95 % confidence intervals). There was a two to six-fold reduction in RSDs between CarboFrit™ liners and glass wool, but the most

dramatic change occurred for 1,2-NQ. Using glass wool, we were not able to consistently identify or quantify 1,2-NQ (**Figure 2.2c**). Similar accuracy and precision was also observed for deactivated dimpled liners with no inlet packing compared with CarboFrit™ liners, so all additional experiments were performed without glass wool.

Method Validation

LC-APCI/MS: Each compound was calibrated with a linear calibration model with a 9-point correlation coefficient (R^2) of 0.99 or better. **Table 2.1** lists the accuracy of individual compounds using the lowest detectable calibration standard as well as the IDL and LOQ calculated. Other APCI methods have slightly lower IDLs (sub ppb), but use less conservative estimates based only on signal to noise ratios*(76)*. Spiked replicates show good accuracy over multiple days (n=6 repeated over 3 days unless otherwise noted), with 18 compounds within 20 % of the true value, and 11 were within 10 % (**Table 2.1**). OPAHs outside of 20% accuracy include: 1,4-BQ (52 %), 9-FLUO (>100 %), AANEQ (35 %), 2me-1,4-NQ-D8 (56 %), and 9-FLUO-D8 (25 %). Accuracy of our method compares well or better than that of another LC method which reported 29-87 % accuracy for 9 OPAHs *(76)*. It is unclear why 9-FLUO has unpredictable responses on our LC-APCI/MS system despite an effective calibration, but this discrepancy underscores the necessity of validating new compounds. In regard to repeatability, 20 compounds showed acceptable variability below 20 % RSD, most of which (14 OPAHs), had RSDs below 15 % (**Table 2.1**). Our repeatability (inter-day RSDs: 4.3-28 %) is comparable or better than that of another similar method *(23)*, and with a method using tandem mass spectrometry (inter-day RSDs: 4.1-17.7 %) *(76)*. Other compounds besides 9-FLUO that showed variability above 20 % RSD were CP[def]PHED (28 %), 1,4-ANTQ (28 %), and AANE (31 %). Additionally, XAN, PNAPO, and CHRO were unable to be ionized using our APCI source. It is important to note that the chromatographic separation described here should be effective for LC-APCI/MS-MS, thus expanding the ability to incorporate more OPAHs as environmental or toxicological data become available.

***GC-EI/MS**:* One unexpected observation during GC OPAH calibration was non-linearity over concentrations within one or two orders of magnitude. Non-linearity was observed despite three separate calibration solutions prepared by two chemists (exemplary compound, 7,12-B[a]ANTQ, shown in **Figure A.1.1 in Appendix 1**). Since non-linearity occurs throughout the calibration curve, detector fatigue is not responsible, nor is cross-contribution likely *(77)* since there are no other ions detected to interfere with quantitation in clean matrices from the internal standard, 2F-FLUO-^{13}C. Quadratic curves had an average linear coefficient (R^2) over 0.99 for all compounds including those OPAHs that were listed as not quantifiable in a previous study *(18)*. Variability between each calibration set is reduced by over 40 % when modeled as quadratic curves compared to linear models using the same data. Though linear models would be preferential, the range for each calibration curve would have to be reduced to just over one order of magnitude (50 ng mL^{-1} to 750 ng mL^{-1}) for the curve to be considered linear (> 0.99 R^2) for 1,4-ANTQ, CP[def]PHEO, 9,10-PHEQ, and 4,5-PYRD, yet would still be below 0.99 for nearly half of the OPAHs in this method (11 out of 26). The result of such a small acceptable calibration range would make quantitation impractical for environmental samples. Therefore, quadratic calibration curves were used for all GC-EI/MS analyses.

All compounds were within ±15 % of the true value, and had less than 10 % RSD on the GC-EI/MS method except for 7,8- and 1,6-B[a]PYRD which did not ionize well under the GC-EI/MS method (**Table 2.2**). The accuracy observed is better than that of the only other GC-EI/MS method published with more ketone-OPAHs (20 % accuracy on one calibration point) *(19)*. Overall precision is excellent, but comparisons of precision to other methods for solvent solutions is difficult since previous GC-MS methods are either qualitative *(28)*, or precision is based using laboratory and instrument variability that are sample specific *(16-19,21,22)*. Despite 1,2-NQ, 4,5-PYRD, and 9,10-PHEQ

performing well on the initial calibration and verification, these three compounds showed considerable variability over time and subsequent analyses. It is unclear why these three compounds show either reduced or no response, but evidence from repeated analyses supports active surface chemistry in the injection port as a potential source of variability. Because variability for these compounds typically *only* occurs with inter-day injections, experiments examining the cause of reduced responses of these OPAHs should take place over the course of multiple days. For this reason, 1,2-NQ, 4,5-PYRD, and 9,10-PHEQ are considered for qualitative purposes on the GC-EI/MS method for environmental demonstrations described below.

OPAH Stability

During the course of the OPAH stability experiment (111, 116 days, LC and GC respectively), all OPAHs were stable with one possible exception, 1,2-ANAPQ, which showed evidence that responses decreased slightly over time (**Figure A.1.2**) or at least were variable on both instrumental methods. Subsequent analyses of several old and newly prepared stock standards showed no response decrease for 1,2-ANAPQ even after a full year (**Figure 2.2d**). Specific causes of reduced or variable responses for 1,2-ANAPQ during the stability study remains unknown, but variability for this specific OPAH has been reported elsewhere *(16)*. The variability of this compound underscores the necessity of running calibration verification samples before and after each batch to monitor system stability on either instrumental method.

Method Demonstration and Comparison

NIST SRM Instrument Comparison: All standard reference materials were able to be successfully quantitated for OPAHs on both instruments and compares well to other published results (**Table A.1.1**). **Figure 2.3** represents chromatograms using each method for diesel particulate matter (SRM 1650b). An unexpected result from this diesel particulate sample was the large response of PNAPO, as well as the presence of

XAN (**Figure 2.3**), neither of which has been previously reported for this SRM at the time of this publication. Suggestive evidence of 1,6-/7,8-B[a]PYRD was seen in diesel and river extracts, although since the values are at or below the LOQ of 55 ng mL^{-1}, this compound is yet to be confirmed in these samples, and is not included in **Figure 2.3**. Reports of PNAPO, XAN, or 1,6-B[a]PYRD have been reported in other environmental samples, including aerosol particulate matter *(25,76)*.

Figure 2.4 displays the average quantitated results of comparable OPAHs in all four matrices from each chromatographic system. Concentrations are prior to any back-calculations from the weight of starting material, dilutions, or surrogate correction so that comparisons between instruments are more easily identified. For individual OPAHs, there is excellent agreement (<20 % difference) for 9-FLUO, CP[def]PHEO, B[a]FLUO, and 5,12-NAPQ between the instrumental methods across all matrices tested. Furthermore there are less than 30 % differences for 9,10-ANTQ-D8 and 7,12-B[a]PHEQ. However, wide discrepancies exist for 2-ethANTQ, which shows poor agreement between the LC-APCI/MS and GC-EI/MS runs (>100 % difference). While overall concentrations in urban dust (SRM 1649b) differ by less than 3 % (GC-EI/MS: 2,513 ng mL^{-1}; LC-APCI/MS: 2,435 ng mL^{-1}), there is only reasonable agreement between the total sum of OPAHs between the two instruments (<50 % difference) due to differences with a few OPAHs as discussed above. Reasons for specific discrepancies between compounds could be due to matrix components that affect quantitation differently on each method.

Evaluation of Quantitation Strategies using Portland Harbor Superfund Passive Sampler Extract*:* All OPAHs that were identified in the original extract using internal standard (IS) quantitation were able to be successfully identified in the series of standard additions (SA). In total, 12 OPAH compounds are identified between methods, with a total of 10 from the LC-APCI/MS, and 8 from the GC-EI/MS (**Figure 2.5**). While all of the data presented in **Figure 2.5** is above instrumental detection limits, 8 of 10

standard addition values for the LC-APCI/MS method are below LOQs. No GC-EI/MS data shown in **Figure 2.5** are below LOQs.

Although conclusions for LC-APCI/MS data are difficult to make considering much of the data is below the LOQ, there are interesting comparisons between quantitation methods. For BANO, both quantitation methods result in values that differ by less than 5 % (SA: 67 ng mL^{-1}; IS: 70 ng mL^{-1}), indicating that there is no interference issues and excellent agreement between quantitation methods. Many other OPAHs have good agreement (differ by 30 % or less) between quantification strategies include AANEQ (29 %), 9,10-ANTQ+1,4-PHED (15 %), CP[def]PHEO (22 %), B[a]FLUO (5 %), 5,12-NAPQ (29 %), and 7,12-B[a]ANCQ (1 %) even though they are at or below LOQ. B[cd]PYRO does not show good agreement between quantitation methods (SI: 13 ng mL^{-1}; IS: 110 ng mL^{-1}). One likely explanation for this discrepancy is matrix enhancement of the 254 m/z ion, which would make the internal standard response higher over that of standard addition. Similar LC-MS matrix enhancement has been described in previous methods *(78)*. Enhancement is also seen for 7,8/1,6-B[a]PYRD while suppression is shown for 2-ethANTQ, both below the LOQ (**Figure 2.5**). The discrepancy for 2-ethANTQ could be ion suppression which is also common in LC-MS data *(78)*. Overall, there is excellent agreement between both quantitation strategies with 7 out of 10 compounds differing by less than 30 %. Because matrix interferences differ from sample to sample, SA quantitation is not usually employed. In this instance, IS quantitation seems reasonable for all but a few OPAHs. Less onerous strategies to improve quantitation accuracy could employ the use of more laboratory surrogates. Recoveries of both 2me-1,4-NQ-D8 and 9-FLUO-D8 were within 30 % of the true value, suggesting that recovery correction might only account for a partial resolution of discrepancies.

For the GC-EI/MS, quantitation methods had good agreement (differed by less than 30 %) for 9,10-ANTQ (11 %), B[a]FLUO (6 %), and FLUO (1 5%). CP[def]PHEO

differed by 35 % between SA and IS estimates. For the other four OPAHs, larger discrepancies exist with SA estimates higher than IS values for the individual OPAHs. The consistent trend on the GM-EI/MS instrument warranted further investigation since passing calibration check standards were analyzed prior to, and after this series of samples, and no obvious signs of suppression were present. The apparent suppression could have been due to either the silicone in the passive sampling device, or from interferences from the deployment in Portland Harbor itself. After simulating silicone background by extracting a non-deployed silicone sampler, results from both standard addition and internal standard quantitation suggest that 1,4-B[c]PHEQ, 7,12-B[a]ANCQ, 5,12-NAPQ, BANO were indeed suppressed (**Figure A.1.3**). Ongoing work in our laboratory is focused on reducing silicone background for passive sampling devices through additional solvent pre-cleaning prior to deployment, and through surrogate correction experiments. Recovery of both 2me-1,4-NQ-D8 and 9-FLUO-D8 was over 90 %, so these surrogates would not have corrected for 1,4-B[c]PHEQ, 7,12-B[a]ANCQ, 5,12-NAPQ, and BANO that were suppressed due to silicone background.

2.5 Conclusions

Improved sensitivities, optimization strategies, and the successful validation of two independent methods containing a large number of OPAHs were described in this work. By utilizing both systems, 24 target OPAHs were able to be quantified in addition to 4 deuterated compounds, with 19 compounds conserved in both methods. Despite surface chemistry difficulties in the injection port with some OPAHs (especially those compounds containing vicinal quinones), the GC-MS method is preferred over the LC-MS method for those compounds that were able to be successfully quantified. Obvious benefits include the additional ions used for identification purposes on the GC-MS method, which drastically reduce the likelihood of false positives that may be present in complex mixtures using a single quadrupole LC-MS. Additionally, the inter-day variability

of even clean standards tended to be less using our GC-MS method. Standard addition experiments showed potential suppression from the environmental sample that was later identified as coming from the silicone of a passive sampling device. Currently, there are very few labeled OPAHs commercially available, and this work highlights the need for improved laboratory surrogates for OPAHs. Ultimately, we hope separation and quantitation strategies provided in this work will provide improved sensitivity, accuracy and reproducibility for OPAH quantitation on LC or GC mass spectrometry instrumentation.

2.6 Acknowledgments

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Figure 2.1 Structures, abbreviations and CAS numbers for OPAHs used in the described methods

Table 2.1 LC-APCI/MS OPAH methods and validation parameters

OPAH Compound	LC/MS Parameters								
	SIM Ion (m/z)	Fragmentor Voltage (eV)	Retention Time (min)	Laboratory Accuracy at 500 ng mL^{-1} (n=17)[a]	Laboratory Variability % RSD (n=17)[a]	Lowest Calibration (ng mL^{-1})	AVG of Lowest Cal (ng mL^{-1}) n ≥ 5	IDL (ng mL^{-1})	LOQ (ng mL^{-1})
1,4-Benzoquinone*	108	140	4.12	760[b]	14[b]	10	11	9.7	49
Chromone	Not Detected	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
1,2-Naphthoquinone	158	75	15.84	590	15	10	11	9.6	48
1,4-Naphthoquinone	158	75	20.67	490	14	10	9.3	7.3	37
9-Fluorenone*	180	100	27.03	QO	QO	10	9.4	11	55
Perinaphthenone	Not Detected	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Acenaphthenequinone	182	80	21.87	510	18	10	9.8	8.5	43
Xanthone	Not Detected	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Cyclopenta[def]phenanthrenedione	204	90	29.10	520	28	10	16.2	11	55
9,10-Phenanthrenequinone	208	105	25.91	500	4.3	10	11	6.1	31
1,4-Anthraquinone	208	105	27.61	470	28	10	15	4.4	22
9,10-Anthraquinone	208	105	28.60	480	5.7	10	11	3.6	18
1,4-Phenanthrenequinone	208	105	28.60						
Benzanthrone	230	90	29.61	470	7.8	10	7.7	6.3	32
Benzo[a]fluorenone	230	90	30.56	460	11	10	8.7	9.4	47
Pyrene-4,5-dione	232	80	28.14	420	10	10	12	6.9	35
Aceanthracenequinone	232	80	28.71	670	31	10	6.0	15	75
2-Ethyl-Anthraquinone	236	110	30.92	440	10	10	12	9.6	48
6H-Benzo[cd]pyrenone	254	105	30.81	440	11	10	9.7	2.6	13
Benzo[c]phenanthrenequinone	258	110	29.62	470	8.2	10	11	5.5	28
5,12-Naphthacenequinone	258	110	31.40	430	16	10	10	26	130
Benz[a]anthracene-7,12-dione	258	105	32.03	470	11	10	8.5	10	50
Benzo[a]pyrene-7,8-dione	282	130	30.98	500	11	10	13	11	55
Benzo[a]pyrene-1,6-dione	282	130	30.98						
Deuterated Compounds and Internal Standard									
2-Fluoro-9-Fluorenone-9-^{13}C	199	80	27.90	IS	IS	IS	IS	IS	IS
1,4-Naphthoquinone-d6	164	70	21.27	410[c]	15[c]	DNM	DNM	DNM	DNM
2-Methyl-1,4-Naphthoquinone-d8*	180	100	24.43	730[c]	16[c]	DNM	DNM	DNM	DNM
9-Fluorenone-d8	188	80	27.17	630[b]	5.7[b]	10	8.3	26	130
9,10-Anthraquinone-d8	216	105	28.76	410	10	10	6.1	9.0	45

Table 2.1 (continued) LC-APCI/MS OPAH methods and validation parameters

[a] Replication presented here represents a set of samples (n=6) ran over 3 days.
[b] Replication presented here represents a set of samples (n=6) ran over 2 days.
[c] Replication presented here represents a set of samples (n=6) ran over a single day.
*Indicates a compound that was considered semi-quantitative for future experiments.
Table abbreviations are as follows: N/A - Not Applicable; QO - Qualitative only; DNM - Did Not Measure; IS - Internal Standard; SIM – Single Ion Monitoring; RSD – Relative Standard Deviation; AVG – Average; IDL – Instrument Detection Limit; LOQ – Limit of Quantitation

Table 2.2 GC-APCI/MS OPAH methods and validation parameters

OPAH Compound	GC/MS Parameters								
	SIM Ion (m/z)	Qualification Ions (m/z)	Retention Time (min)	Laboratory Accuracy at 500 ng mL^{-1} (n=18)[a]	Laboratory Variability % RSD (n=18)[a]	Lowest Calibration (ng mL^{-1})	AVG of Lowest Cal (ng mL^{-1}) n ≥ 5	IDL (ng mL^{-1})	LOQ (ng mL^{-1})
1,4-Benzoquinone	108	54, 82	4.75	550	5.6	5	5.8	0.49	2.5
Chromone	146	118, 92	11.62	510	4.7	5	5.7	0.89	4.5
1,2-Naphthoquinone*	130	102, 76	14.41	480	5.6	100	95	36	180
1,4-Naphthoquinone	158	130, 104	11.90	480	4.8	5	6.9	0.45	2.3
9-Fluorenone	180	152, 151	16.30	470	4.6	5	6.2	0.20	1.0
Perinaphthenone	180	152,151	18.81	490	5.1	5	5.3	0.89	4.5
Acenaphthenequinone	126	154, 182	18.23	450	9.1	50	54	11	55
Xanthone	196	168, 139	18.14	470	4.7	5	6.3	0.38	1.9
Cyclopenta[def]phenanthrenedione	204	176, 205	21.06	460	5.0	5	7.5	0.21	1.1
9,10-Phenanthrenequinone*	180	152, 208	23.15	500	8.1	50	51	1.5	7.5
1,4-Anthraquinone	208	152, 126	21.03	470	4.8	10	16	5.6	28
9,10-Anthraquinone	208	180, 152	19.89	470	4.9	5	7.9	6.9	35
1,4-Phenanthrenequinone	208	152, 126	19.83	460	5.1	10	11	0.86	4.3
Benzanthrone	230	202, 200	28.19	520	4.9	5	6.8	0.78	3.9
Benzo[a]fluorenone	230	200, 231	25.96	490	5.1	5	7.2	0.45	2.3
Pyrene-4,5-dione*	204	232, 176	28.79	440	9.3	50	52	11	55
Aceanthracenequinone	204	176, 232	28.65	440	7.7	250	250	27	135
2-Ethyl-Anthraquinone	236	221, 193	23.67	480	5.1	5	7.4	0.35	1.8
6H-Benzo[cd]pyrenone	254	226, 113	33.31	550	5.6	5	7.6	1.1	5.5
Benzo[c]phenanthrenequinone	229	258, 257	29.63	500	6.7	5	6.7	1.7	8.5
5,12-Naphthacenequinone	258	202, 230	30.80	510	7.6	5	7.6	1.3	6.5
Benz[a]anthracene-7,12-dione	202	258, 200	29.58	500	6.5	5	7.3	0.85	4.3
Benzo[a]pyrene-7,8-dione	Not Detected	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Benzo[a]pyrene-1,6-dione	Not Detected	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Deuterated Compounds and Internal Standard									
2-Fluoro-9-Fluorenone-9-^{13}C	199	170, 169	15.697	IS	8.7	IS	IS	IS	IS
1,4-Naphthoquinone-d6	164	136, 108	11.852	500	5.4	5.0	6.0	0.32	1.6
2-Methyl-1,4-Naphthoquinone-d8	180	152, 122	13.153	480	4.7	5.0	6.0	0.42	2.1
9-Fluorenone-d8	188	160, 158	16.236	490	4.6	5.0	5.8	0.18	0.90
9,10-Anthraquinone-d8	216	188, 160	19.81	450	4.4	5.0	7.2	0.65	3.3

Table 2.2 (continued) GC-EI/MS OPAH methods and validation parameters

[a] Replication presented here represents a set of samples (n=6) ran over 3 days.
*Indicates a compound that was considered semi-quantitative for future experiments
Table abbreviations are as follows: N/A - Not Applicable; QO - Qualitative only; DNM - Did Not Measure; IS - Internal Standard; SIM – Single Ion Monitoring; RSD – Relative Standard Deviation; AVG – Average; IDL – Instrument Detection Limit; LOQ – Limit of Quantitation.

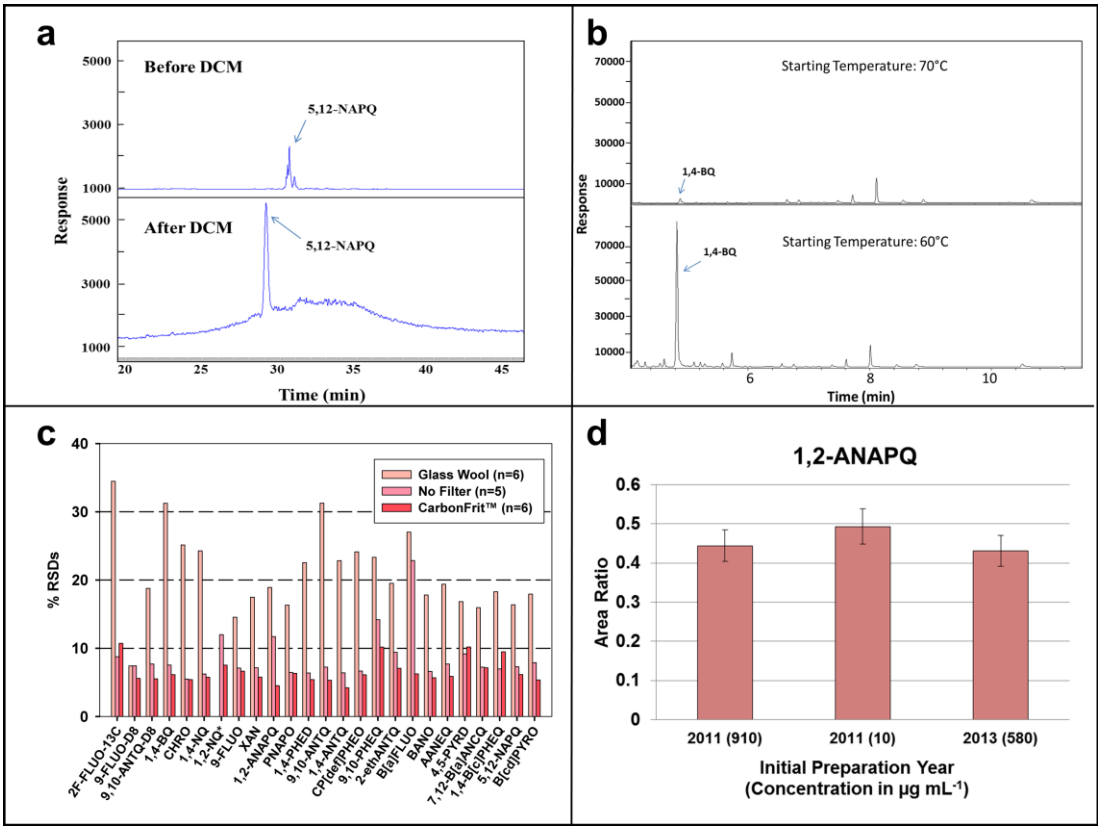

Figure 2.2 Results of analytical investigations of OPAHs: a) five-fold signal improvement after adding 1% dichloromethane (DCM) to LC-MS solvent system for 5,12-napthacenequinone, b) four hundred-fold enhanced peak response for 1,4-benzoquinone with an improved temperature profile in the GC-MS method, c) two to six-fold reduction in relative standard deviations between injection liner configurations, d) stability of acenaphthenequinone responses after internal standard normalization (area of target over the area of the internal) on the GC-EI/MS. Error bars correspond to instrumental variation from **Table 2.2**.

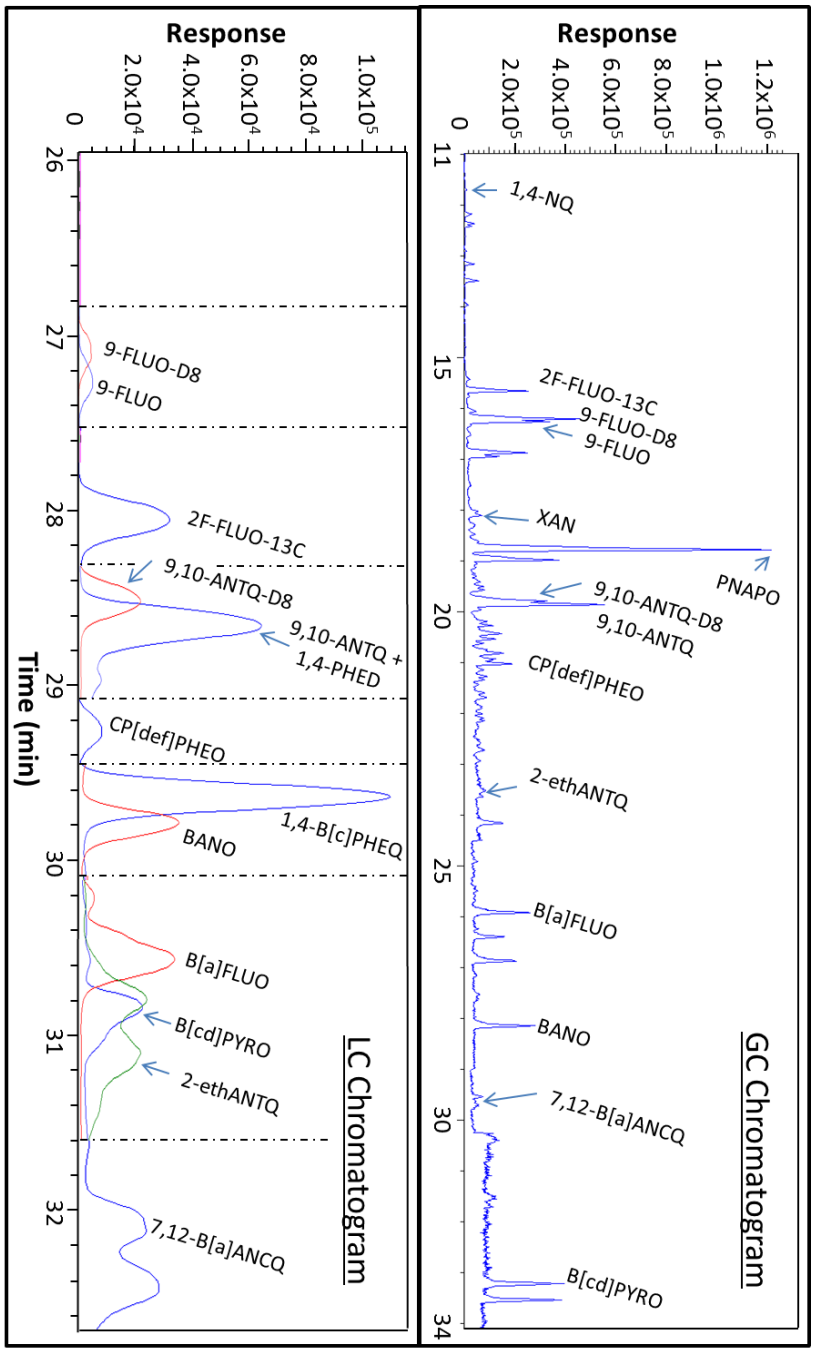

Figure 2.3 Chromatograms from both instruments for NIST SRM 1650b (diesel particulate matter). The GC chromatogram is displayed as a total ion chromatogram (TIC), while the LC figure is displayed as extracted ion chromatograms (EICs). The vertical breaks (dashed lines) in the LC chromatograph represent EICs from one or more ions during the run. The LC chromatogram has been scaled evenly between breaks in response (y-axis) and time (x-axis).

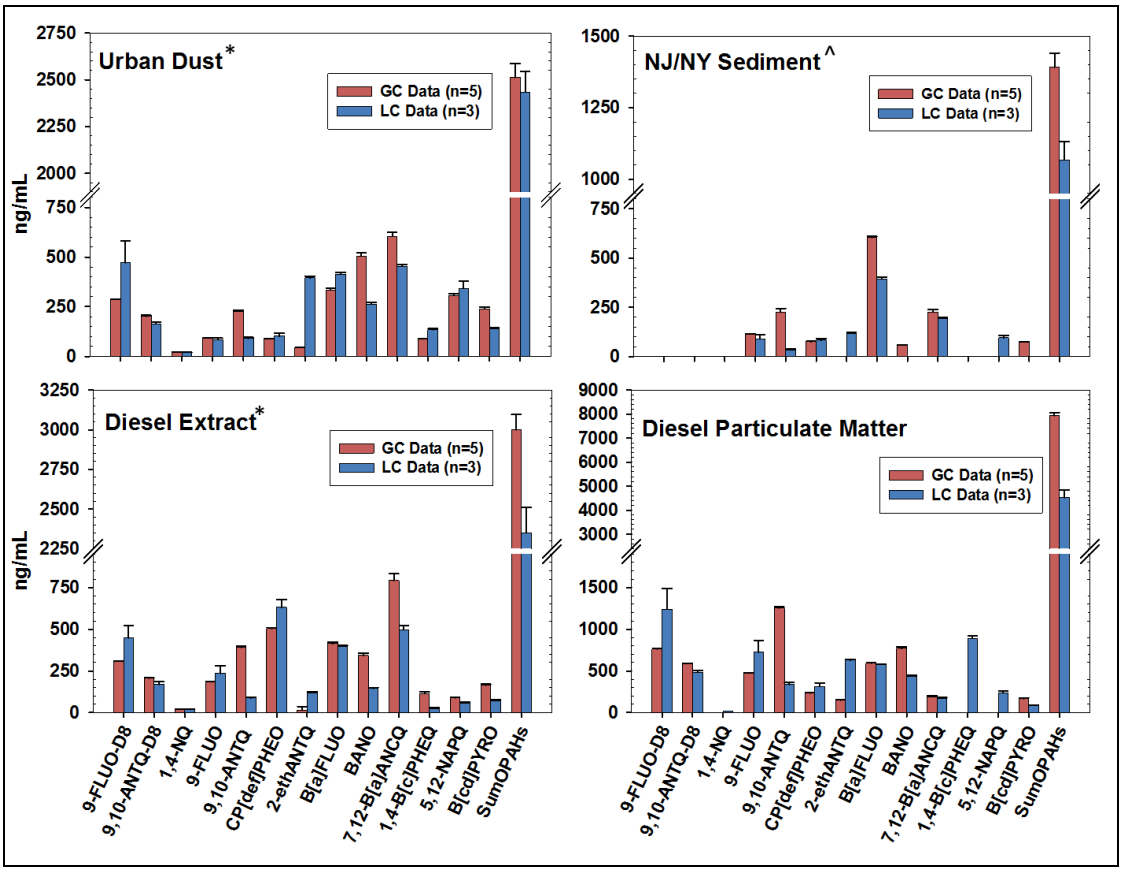

Figure 2.4 Comparison of GC and LC data for SRM extracted material. GC data is presented as the average and one standard deviation of 5 runs over 2 days, while LC Data is from 3 runs over 2 days. Only comparable data is represented here, target compounds that were unique to each system are not shown. No data has been corrected for recovery in order to directly compare differences between chromatograph systems. *Extract was diluted by 1/3. ^Extract was diluted by 1/10.

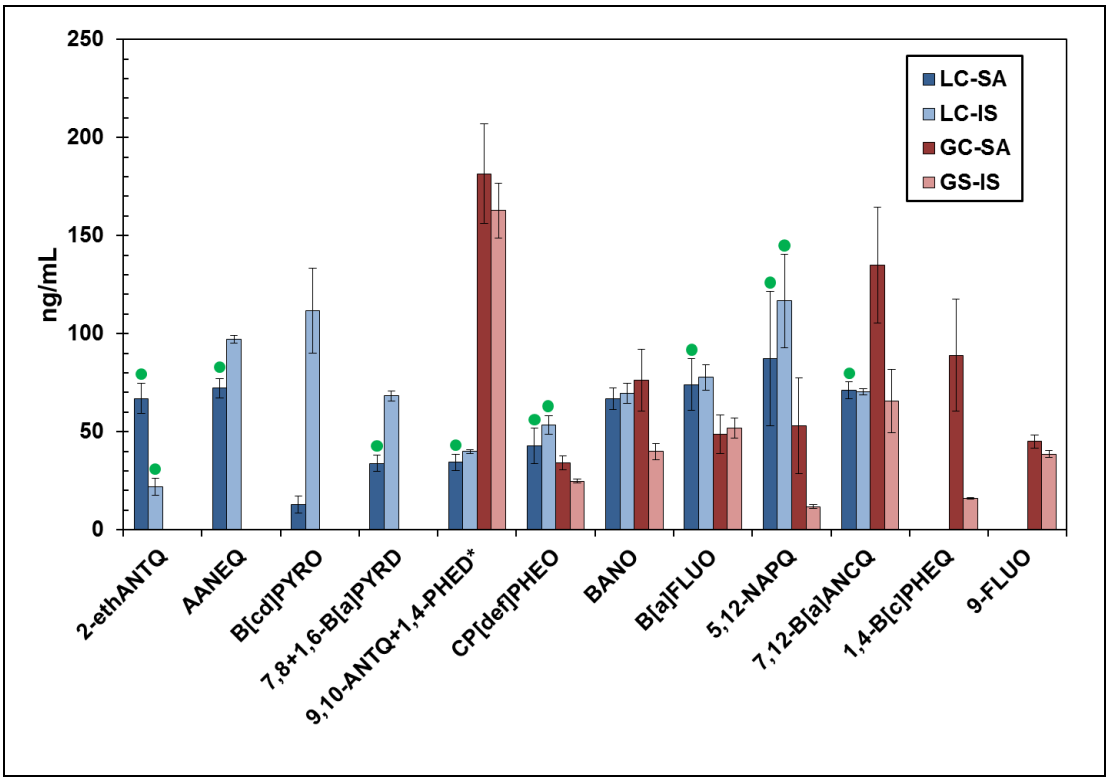

Figure 2.5 Comparison of standard addition and internal standard quantitation for both methods. Standard deviation for all values are the result of replication (n=3) on the instrument, with the addition of standard addition variability incorporating slope and intercept standard deviations from the linear regression performed for each analyte. Internal standard quantitation below LOQ is marked with a *circle*. If the lowest dilution of the standard addition series was below the limit of quantitation, the final value for that regression was also marked with a circle. Abbreviations: LC – Liquid Chromatography; GC – Gas Chromatography; IS – Internal Standard Quantitation; SA – Standard Addition Quantitation; LOQ – Limit of Quantitation (5 times the IDL). *denotes 9,10-ANTQ and 1,4-PHEQ co-elute on the LC-APCI/MS method, but are separated on the GC-EI/MS method. Values for the GC represent 9,10-ANTQ only.

Chapter 3 - Improvements in Pollutant Monitoring: Optimizing Silicone for Co-Deployment with Polyethylene Passive Sampling Devices

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3.1 Abstract

Sequestering semi-polar compounds can be difficult with low-density polyethylene (LDPE). By using silicone, pollutants can be targeted with lower log K_{ow} values. In this work, optimized methods for cleaning, infusing reference standards, and extraction are reported along with field comparisons of five silicone materials. In a final field demonstration, the most optimal silicone material is coupled with LDPE in a large-scale study to examine polycyclic aromatic hydrocarbons (PAHs) and oxygenated-PAH (OPAHs) in a Superfund site. OPAHs exemplify a sensitive range of chemical properties to compare polymers (log K_{ow} 0.2-5.3), and also represent transformation products of the more commonly studied parent PAHs. On average, while polymer concentrations differed nearly 7-fold, water-calculated values were more similar (about 3.5-fold or less) for both PAHs (17) and OPAHs (7). Individual water concentrations of OPAHs differed dramatically between silicone and LDPE, highlighting the advantages of choosing appropriate polymers and optimized methods for pollutant monitoring.

3.2 Introduction

Many benefits of passive sampling are practical, whether it is cost, ease of use over grab samples, concentrated extracts over diffuse matrices, or time-weighted averages over the deployment period *(30,35,37,79)*. Another important benefit is that passive sampling concentrations represent bioavailable contaminants in the sample media *(39)*. One challenge with passive sampling is choosing a receiving phase among the many diverse options that exist. For example, at least 22 different types of materials, sorbents, or solvents are reported as receiving phases for passive sampling in a recent review *(37)*. Some PSDs specialize in targeting polar or non-polar compounds, and some materials can be used in tandem with others to broaden the total range of sequestered compounds *(48,80,81)*. In addition to compound selectivity, considerations for using PSDs derive from previous development of uptake kinetics and published laboratory methods *(37,82)*. One of the most commonly used passive samplers is low-density polyethylene (LDPE) due to the low cost of the material, hydrophobic properties for targeting many persistent organic pollutants (POPs), and available partitioning and sampling rate estimates *(83-85)*.

However, LDPE does not sequester low K_{ow} compounds as well as another polymer, silicone *(48,82)*. Silicone has become an increasingly popular passive sampler in the past 10 years, and has been compared with LDPE to see differences in sequestration of target chemicals *(47,48)*. Although quantitative comparisons between aqueous concentrations were similar (< 2 to 3-fold among PAHs and PCBs), a dramatic increase of absorbed analytes was seen using silicone over LDPE for compounds with log K_{ow} values lower than 6 *(48)*. More efficient absorption of analytes into the polymer can result in several advantages, including greater flexibility in deployment times, lower detection limits, and applications to bioassays due to greater concentrations in samples. Sequestration differences between LDPE and silicone can be partially explained by the structural make-up of each polymer. Silicone is made of a silicon-oxygen backbone with

various functional groups bonded to silicon such as methyl, phenyl, vinyl, or fluoro constituents *(35,82)*. In contrast, LDPE consists of carbon and hydrogen *(82)*, which gives this polymer a more hydrophobic property. Ultimately, both polymers yield more accurate data than other passive sampling devices *(47)*, so advantages of using one or the other depend on targeted compounds. Unlike previous comparisons of silicone and LDPE which focused on POPs *(47,48)*, this research includes compounds that are transformation products of pollutants. One class of compounds that is well suited for a comparison between silicone and LDPE is oxygenated-PAHs (OPAHs). OPAHs are degradation products of PAHs *(9)*, and are emerging contaminants of interest that have log K_{ow} values less than 6 for 22 previously studied compounds *(86)*. Other classes of pollutants, such as pesticides, span a wide range of chemical properties that are also be beneficial for polymer comparisons. By addressing data gaps through monitoring emerging compounds of interest using passive samplers, this research can highlight differences in silicone and LDPE and ultimately assess a greater range of contamination. Considering the chemical structural of the silicone polymer and previous evidence illustrating polymer differences in sequestration, silicone should sequester higher concentrations and a greater range of OPAH compounds than LDPE.

Therefore, our objective focused on three aims: first, to optimize silicone cleaning methods to reduce background chromatographic interferences, and to infuse silicone with labeled internal standards for uptake rates and water concentration estimates. Second, we compared five silicone polymers in a field application at a Portland Harbor Superfund site with a history of POP (including PAH and pesticide) contamination *(33,43)*. Sequestration data was used to select silicone polymers best suited for co-deployment studies with LDPE. Finally, the optimal silicone was compared with LDPE for PAHs and OPAHs. By optimizing silicone passive samplers to be used in tandem with LDPE, this work provides a field validated method for quantification of a wide range of contaminants including PAHs, OPAHs, and pesticides.

3.3 Methods

Analyte information

OPAH, PAH, and single pesticide standards were bought from Sigma Aldrich (St. Louis, MO), Chiron (Trondheim, Norway), and Fluka (part of Sigma-Aldrich). Pesticide and PAH mixes were purchased from Accustandard (New Haven, CT). Labeled standards used as performance reference compounds (PRCs), laboratory surrogates, or instrument internal standards were obtained from either CDN Isotopes (Pointe-Claire, Quebec, Canada), or Cambridge Isotope Laboratories (Tewksbury, MA). A complete list of all quantitative analytes including surrogates, PRCs, and internal standards is given in Appendix 2 (**Table A.2.1**). All solvents were Optima-grade or equivalent (Fisher Scientific, Pittsburgh, PA), and all laboratory water used for infusions or post-deployment cleaning was filtered through a Barnstead D7389 purifier (Dubuque, IA).

Polymer construction

A total of five silicone polymers were purchased from three companies: Stockwell Elastomerics Inc. (Philadelphia, PA), Altec Products Limited (Bude, Cornwall, UK), and CS Hyde Company (Lake Villa, IL) (**Table 3.1**). Silicone was purchased in square-yard rolls, and strips were cut from the sheet using a table cutter/trimmer (Fletcher-Terry Company, Farmington, CT). The AteSil™ silicone was purchased as talc-free silicone in 30 x 30 cm sheets. Three AteSil™ strips were cut and used together to obtain approximate dimensions of the other polymer strips. All deployed strips were of similar dimensions, approximately 91 cm x 2.6 cm, although thickness differed between materials (**Table 3.1**). Random subsets of strips were weighed during construction to determine variability, and averages were used to normalize absorption data (**Table 3.1**). Polyethylene strips were cut from pre-sized layflat tubing (width approximately 2.7 cm) at 110 cm, and loops were formed on each end for deployment after heat-sealing each end. Total length of LDPE strips were approximately 100 cm.

Laboratory optimization: pre-cleaning, infusion, post-deployment cleaning, and extraction

Before deployment, silicone was cleaned with solvents to remove chromatographic interferences from the curing process of the polymer *(82)*. Initially, silicone extraction and pre-cleaning experiments used ethyl acetate since it does not severely impact the integrity of the silicone itself *(82)*, and ethyl acetate is a solvent with both polar and non-polar properties that might be conducive to OPAHs. In addition, to exploit inherent differences in each polymer, ethyl acetate was used as the primary solvent for silicone, while the more non-polar hexane was used for LDPE cleaning as previously described *(83)*. Pre-cleaning experiments for silicones were adapted from several studies *(44,53,82,84,87)*. Ultimately, sufficient reduction of siloxane background was only achieved with a combination of three extraction periods of 1:1, hexane:ethyl acetate, followed by two more periods of 1:1 methanol:ethyl acetate. Roughly 65 g of silicone was placed into an amber jar (1L) before the mixed solvents were added to fill each container. Each extraction period was at least 2 hours, but no more than 14 hours (overnight). Samples were shaken at approximately 60 rotations per minute (rpm) in a water bath at 40 °C (New Brunswick Scientific, Edison, NJ). Once an adequate pre-cleaning method was finalized, a secondary experiment evaluated the effectiveness of using recycled solvents to reduce waste. Silicone strips were cleaned with a portion of solvents re-used from a previous exchange (see **Appendix 2, Figure A.2.1** for more details). All polymers were dried under filtered vacuum in either sealed glassware or stainless steel kegs (AEB Kegs, Delebio SO, Italy).

Deployed polymers for the final comparison were spiked with performance reference compounds (PRCs) used to estimate *in-situ* sampling rates in order to calculate water concentrations *(84,88)*. Infusion solutions were modified to a 50% mixture of methanol/water rather than 80% used in a previous study *(84)*. Increasing the water

content increases the fugacity of the PRC compounds into the silicone, and reduces the total amount of compound needed for the infusion process. Briefly, 5-6 strips (or 60-90 g of silicone) were placed into a 1 L amber glass jar and filled with 750 mL of methanol/water (1:1, v:v). PRC compounds were spiked into the solution and allowed to equilibrate with the silicone for 3 days at 60 rpm and 40°C. Fluorene-d10, benzo[b]fluorenthene-d12, p,p-DDE-d4, and 9,10-anthraquinone-d8 were used for both silicone and LDPE, and spiking concentrations were adjusted for differences in partitioning, polymer mass, and length of deployment *(84)*. Polyethylene was infused with PRCs at 4 to 100 µg per strip directly spiked within the tubing before sealing the other end of the strip.

After deployment, each polymer was cleaned with ambient waters to remove any surface sediment or biological material on the polymer (**Figure 3.1**). Once in the laboratory, silicone was rinsed further with filtered water and isopropanol, while LDPE was rinsed with water, dilute hydrochloric acid, and isopropanol based on previous work *(41)*. Post deployment-cleaned strips were stored at -20 °C until extraction. Laboratory surrogates (**Table A.2.1**) were spiked into amber jars at 500 ng/mL before extraction. Individual silicone strips were extracted with two sequential rounds of 100 mL of ethyl acetate on an orbital shaker set at 60 rpm (ambient temperature), and the total extraction time was 18 hours. LDPE was extracted with hexanes in a similar fashion *(83)*. All extracts were quantitatively concentrated to 1 mL using closed cell evaporators (TurboVaps®, Biotage, Charlotte, NC), and transferred to chromatography vials. Extracts were stored at 4 °C until analysis.

Site characterization

Portland Harbor Superfund is located in downtown Portland, OR, and stretches approximately nine miles along the Willamette River. Contaminants of concern at this site include PCBs, dioxins, PAHs, pesticides, and heavy metals *(89)*. In 2010, five silicone

polymers were deployed at river mile (RM) 3.5 west (W), while in 2011, just three silicones were deployed along with LDPE (**Figure 3.2**). Both 2010 and 2011 deployments included RM 3.5W (**Figure 3.2**, yellow star). Deployment took place from September 2-30, 2010 (28 days), and September 1- 22, 2011 (22 days). Water cages were purchased from Environmental Sampling Technologies, Inc. (St. Joseph, MO) with all polymers co-deployed within the same cage, and multiple cages deployed at each site in both sampling years. The deployment system consisted of an anchor, steel cable, water cages, and two buoys: one for buoyancy, and another on top for retrieval *(43)*. Each cage was approximately 2.5 meters off the bottom of the river.

Analytical methods

Specific method details can be found for PAH *(41)*, OPAH *(86)*, and pesticide *(90)* analyses published previously. Internal standards for each method were spiked into extract aliquots just prior to instrumental analyses. A gas chromatograph (GC) with an Agilent DB-5 column (30 m length, 0.25 mm inner diameter, 0.25 µm film thickness) was used to analyze OPAHs and PAHs (2010 deployment), while an Agilent DB-XLB (30m, 0.25mm, 0.25 µm) and a DB-17MS (30m, 0.25mm, 0.25 µm) was used to analyze pesticides with dual column confirmation *(90)*. An Agilent Select PAH column (30m, 0.25mm, 0.15 µm) was used for PAHs in the 2011 deployment. The OPAH and PAH methods used mass spectrometry (MS) detection (model 5975B, Agilent), while the pesticide method utilized dual electron capture detection (model 6890N, Agilent). All compounds were calibrated with calibration curves of five points or more, and had correlations of 0.99 or better. Contaminant screening for additional compounds was performed with GC/MS retention time locking Automated Mass Deconvolution Identification Software (AMDIS) in conjunction with created and purchased libraries totaling 1,180 unique compounds. Identification and confirmation criteria has been described previously *(91)*, but each compound had at least a 60% spectral match before additional confirmation criteria were used for each qualitative determination.

Quality Control

Including field, laboratory, cleaning, and instrumental blanks, over 40% of the analyzed samples were for quality control (QC) purposes. During polymer construction, at least two strips were analyzed from each batch to assess adequate removal of chromatographic interferences. If the highest background peak had an area less than 15 fold of a 500 ng/mL spiked internal standard, then that background level was considered adequate. Both strips had to pass this criterion to allow a polymer batch to be used. Each trip to Portland Harbor included field blanks to monitor contamination from travel or field processing. During post-deployment cleaning, non-deployed strips were used to monitor any contamination prior to freezer storage (-20 °C). When samples were extracted, laboratory reagent blanks accompanied each batch or day of extraction. The final type of QC samples was a verification standard, which included all target compounds for the appropriate method. Compounds were verified +/- 20% of the true value for at least 90% of the target list before samples were analyzed. The reporting limit was set as the average of all blank samples from field and laboratory plus three times the standard deviation. Concentrations below the reporting limits were not included in results.

Calculated Water Concentrations

Typically for LDPE and silicone, sampling rates (R_s) are determined through *in-situ* calibration with PRCs *(84,88)*. Partition coefficients (K_{sw}) for PAHs were obtained from the literature for LDPE *(85)* and silicone *(92)*. Because there is not a compound specific model for estimating OPAH partition coefficients, PAH models based on K_{ow} were used since that parameter would be more sensitive to OPAH compounds than molecular weight (ex: log K_{ow}s: OPAHs - 0.2 to 5.3; PAHs – 3.3 to 7.3, MW: OPAHs - 108 to 282; PAHs – 128 to 302). All partition coefficient models have above 0.88 r^2 correlations whether K_{ow} or molecular weight chemical parameters were used *(85,92)*. For LDPE

sampling rate (R_s) estimates, we used an empirical uptake theory with compound-specific adjustments for target compounds *(32)*. This model was originally based on LDPE filled with triolein (called semi-permeable membrane devices, or SPMDs), but previous work showed little differences in sampling rates between SPMDs and LDPE *(93)*. Silicone R_s values were estimated from an empirical model as well *(94)*. Final water concentrations were determined making no assumptions about stages of uptake at time of retrieval *(32)*. Additional details and final equation are in Supporting Information.

3.4 Results and Discussion

Laboratory Optimization of Silicone

Silicone background was ultimately reduced to similar levels as in LDPE, but the process was iterative (see Appendix 2 for more details). Although polymer cleaning results are rarely reported, this methodology compares well with others that rely on solvent exchanges *(53,82,84)*, and it is faster (≤ 48h) than soxhlet extraction methods (90h) *(52,87)*. Moreover, solvents could be effectively recycled (both hexane/ethyl acetate and methanol/ethyl acetate mixtures) between batches of silicone (**Figure A.2.1B**). By using this recycled solvent, the total solvent use is reduced by 20% (**Figure A.2.2**). In addition, the background of SS silicone utilizing the recycled solvent method was similar to LDPE (**Figure A.2.1B** – green and black chromatograms, respectively). An additional benefit is that the optimal background is achieved without relying on post-extraction silica cleanup used in other silicone work *(44,52,84)*, and the silicone extract can be easily integrated into zebrafish bioassays (manuscript in preparation).

The PRC infusion process resulted in excellent precision across different strips and batches, with average relative standard deviations (RSDs) of <13%. This repeatability compares well with other published infusion RSDs of 10% or less *(84)*. The efficiency of the infusion after modifying the methanol/water ratio was calculated by

dividing the average amount in the silicone by the amount in the initial infusion solution. Infusion efficiencies of PRCs ranged from 20 ± 7 % (9,10-anthraquinone-d8) to 111 ± 11% (p,p'-DDE-d4), indicating that the infusion process was successful transferring most, if not all, of the compounds into silicone strips. Extraction efficiencies were not measured in this study, but assumed to be adequate and similar to the 96% extraction efficiency reported using other silicone with the same methodology for PAHs *(91)*.

Initial Field Comparison of Five Silicones

During field retrieval, all silicone polymers had minimal biofouling after a few seconds of physical agitation with ambient water (**Figure 3.1C-G**). In total, 25 PAHs were identified among all polymers (**Figure 3.2**). Polymers were first compared using PAHs due to analytical methods available at that time, and since PAHs are still contaminants of concern in Portland Harbor *(33)*. Concentrations were normalized for each silicone by mass, and AA sequestered roughly 2 fold more ΣPAHs than other silicone polymers (**Figure 3.2**). However, both AA and CT silicone were heavily degraded during the extraction process, leaving behind silicone residue in both glassware and instrumentation. The leftover residue likely resulted in the very low PAH surrogate recoveries seen for both AA (2-11%) and CT (2-43%), which contrasts with the higher recoveries seen with ST (70-130%), SS (62-138%), and CS (73-130%). Despite the common use of AA as a silicone PSD *(82,87,92,94)*, the other types of silicone were substantially easier to extract, and resulted in better recoveries and precision of analytes (**Figure 3.2**). The siloxane background of AA and CT also interfered with full scan analyses. Therefore, only ST, SS, and CS were further evaluated for qualitative sensitivity of low K_{ow} compounds which can be seen in the Supporting Information. Overall, 30 compounds were identified between polymers (**Table A.2.2**), and LDPE did not sequester any compounds below a log K_{ow} value of 4.9, which is similar to previous field data *(47,48)*. Because sequestration was similar between the three silicones and advantages

among them were not immediately apparent, ST, SS, and CS were deployed along with LDPE the following year.

Ideal Silicone Polymer Selection and PAH Comparisons with LDPE

Six different field sites were sampled using LDPE, SS, ST, and CS polymers to further assess and compare silicones to LDPE. Samples were analyzed using quantitative methods for PAHs, OPAHs, and pesticides (see **Table A.2.1**). Laboratory surrogate recoveries after extraction varied widely among each method. For silicones, pesticide recoveries ranged from 13-113%, averaging 60%. PAH recoveries ranged from 35-185%, and averaged 94%. OPAH recoveries performed well, ranging from 72-140% with an average of 105%. Recoveries of surrogates in LDPE extractions were similar for pesticides (34-86%) and PAHs (30-98%), and like silicone, the best recoveries were for OPAHs, which ranged from 69-110% with an average of 88%. Lower recoveries (outside of ± 30% of the true value) were almost always associated with more volatile surrogates. For instance, most low recoveries for PAHs were for naphthalene-d8, and for pesticides, all low recoveries were attributed to tetrachloro-m-xylene. Additional variability may be due to the study size (> 70 samples) and multiple weeks of extractions. However, most recoveries were within 30% of the true value, and for comparison purposes, recoveries were similar for each method across polymers, so concentrations were assumed to be affected similarly across the values reported below.

Concentrations of three pesticides identified in Portland Harbor predictably differed between LDPE and silicone based on log K_{ow}, and were consistent with earlier AMDIS results. Specifically, **Figure 3.3** illustrates all silicones having greater amounts sequestered (ng/g PSD) of endosulfan sulfate (log K_{ow} 3.7) compared with LDPE. This is in contrast with p,p'-DDD, which was greater in LDPE and more hydrophobic (log K_{ow} 6.0). Chlorpyrifos was more variable among the polymers (**Figure 3.3**), with a log K_{ow} (4.96) value between that of the other compounds. One goal of this research was to develop

samplers for co-deployment, and the pesticide and AMDIS data suggests that the methodology successfully exploited inherent differences in polymers initially reported in other work *(48,82)*. While ST had the highest amount of endosulfan sulfate sequestered in the polymer, it was more difficult to use in the field and laboratory due to a tendency to adhere to metal and glass surfaces when dry, and had higher variability within a complementary range of K_{ow} sequestration (**Figure 3.3**). Therefore, SS silicone was chosen as the best silicone coupled with LDPE since it had the highest precision across all field testing and chemical compound classes. However, it is acknowledged that there is little difference between either silicone sponge material overall (SS or CS).

In the final comparison using SS silicone and LDPE, PAH data was evaluated to see if differences in absorption or extraction methodology would be reconciled after calculations to water concentrations. In **Figure 3.4A**, ΣPAH concentrations in the SS silicone polymer (ng/g) are about 7 fold lower than in LDPE. While acknowledging differences in solvents which could impact extraction efficiency, this was surprising considering previous evidence showing much higher concentrations of PAHs in silicone over LDPE *(48)*. Regardless, overall differences were reconciled to average 3.5-fold or less (individual or ΣPAH) once both polymer extracts were calculated to water concentrations in ng/L (**Figure 3.4A**). Moreover, this nominal 3-fold difference is consistent with other PAH silicone and LDPE data from aqueous field deployments *(48)*. LDPE is clearly the better polymer for PAHs using this methodology, as it sequesters PAHs at higher concentrations and likely has more accurate results than silicone given the polymer-specific partition coefficients for polyethylene. Although silicone partitioning coefficients were shown to vary little (on a log scale) in a multi-silicone comparison *(82)*, small differences in these estimates could explain the gap in quantitation for this work. Future work would be improved by empirically determining K_{sw}s for SS silicone. Overall, both polymers consistently sequestered 17 PAHs of varied molecular size and physicochemistry (log K_{ow} range: phenanthrene – 4.56 to

indeno(1,2,3-c,d)pyrene - 6.58). The ratio of individual analytes was conserved between polymers, with phenanthrene, fluoranthene, and pyrene comprising the majority of ΣPAH amounts (56 to 84% in silicone, 67 to 81% in LDPE). Although LDPE sequesters more PAHs, both polymers in this field study would have resulted in similar descriptions of field sites if used solely for Portland Harbor characterization. Both polymers showed elevated levels of PAHs within the Superfund (sites RM 3E to 7W) as compared to outside the area (Columbia and RM 14W sites, **Figure 3.4A**). Results of replication are also similar (**Figure A.2.3A**), with RSDs averaging 7% for LDPE and 11% for SS silicone across field sites.

Final Polymer Comparisons with Emerging Oxygenated-PAHs

OPAHs are an emerging concern in PAH contaminated areas *(9)*, and represent a good example of the physicochemistry range that might be sensitive to differences between silicone and LDPE (log K_{ow} 0.2 – 5.3). The data in **Figure 3.4B** represents some of the first aqueous concentrations of OPAHs at a Superfund site using either LDPE or silicone passive samplers, and this data represents some of the first evidence showing these compounds having similar magnitudes to PAHs in an aqueous concentration (**Figure 3.4**). Comparisons between ΣPAH and ΣOPAHs have been shown to be similar among several other matrices *(18)*, and in a very recent publication, concentrations of 9-fluorenone and 9,10-anthraquinone were found to be higher than corresponding PAH homologues in waste, river, and effluent waters *(95)*. Overall, ΣOPAHs are similar for both polymers, and 4 out of 6 sites do not significantly differ ($p \geq 0.44$ from t-tests, see **Figure A.2.3B**). In contrast to PAHs, the amount of OPAHs sequestered in each polymer is similar despite the log K_{ow} range from 3.4 (9,10-anthraquinone) to 4.8 (5,12-naphthacenequinone) (**Figure 3.4B**). While it has long been demonstrated that K_{ow} alone cannot account for uptake differences observed in model passive samplers *(96-98)*, the use of additional parameters to more accurately and precisely model uptake has been elusive. As an example, out of several physiochemical parameters (molecular weight, polar surface

area, Van der Waals volume, C:H ratio), a regression using K_{ow} to predict OPAH sequestration has a model coefficient (R^2) of just 0.08, while one using a ratio of Van der Waals volume over the polar surface area is a slightly better predictor of partitioning (R^2 = 0.20, see **Figure A.2.4**). Clearly, more work is needed to predict absorption between polymers, but future studies might benefit from using these or other physiochemical parameters.

Perhaps the most interesting OPAH results are differences observed between polymers for individual OPAH concentrations. Specifically, benzofluorenone and 7,12-benz[a]anthracenequinone (**Figure 3.4B**) differed dramatically between polymers by 50% and 109%, respectively. Differences persist even after calculating water concentrations, but determining specific partitioning coefficients might rectify some of these discrepancies. Like the ΣPAHs, the ΣOPAHs from either polymer suggest higher concentrations within the Superfund site than outside of it. However, individual contributions to these ΣOPAHs would indicate that 9,10-anthraquinone and benzofluorenone would be the primary components based on LDPE results (averaging 83% of the total), while no individual OPAH comprised more than 25% of ΣOPAHs in SS silicone. Four to five individual OPAHs are needed to achieve 83% or more of ΣOPAHs in the silicone at any site. In this respect, the original hypothesis is supported because silicone sequesters more individual OPAHs than LDPE. In fact, fluorenone and 5,12-napthacenequinone are below the reporting limit for LDPE at all sites except RM 3E. The difference between polymers is critical, because early evidence suggests that there are large differences between individual OPAH toxicities *(99)*. If remediation or toxicity concerns are important at a contaminated area, then methods that capture a large range of individual OPAHs will have additional value. Ultimately, both PAHs and OPAHs were quantitated using both polymers, but silicone appears more appropriate for OPAHs given the greater sensitivity for individual compounds (especially 9-fluorenone, benzanthrone, and 5,12-naphtacenequinone). In total, this work advances methods for

using silicone passive samplers alone or in conjunction with LDPE, provides information on analytical criteria for passive sampling choices, and provides valuable real world OPAH information for this emerging compound class.

3.5 Acknowledgements

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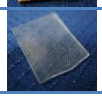
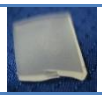
Table 3.1 Silicone and LDPE polymers by manufacturer, abbreviation, and physical information

Supplier	PSD Material (Abbreviation)	Depiction	Strip Surface Areas (cm^2)	Strip Volume (cm^3)	Strip Weight (n = 5, g)
Stockwell Elastomerics	Silicone Sponge (**SS**)		~480	~18	15.5 ± 1.4
Stockwell Elastomerics	Thin Translucent Sheet (**ST**)		~480	~7.2	7.87 ± 0.34
CS Hyde	Commercial-Grade Sponge (**CS**)		~480	~18	17.6 ± 0.10
CS Hyde	Translucent Sheet (**CT**)		~480	~18	27.9 ± 0.26
Altec Products Limited	AlteSil™ (**AA**)		~470	~11	15.4 ± 0.33
Brentwood Plastic, Inc.	Low density polyethylene (**LDPE**)		~540	~5.1	4.82 ± 0.08

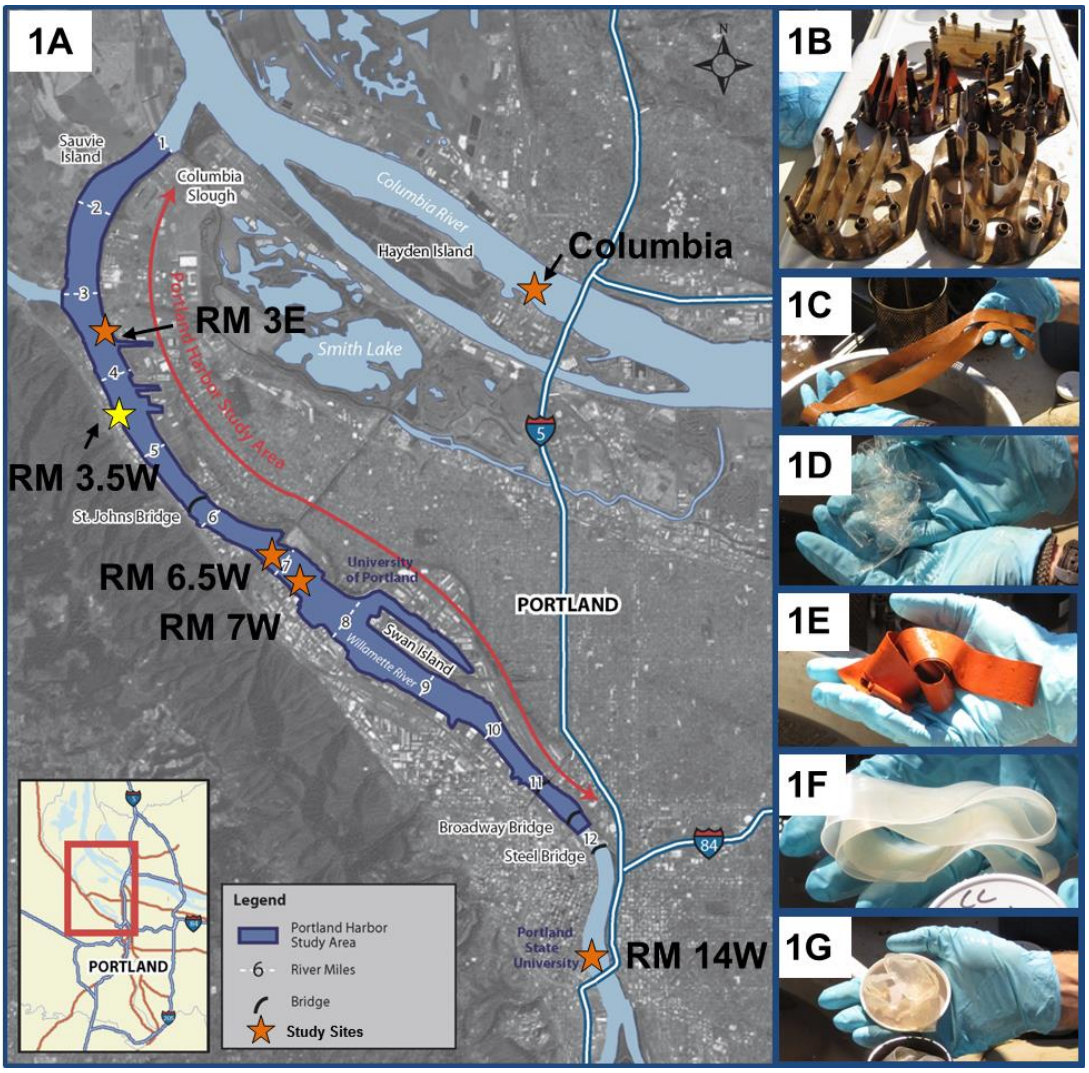


Figure 3.1 Field deployment of multiple polymers in Portland Harbor Superfund, OR: 1A) *stars* represent field sites in and outside of the Superfund in 2011, and the *yellow star* (RM 3.5W) was a deployment site in 2010 and 2011; 1B) silicone polymers immediately after deployment in 2010; 1C-G) polymers after deployment before storage in amber jars: SS, ST, CS, CT, AA, respectively, see **Table 3.1** for abbreviations. Map of Portland Harbor Superfund courtesy of U.S. Environmental Protection Agency.

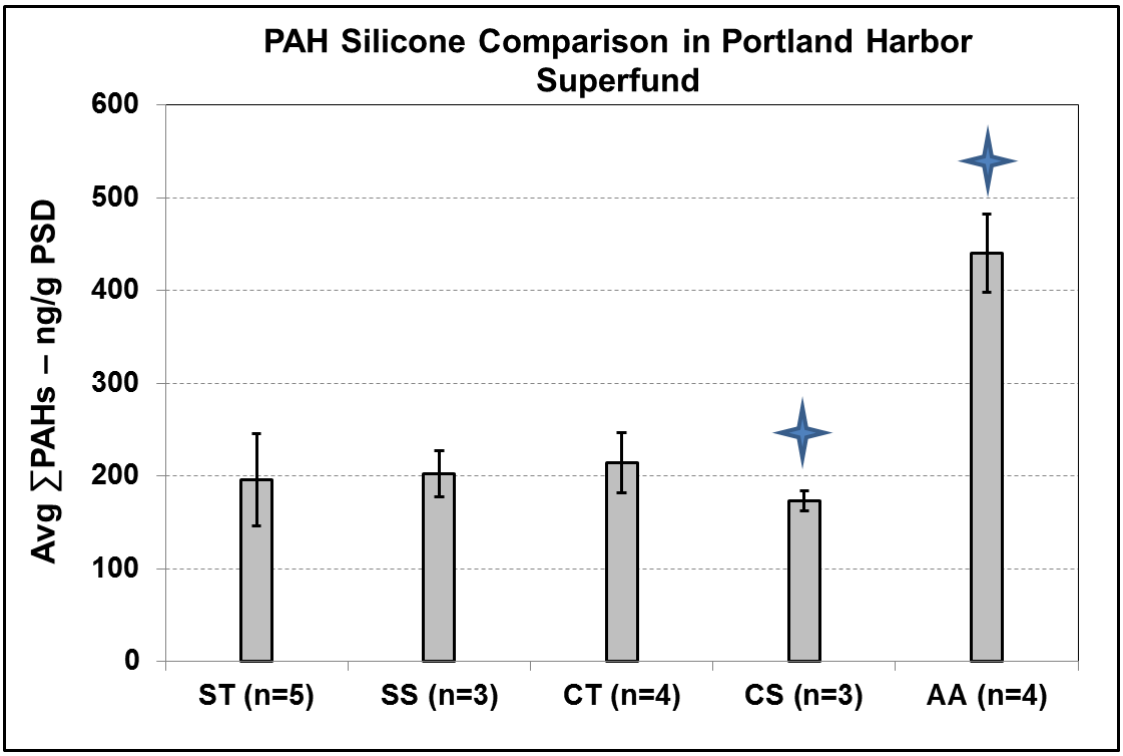


Figure 3.2 Average summation of 25 PAHs from all silicone in 2010 at RM 3.5W. Concentrations were normalized to polymer mass to highlight differences between silicone polymers. Blue stars indicate severe degradation of polymer during extraction.

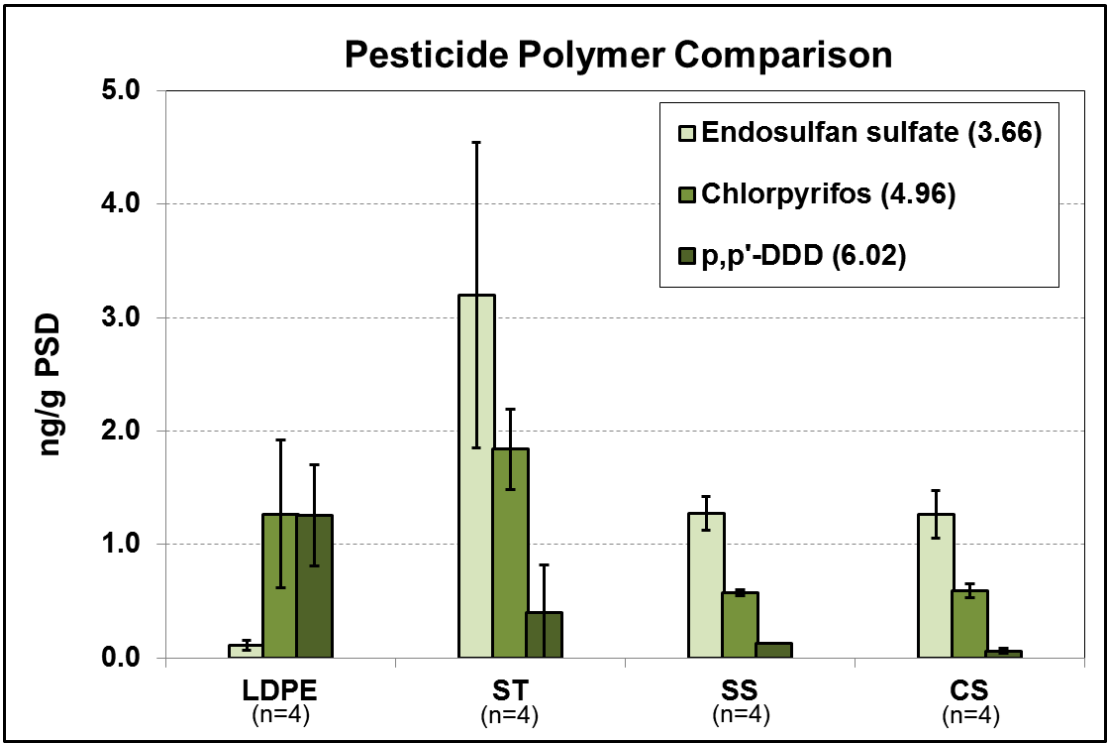


Figure 3.3 Average concentrations of three pesticides found in Portland Harbor RM 14W, 2011. Concentrations are normalized per mass of each PSD to highlight sequestration differences. Numbers in parentheses after compound names represent log K_{ow} values.

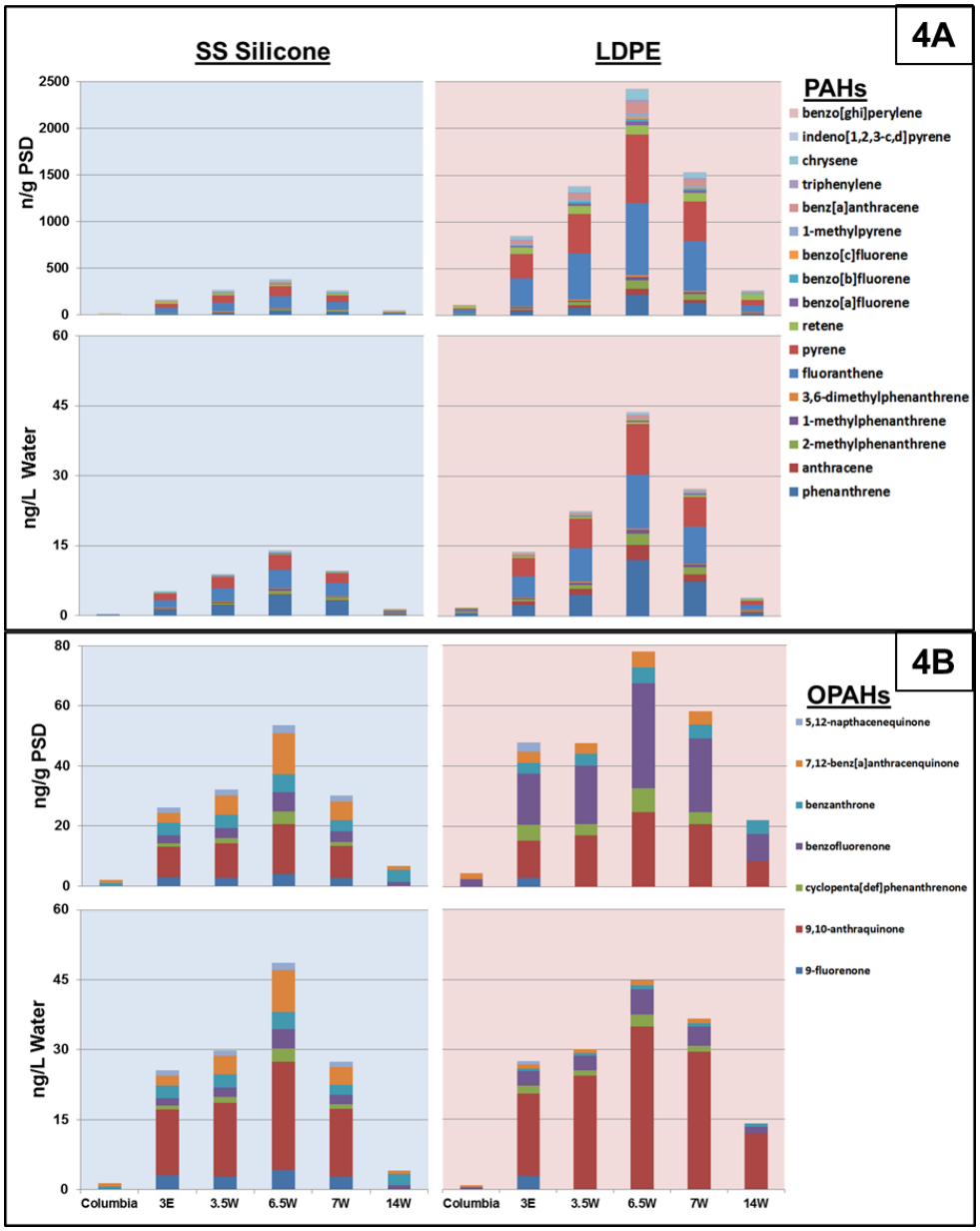


Figure 3.4 Average (n=3) individual PAH and OPAH concentrations before (ng/g PSD) and after water calculations (ng/L) for SS Silicone (blue background) and LDPE (light red background) for six Portland Harbor sites in 2011. 4A) PAH concentrations are consistently higher in LDPE before and after back-calculation although individual contributions are similar. 4B) OPAH concentrations are more similar than PAHs before and especially after back-calculation between polymers, although individual contributions are more disparate. Fluorenone and 5,12-napthacenequinone are below the reporting limit for LDPE at all sites except RM 3E.

Chapter 4 - Silicone Wristbands as Personal Passive Samplers

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4.1 Abstract

Active-sampling approaches are commonly used for personal monitoring, but are limited by energy usage and data that may not represent an individual's exposure or bioavailable concentrations. Current passive techniques often involve extensive preparation, or are developed for only a small number of targeted compounds. In this work, we present a novel application for measuring bioavailable exposure with silicone wristbands as personal passive samplers. Laboratory methodology affecting pre-cleaning, infusion, and extraction were developed from commercially available silicone, and chromatographic background interference was reduced after solvent cleanup with good extraction efficiency (>96%). After finalizing laboratory methods, 49 compounds were sequestered during an ambient deployment which encompassed a diverse set of compounds including polycyclic aromatic hydrocarbons (PAHs), consumer products, personal care products, pesticides, phthalates, and other industrial compounds ranging in log K_{ow} from -0.07 (caffeine) to 9.49 (tris(2-ethylhexyl) phosphate). In two hot asphalt occupational settings, silicone personal samplers sequestered 25 PAHs during 8- and 40-hour exposures, as well as 2 oxygenated-PAHs (benzofluorenone and fluorenone) suggesting temporal sensitivity over a single work day or week ($p<0.05$, power = 0.85). Additionally, the amount of PAH sequestered differed between worksites ($p<0.05$, power = 0.99), suggesting spatial sensitivity using this novel application.

4.2 Introduction

Whether through work-related exposure, or interactions with the ambient environment, people are exposed to a complex mixture of natural and man-made chemicals. Chemical exposure may occur through dermal, oral, or inhalation pathways, and compounds such as polycyclic aromatic hydrocarbons (PAHs), pesticides, dioxins, and polychlorinated biphenyls have been studied for decades.*(100)* However, linking mixed chemical exposures to health effects is often difficult given the diversity of compounds and often low levels of exposure.*(101,102)* Even in occupational circumstances where many chemicals of concern are identified, linking exposure to biological endpoints is challenging given the long latency of some diseases, the magnitude of the potential doses or interactions, and other confounders with exposure such as life behaviors and genetic variability.*(102,103)* Because of this complexity, there is now a push to capture life-course environmental exposures from before birth onwards recognized as the "exposome".*(104)* To understand linkages between the exposome and resulting toxicity, researchers are developing new technologies and methods to characterize exposure to an ever larger range of compounds. Often however, environmental data are gathered from single time points which may not reflect average exposures in profile or magnitude. In contrast to single time point samples, active and passive sampling has been used to monitor PAHs and other organic chemicals with stationary and personal samplers.*(49,50,105,106)* Although stationary samplers are used in several occupational studies, personal samplers have the advantage of being more relevant to an individual.*(50)* The most common personal samplers are active devices that pump air through filters that are extracted for target compounds. However, active personal devices are relatively expensive, require energy, and ultimately limited to implement on a wide scale.*(50)* An alternative to active monitoring is passive sampling.

A report of a personal passive sampler was first published in 1973,*(49)* but most growth in passive sampling and personal monitoring has occurred within the last

decade.*(30)* Passive sampling devices (PSDs) are used to sequester organic molecules through passive diffusion from water or air, and provide time-weighted averages of chemical concentrations.*(34)* Because chemicals continually accumulate in PSDs, the sensitivity of analytical detection is increased, and samplers represent time-averaged concentrations rather than episodic contamination.*(34)* PSDs have been used for personal monitoring starting with water vapor and SO_2 measurements,*(49)* and have expanded to include organic contaminant classes like PAHs and PCBs in recent years.*(50)* Materials used in passive sampling vary widely, and have included simple matrices like activated carbon,*(107)* as well as complex polymers like polyethylene and silicone.*(30)*

Historically, most personal monitoring samplers measure only one or a few compounds,*(108)* but recently the applications of PSDs have expanded to entire compound classes in order to assess chemical mixtures. One recent example includes polyurethane foam (PUF) used as personalized passive samplers.*(51)* While this material successfully sequesters hydrophobic PAHs, PCBs, and certain pesticides,*(50)* it is unclear whether these samplers would be able to target less hydrophobic compounds and more volatile pesticides.*(109)* In addition, it is unclear if future work will be able to exclude non-target particulate sizes with a protective surface.*(51)* In contrast, compounds reported in silicone PSDs represent only the vapor phase, which may encompass 34-86% of the toxicological dose of PAHs in industrial exposures.*(54)* We wanted to demonstrate a PSD that can be used to measure PAHs and volatile organic compounds, but also one that captures personal care products, pesticides, and other compounds of emerging concern with a wide range of physicochemistry. Silicone is known to absorb a wide range of compounds in field applications from volatile benzene, toluene, ethylbenzene, and xylene compounds to more hydrophilic contaminants containing hydroxyl, ketone, or carboxyl groups.*(35,48)*

We hypothesized that silicone material in commercial wristbands could be modified for use as a personal passive sampler in much of the same way silicone is used and demonstrated in environmental studies. By using wristbands as a personal passive sampler, it would have advantages as compared with active samplers mentioned previously, and result in data that represents time-weighted, vapor-phase concentrations. Our objectives were threefold: modify commercially available wristbands for analytical extraction, identification, and quantitation of target compounds; demonstrate sequestration of a wide physiochemical range to broaden potential usage of the personal PSD; and finally, present quantitated data in real-world occupational settings to examine if samplers provide useful sensitivity and selectivity in this novel application.

4.3 Experimental Section

Wristband and pre-cleaning experiments.

All solvents were Optima-grade (Fisher Scientific, Pittsburg, PA) or equivalent, and all laboratory glassware or other tools were solvent-rinsed before use. Any water used in post-deployment cleaning or initial washes of commercial silicone was filtered through a Barnstead D7389 purifier (Dubuque, IA). Commercially available silicone bracelets were purchased in two sizes (width: 1.3 cm and 2.5 cm; inner diameter: 6.4 cm and 6.7 cm respectively; 24hourwristbands.com, Houston, TX), and were used in several configurations throughout the study (**Figure 4.1**). Weights of smaller width wristbands were similar regardless of pigmentation (orange: 5.67 ± 0.02 g; clear: 5.68 ± 0.02 g; orange/white: 5.71 ± 0.02 g; n= 15 for each color). Larger 2.5 cm wristbands weighed 10.38 ± 0.02 g, but only the smaller sized wristbands were used in quantitative work described below. Before deployment, oligomers and other material that might interfere with future chemical analyses were reduced with various solvents in material/solvent ratios similar to other published work.*(44,53,82,84,87)* After several experiments to optimize the process with less solvents or cleaning time, the final procedure used

nominally ≤ 65 g of silicone in 800 mL of mixed solvent for 5 exchanges. A mixture of ethyl acetate/hexane (1:1, v:v) was used for the first three exchanges, and ethyl acetate/methanol (1:1, v:v) was used for the last two exchanges. Each exchange occurred after a minimum of 2.5 hours at 60 rotations per minute (VWR orbital shaker, Radnor, PA). Afterwards, solvent-cleaned wristbands were placed in stainless steel canisters (AEB Kegs, Delebio SO, Italy) and dried under PUF filtered vacuum (≤ 3 days). Dried wristbands throughout the study were stored in either amber glass jars or in polytetrafluoroethylene (PTFE) air-tight bags (**Figure 4.1b-c**) at 4°C until needed. Prior to occupational field deployment, two wristbands from a batch of pre-cleaned silicone were assessed to ensure cleaning processes were adequate for quantitative analyses. Specifically, if the highest background peak had an area less than 15 fold of a spiked internal standard of 500 ng/mL, then that background level was considered adequate for deployment and quantitative analysis.

Extraction and exposure optimization.

Reports of extraction of silicone vary widely from single soaking periods, to extended soxhlet extraction over 90 hours.*(52,87)* To determine an adequate extraction method, pre-cleaned silicone wristbands were infused with four deuterated PAHs similar to a previous method.*(84)* Briefly, acenaphthylene-D8, fluorene-D10, phenanthrene-D10, and pyrene-D10 were pipetted into a 1 L jar filled with approximately 50-100 g of silicone and a methanol/water (1:1, v:v) solution. Compounds were allowed to equilibrate for three days since the ratio of methanol/water used was 1:1 rather than 4:1 as originally described.*(84)* Using a 1:1 ratio requires less deuterated compounds in the infusing solution since more will partition to the silicone. Wristbands were dried as previously described, and then three rounds of extraction at two time periods of either 2 or 24 hours were used to examine efficiency (Appendix 3 - **Figure A.3.1**).

Post-deployment cleaning consisted of two rinses with purified water, and one rinse with isopropyl alcohol to reduce any water residue and further remove surface particulates (**Figure A.3.2**). Field samplers were extracted twice with 100 mL of ethyl acetate on an orbital shaker at 60 rotations per minute (VWR) for nominally 2 hours each time. Both rounds of extraction were combined and reduced to 1 mL (measured with pre-marked glassware) with closed-cell evaporators (Biotage LLC, Charlotte, NC). Samples were transferred and stored in amber chromatography vials at 4°C.

To examine whether PAHs would degrade after sorption to the wristband, or if field/handling conditions would influence exposure concentrations, we again infused wristbands with several PAHs (fluorene-d10, benzo[b]fluoranthene-d12, fluorene, pyrene and benzo[b]fluoranthene) and either exposed outdoors (in sun or shade) or within PTFE storage bags at approximately -20°C, 23°C and 35°C. Additional details are described in the Supporting Information. Silicone PSDs were extracted and stored as described above.

Instrumental analysis.

Samples screened for 1182 chemicals of concern were analyzed using retention time locking automatic mass spectral deconvolution and identification software (AMDIS) on an Agilent 5975B gas chromatograph-mass spectrometer (GC-MS) with a DB-5MS column (Agilent) at an electron impact mode of 70 eV. The spectra were compared against in-house and purchased libraries of compounds that included pesticides, polychlorinated biphenyls (PCBs), parent and substituted PAHs, pharmaceuticals, phthalates, as well as other compounds. Prior to PAH and OPAH instrumental analyses, perylene-d12, and fluorofluorenone-C^{13} were spiked at 500 ng mL^{-1} as internal standards, respectively. Instrument parameters to analyze PSD extracts for 33 PAH compounds and 22 OPAHs have been described previously.*(41,86)* Analyses were

performed on the same GC-MS and column described above but in selective ion mode rather than full scan. In addition, deuterated homologues of PAHs (7) and OPAHs (2) were used during the extraction process to monitor potential losses in the laboratory.*(41,86)* For PAHs, sample concentrations were determined by the relative response of deuterated surrogates to target analytes in a 9-point calibration curve with correlation coefficients for each analyte greater than 0.98. OPAHs were also quantitated with a 9-point calibration curve with correlation coefficients >0.99, but were not recovery corrected due to the availability of appropriate surrogates.*(86)*

Ambient demonstration.

To determine if silicone wristbands could sequester a wide range of organic compounds, a public query was made to collect volunteers. Participants were instructed to wear a wristband continuously for 30 days including bathing, sleeping, or other activities. A total of 30 pre-cleaned and dried wristbands were placed inside 3 amber jars, and metal tongs were used by participants as they took one or two wristbands to wear. A sign-out sheet was used to track the number of wristbands a participant took (1 or 2), but no surnames or personal information was asked or collected during this initial demonstration. At the end of the 30 day period, small (250 mL) amber jars were used to collect each individual wristband and were stored at -20°C until post-deployment cleaning and extraction. In addition, three non-deployed wristbands were placed inside amber jars at room temperature to serve as controls for potential laboratory or processing contamination.

Occupational Application.

To meet our final objective, we deployed silicone PSDs to roofers using hot asphalt since occupational environments represent relevant exposures, and we focused on PAH quantitation since this compound class is of toxicological concern for this occupation.*(102,103)* Our occupational study was approved by the institutional review

board (IRB) of Oregon State University, and roofers were recruited to wear the silicone personal samplers while working with hot asphalt. To see if reduced skin contact would improve chemical analyses, each roofer wore three designs of silicone personal samplers simultaneously: a single wristband like the initial ambient study, a cut wristband pinned as a lapel on a shirt collar, and a stacked wristband in which an inner silicone band protected the outer band from sweat and oils (**Figure 4.1a**). Hereafter, each configuration will be referred to as either single, lapel, or stacked, respectively. In the first setting, three workers wore PSDs for both a single day (approximately 8 hours), and for a representative workweek (32-39 hours) while refurbishing a roof at an active worksite. Due to availability, only the single and lapel configurations were worn for 8 hours while all three configurations were worn for the representative workweek. Both the single and multiday deployments began on the same day. At the second site, five pre-apprentice roofers wore all three silicone PSD configurations throughout an 8-hour shift at a training facility. Before either deployment, each sampler was placed into pre-labeled PTFE bags (**Figure 4.1b-c**). Nitrile gloves were used before and after each shift by non-participants when handling PSDs. In the case of the multi-day deployment, PSDs were returned at the beginning of the next available shift after overnight storage at 4°C. Travel blanks consisting of pre-cleaned silicone PSDs in PTFE bags were used at each setting and type of deployment (single or multi-day). Additional roofing information is available in Supporting Information.

Quality control and statistics.

Over 40% of instrumental samples were for quality control (QC) purposes. QC samples not already mentioned included instrument check standards ran before and after each set of samples (every 10 or less) as well as laboratory solvent blanks. PAHs and OPAHS in check standards had to be within 20% of the true value before samples would be allowed to proceed with analysis. Non-deployed wristbands were used during post-deployment cleaning to ensure there was no contamination or compound carryover

between samples. For AMDIS analysis, only compounds above a 60% mass spectral match were considered for chemist review. Deconvoluted results are compared against reference spectra for each target analyte, and if multiple lines of evidence (ex: correct ratios of ions, larger ions more representative of the parent ion, and retention time match) are present, then an analyte is considered as identified in the sample. Any compounds identified in controls or laboratory blanks were removed from the initial ambient demonstration since AMDIS results are descriptive as presented. Any quantitated compounds in blanks from PAH or OPAH methods are described in the results section.

Multivariate statistics were performed on ambient data using R statistical software (R development core team, Vienna, Austria). Identification data was converted into binary values, and a non-metric multidimensional scaling model was used to graphically represent the data with Jaccard distance. For occupational comparisons, after normality and equal variance tests passed criteria, parametric t-tests were performed in Sigmaplot (Systat Software Inc., San Jose, CA) with an assumed alpha value of 0.05. The power and p-value for the t-tests are listed for each result described below. In this demonstration, PAHs were not back-calculated to atmospheric concentrations since meaningful comparisons could already be made and address our original objectives of sensitivity and selectivity in a real-world exposure.

4.4 Results and Discussion

Laboratory method development.

Initially, silicone background was reduced similar to other methods in either solvents used or extraction times.*(44,53,82,84)* While this initial methodology allowed compounds to be identified without post-extraction silica cleanup used in other work,*(44,52,84)* improvements were sought to further reduce cleanup time and siloxane background (**Figure 4.2a**). After optimization experiments, cleanup was improved by

incorporating hexanes in addition to ethyl acetate and methanol. Additionally, a reduction of pre-cleaning time was achieved in under two days versus five (**Figure 4.2b**). After the cleanup method was finalized, all compounds reported in this work (PAHs and OPAHs) were spiked with silicone wristbands, extracted through the laboratory procedure, and quantitated within 26% of the true value (**Figure 4.2c**).

In extraction efficiency experiments, over 90% of the total amount of acenaphthalene-D8, fluorene-D10, phenanthrene-D10, and pyrene-D10 were extracted with the first round of ethyl acetate (**Figure A.3.1**). Less than 6% and 5% were extracted with a second and third round respectively. Variability of infused wristbands used for these extraction experiments had less than 13% relative standard deviation across all time points and compounds. PAHs with lower hydrophobicity had lower extraction efficiency over the first round of solvent, but all four compounds were ≥96% of the final extracted amount after two rounds of extraction. The total amount of compounds did not differ whether treatments were 2 or 24 hours (892 ± 60 ng/mL or 878 ± 47 ng/mL, respectively).

In the sun/shade experiment, we did not observe any statistical difference between PAHs over a four-hour period (**Figure A.3.4**). This preliminary evidence suggests there was no photo-degradation of 5 PAHs once sequestered into the PSD, which is consistent with a previous observation that PAHs sorbed to fly ash have reduced photo-oxidation.*(110)* Further study would be needed to examine PAH stability of longer time periods and varied irradiance, but for the purposes of this initial paper, potential degradation of sorbed analytes was not of concern. Additionally, no difference was observed among transport temperatures in PFTE bags (**Figure A.3.5**). Our data suggests that transportation in PTFE bags with temperatures as high as 35 ^{o}C and transport times up to 72 hours does not affect target analyte recovery. Stability during

PFTE transport is consistent with similar work with PAHs and pesticides in polyethylene passive samplers (*manuscript submitted*).

Ambient demonstration

A wide range of compounds were identified from the ambient wristband extracts from 22 participants, with log K_{ow} properties ranging from -0.07 (caffeine) to 9.49 (tris(2-ethylhexyl) phosphate) listed in **Table 4.1**. In contrast, a recent publication required that two PSDs materials together in an environmental deployment were needed to obtain a similar range of chemistry (K_{ow}: caffeine -0.07 to DDT 6.91).*(81)* In total, 49 different compounds were identified in our study, including PAHs, consumer and personal care products, pesticides, phthalates, and other industrial compounds (**Table 4.1**). Most individual compounds were PAHs, or consisted of industrial compounds typically used as flame retardants, plasticizers, or used in synthetic material manufacturing (**Table 4.1**).*(111)* The two most detected compounds are diethyl phthalate (all samples) and tonalide (20 of 23), both of which are used in personal care products like fragrances or cosmetics.*(111)* Home-use pesticides like N,N-diethyl-m-toluamide (DEET) and fipronil (pet flea medicine) were identified in several samples as well as consumer product ingredients like caffeine and nicotine. Many of the individual compounds listed in **Table 4.1** have been previously sequestered in environmental studies using silicone,*(48,112)* and all of the compound classes have been associated with human exposures through previous research.*(101,113,114)* Overall, results from these personal silicone samplers represent a wide diversity of bioavailable compounds, and appear to be different among individual participants using the non-metric multidimensional scaling model (**Figure A.3.3**). Further enhancements and separation of unique profiles of exposure should be possible once squalene and free fatty acids detected from full scan analysis are reduced by minimizing skin contact (in placement or duration as demonstrated in the occupational study).

Along with prominent skin components, caffeine and other relatively non-volatile compounds in **Table 4.1** were likely taken up through direct contact, and wristband passive samplers may be beneficial in cases where less volatile metabolites or unchanged parent compounds are targeted in human exposure estimates. However, further evidence of this route of exposure is needed before studies may exploit this potential sampling attribute while separating out interferences from skin components. For the purposes of the occupational study discussed below, several silicone configurations (single, lapel, and stacked) were used to evaluate changes, if any, with skin contact during these shorter exposure periods.

Occupational PAH results

A total of 8 roofers wore silicone passive samplers, with 3 at an active worksite (#1-3, **Figure 4.3a**), and 5 at the training center (#4-8, **Figure 4.3a**). No discomfort or work interference was reported from the samplers regardless of configuration. All extracted samplers contained measurable levels of PAHs, 12 of which are on the EPA priority list.*(115)* In addition, two OPAHs (benzofluorenone and fluorenone) were detected and quantifiable in both occupational settings. OPAHs are not typically monitored in asphalt exposures, so this represents some of the first evidence of a potential data gap in occupational exposure. Total PAHs ranged from 230 to 4,600 ng/PSD (**Figure 4.3a**) and trip blank PAH concentrations were all below 11 ng/PSD. Therefore, wristbands were extremely sensitive even after only 8-hours of exposure, and individual PAH concentrations from silicone PSDs exceeded instrument detection limits from 2 to over 1400 fold. In addition, blanks had PAHs below detection for 31 of the 33 PAHs measured, with only naphthalene or 2-methylnaphthalene as background PAHs. However, levels of these two PAHs in blanks were negligible, considering average background from either PAH was nominally 3 fold lower or more than any individual's deployed sample. There were no detectable OPAHs in any blank. Individual PAH

surrogate recoveries ranged from 53-122% (average = 91%, median = 94%) while OPAH surrogate recoveries ranged 64-120% (average = 83%; median = 82%).

While the chromatography was easier to interpret for lapel and stacked designs, all PAHs and OPAHs were able to be identified and accurately quantified in all three configurations at all exposure durations. Although sample sizes are small at either site, there is no statistical difference between configurations ($p>0.05$, power < 0.8, **Figure A.3.6**). However, in some cases both single and stacked designs had lower concentrations than lapels for some roofers (participants #5, and 7, **Figure 4.3a**), and after reviewing participant questionnaires it was determined that these wristbands (either in stacked or single configurations) were worn underneath protective clothing. In the case of participant 8, who had a lower value for the lapel than other roofers, the survey data indicated that this lapel was covered as well. Not all discrepancies can be explained with protective clothing information (participant 4's stacked wristband was reportedly covered yet is the highest value for that individual), but in future occupational applications, it would be important to indicate how the sampler should be worn with respect to personal protection equipment. Even though PSDs are sequestering the vapor phase, it is likely that personal protection equipment impacts the level of exposures seen by restricting air flow with respect to a non-covered PSD. Further study would be needed to explore this idea, and this potential application of evaluating the effectiveness of protective clothing. Ultimately however, designs did not significantly differ, and all PSDs from each individual were pooled together to observe the trends described below.

Regarding temporal sensitivity, there was a significant difference between single day or multiday exposures ($p<0.05$, power = 0.85, **Figure 4.4a**). Interestingly, 22 out of 23 PAHs and OPAHs detected in the 40 hour deployment were also detected in the 8 hour deployment, further illustrating the capability of the sampler for typical 8 or 10

hour time-weighted averages. Benzo[a]pyrene was not detected in the 8 hour deployment, but benzo[e]pyrene was quantitated, refuting the inference that larger PAHs would not be able to be detected in a shorter exposure period. Additionally, because benzo[a]pyrene was just above reporting limits after nominally 40 hours of exposure, it is likely that this compound was too low for our methods to quantitate at 8 hours, rather than a failure of the sampler itself. Phenanthrene and alkylated phenanthrenes were the most common and most abundant individual PAHs (**Figure 4.4b-d**). Other atmospheric PAH profiles report the prominence of phenanthrene in hot asphalt exposure,*(116)* and it has the highest emission rate out of 14 PAHs measured in working asphalt.*(103)* Unexpectedly, naphthalene and alkylated homologues (**Figure 4.4c-d**) were higher in 8-hour over 40-hour deployments. Differences in compound equilibrium between silicone and the atmosphere could explain naphthalene concentrations over time, and it is known that naphthalene is difficult to interpret with work-related exposure due to confounders such as cigarette smoking.*(117)* In fact, participants 2 and 3 did report cigarette use, while participant 1 did not. However, due to the small sample size, we are reluctant to over interpret the results here.

At both occupational sites personal silicone samplers were worn for approximately one 8-hour work day. While this small study cannot examine specific differences between worksites, we make the following casual observation about spatial sensitivity as detected by our passive sampler. Individuals at the rooftop site had similar profiles of PAH exposure, but differed in magnitude between participant 1 and participants 2-3 (**Figure 4.4b-d**). Survey information indicated participant 1 was a safety monitor at the worksite, while the other two participants were journeymen roofing professionals that reported directly handling hot asphalt. In another example, exposures were compared between occupational settings, and a significant difference was seen between study sites ($p<0.05$, power = 0.99, **Figure 4.3b**). The training center had a higher average PAH concentration than at the worksite (training center: 3040 ± 1090

ng/PSD; worksite: 800 ± 570 ng/PSD). Survey reports indicated that while hot asphalt was used in a similar manner at both sites, there was a difference between work enclosures. At the training center, hot asphalt is used to build a simulated roof at ground level in a semi-enclosed outdoor space. In contrast, hot asphalt was used on the rooftop only after the old roofing material was taken out, reducing some of the asphalt exposure. Taken together, spatial evidence supports the use of silicone wristbands as sensitive personal monitoring PSDs for exposures in a real world application. However, since we did not expect this level of sensitivity, additional work should be carried out to explore more specific differences between individual exposures.

Silicone personal samplers present an innovative sampling technology platform producing relevant, quantifiable data. By using these passive samplers, an atmospheric, time-weighted average concentration over an exposure period can be compared with exposure limits and compliance measurements through *in situ* calibration. Future work using isotope-labeled performance reference compounds to obtain *in situ* sampling rates will be done by infusing these compounds into PSDs prior to use. *(84,92,118,119)* Studies utilizing this sampler are currently underway, and we hope this easy-to-wear and dynamic application of silicone may become a valuable tool to address challenges of the exposome and mixture toxicity.

4.5 Acknowledgements

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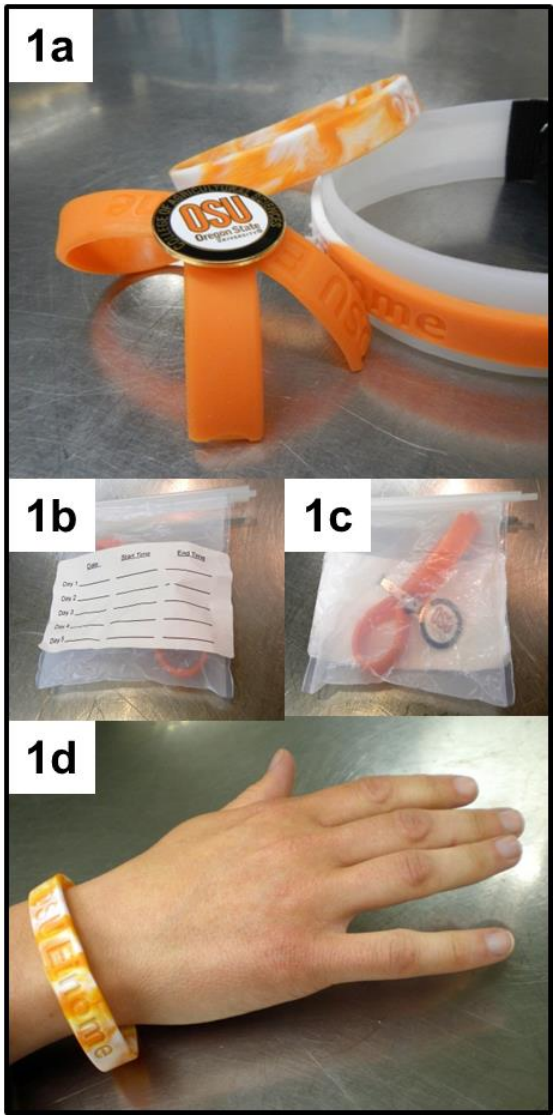

Figure 4.1 Examples of silicone personal sampling samplers. a) Configurations of wristbands used in the study including a "single" wristband, one cut and worn as a "lapel", and as a "stacked" wristband in which only the outer band was analyzed; b-c) bags used for transport that were attached to track participant ID and exposure time in the occupational deployments; d) single wristband deployment (debossed writing as pictured: "OSU EINOME" – Oregon State University Environmental Integrated Organic Monitor of Exposure)

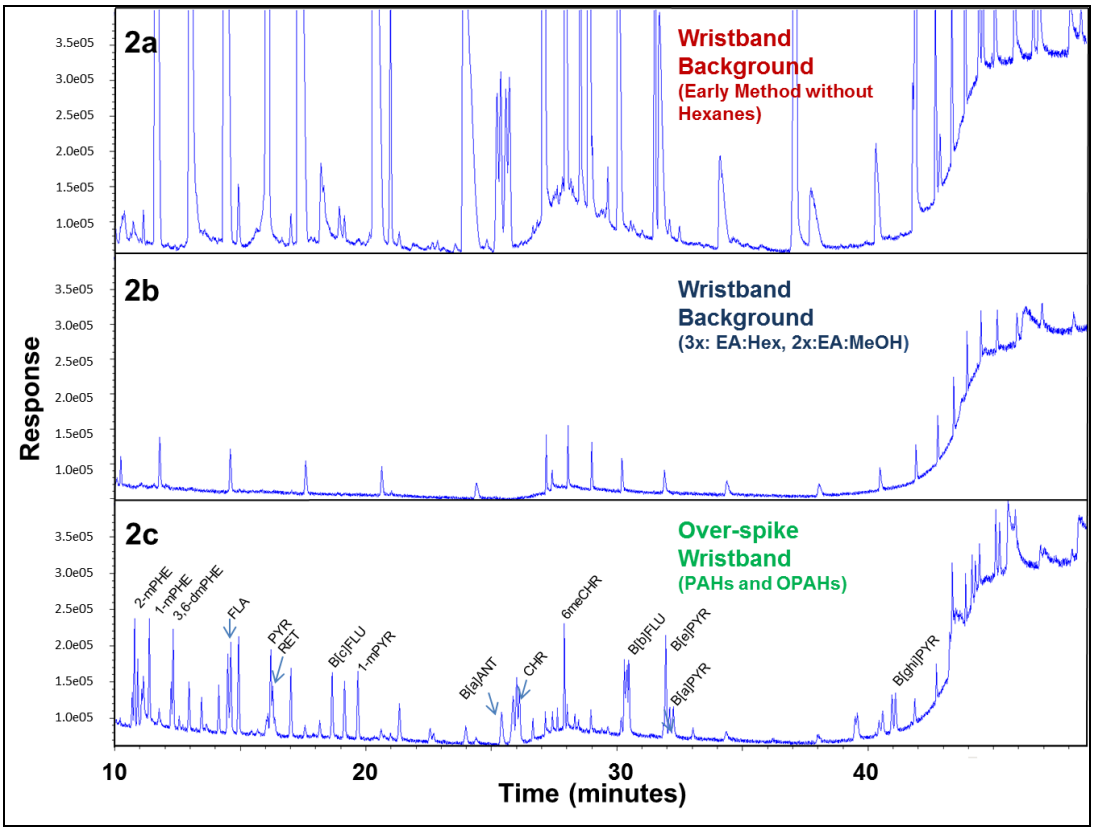

Figure 4.2 Total ion chromatograms of wristband extracts through stages of cleaning and over-spike on the GC-MS. All chromatograms are scaled equally to easily show differences in chromatograms. a) A wristband background with 5 rounds of ethyl acetate/methanol. b) The addition of hexane to solvent pre-cleaning drastically reduced total background inferences. Peaks here were identified as forms of siloxanes from mass spectral comparisons to NIST libraries. c) Notable peaks of the over-spike chromatogram are labeled with corresponding PAH abbreviations.

Table 4.1 Compounds identified from AMDIS spectra against chemical libraries during ambient exposures estimated values

Groups	Compounds	CAS	log K_{ow}	# of WBs	Possible Use or Occurrence
PAHs	1-methylnaphthalene	90-12-0	3.9	16	Petrogenic and pyrogenic sources
	anthracene	120-12-7	4.5	6	Petrogenic and pyrogenic sources
	fluorene	86-73-7	4.2[a]	5	Petrogenic and pyrogenic sources
	1,6-dimethylnaphthalene	575-43-9	4.3[a]	4	Petrogenic and pyrogenic sources
	1-methylphenanthrene	832-69-9	5.1[a]	3	Petrogenic and pyrogenic sources
	1,2-dimethylnaphthalene	573-98-8	4.3[a]	2	Petrogenic and pyrogenic sources
	acenaphthylene	208-96-8	4.1	1	Petrogenic and pyrogenic sources
	pyrene	129-00-0	4.9	1	Petrogenic and pyrogenic sources
	retene	483-65-8	6.4[a]	1	Petrogenic and pyrogenic sources
Consumer Products	tonalide	1506-02-1	5.7	20	Fragrance in cosmetics, and cleaning products
	carvone	99-49-0	3.1[a]	14	Oil of caraway seeds, used in perfumes, soaps
	triclosan	3380-34-5	4.8	9	Active agent in deodorants and antiseptics
	caffeine	58-08-2	-0.1	6	Component of coffee, sodas, and other beverages
	nicotine	54-11-5	1.2	4	Active ingredient in tobacco products
	eugenol	97-53-0	2.5	4	Clove perfumes, essential oils, dental medicine
	celestolide	13171-00-1	5.9(est)[b]	2	Musk fragrance in cosmetics or perfumes[a]
	musk ketone	81-14-1	4.3	1	Fragrance in cosmetics, perfumes
	phantolide	15323-35-0	5.9(est)[b]	1	Musk fragrance[a]
	phthalimide	85-41-6	1.2	1	Used in dyes, fungicide
Pesticides	benzyl benzoate	120-51-4	4.0	18	Acaricide and Insecticide
	N,N-diethyl-m-toluamide	134-62-3	2.0	11	Insect and acarid repellant (DEET)
	promecarb artifact	3228-03-3	3.5(est)[b]	6	Possible metabolite of an insecticide
	methoprene	40596-69-8	5.5	5	Broad spectrum insecticide
	fipronil	120068-37-3	4.0	3	Insecticide designed for pet use
	fipronil-sulfone	120068-36-2	4.4(est)[b]	2	Metabolite of fipronil[c]
	fipronil, desulfinyl-	111246-15-2	4.2(est)[b]	1	Photodegredate of fipronil[c]
	trifluralin	1582-09-8	5.3	1	Pre-emergent herbicide
Phthalates	diethyl phthalate	84-66-2	2.5	23	Vehicle for fragrances and cosmetics
	butyl benzyl phthalate	85-68-7	4.7	19	Plasticizer for floor tile, foams, carpet backing
	di-n-octyl phthalate	117-84-0	8.1	11	Plasticizer for cellulose and vinyl resins
	di-n-hexyl phthalate	84-75-3	6.8	9	Used in making dip-molded plastics
	dicyclohexyl phthalate	84-61-7	6.2(est)	6	Plasticizer for cellulose, and chlorinated rubber
	dimethylphthalate	131-11-3	1.6	5	Plasticizer for cellulose and vinyl resins
Industrial Compounds	benzophenone	119-61-9	3.2	19	Used in paints, cosmetics, pesticides, medicine
	triphenyl phosphate	115-86-6	4.6	15	Fire retardant and plasticizer
	tris(2-butoxyethyl) phosphate	78-51-3	3.8	4	Plasticizer in rubber gaskets and floor products
	tributyl phosphate	126-73-8	4.0	5	A fire retardant, plasticizer, antifoaming agent
	2-methylphenol	95-48-7	2.0	4	A solvent, disinfectant and chemical intermediate
	tris(2-chloroethyl) phosphate	115-96-8	1.4	3	Flame-retardant plasticizer in vinyl resins, used in carpet backing or upholstery
	tris(2-ethylhexyl) phosphate	78-42-2	9.5[a]	3	Flame-retardant plasticizer in vinyl resins, and antifoaming agent
	o-tricresylphosphate	78-30-8	6.3	2	Flame-retardant plasticizer in resins and coatings
	triethylphosphate	78-40-0	0.8	2	As a solvent/plasticizer in cellulose gums
	o-phenylphenol	90-43-7	3.1	2	A citrus fungicide and lumber disinfectant
	m-cresol	108-39-4	2.0	2	In synthetic resins, disinfectants, fumigants, photographic developers, explosives
	p-tricresylphosphate	78-32-0	6.3	1	In cellulose, vinyl and rubber products, also a sterilizing agent for surgical instruments
	2,4-dimethylphenol	105-67-9	2.3	1	A disinfectant, fungicide, sanitizer, and virucide
	4-methylphenol	106-44-5	1.9	1	Used in resins, petroleum, photography, paints

Table 4.1 (continued) Compounds identified from AMDIS spectra against chemical libraries during ambient exposures estimated values

[a]National Library of Medicine - (NLM 1993).
[b]Estimated from EpiSuite EPA (EPIWEB 4.1)
[c]National Pesticide Information Center – (NPIC 2009)
Unless otherwise noted, log K_{ow} and compound occurrence information was acquired from the Hazardous Substances Data Bank by the National Library of Medicine.
Abbreviations: WBs - wristbands, est – estimated values

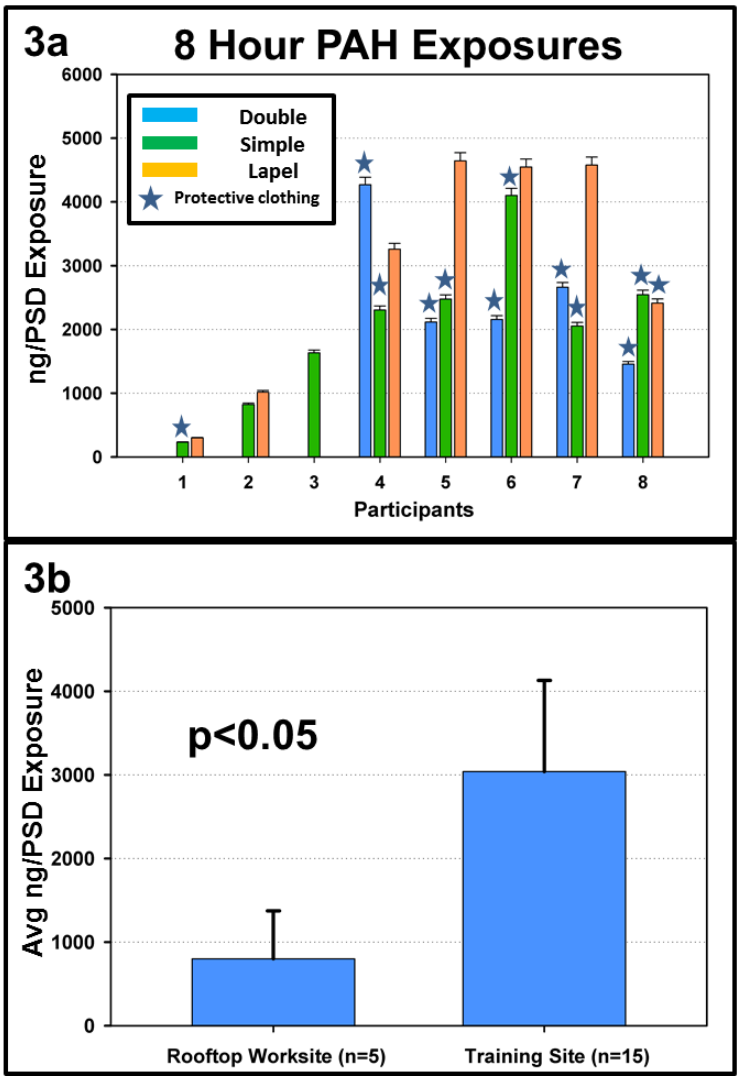

Figure 4.3 Three silicone passive sampler designs sequestering PAHs in a single work day. a.) All samplers from both occupational settings are pictured including those from #1-3 at a rooftop worksite, and #4-8 at the training facility. The lapel corresponding to #3 was lost during the field deployment. Standard deviations here are derived from non-deployed wristbands (n=5) representing laboratory and instrumental variability spiked with all target PAHs (average RSD: 2.30%). Blue stars represent silicone passive samplers that were reported as covered with protective clothing during exposure. b) Overall exposures between sites differed significantly over an 8 hour work period ($p<0.05$). Standard deviations here are the result of all samplers pooled together from each participant.

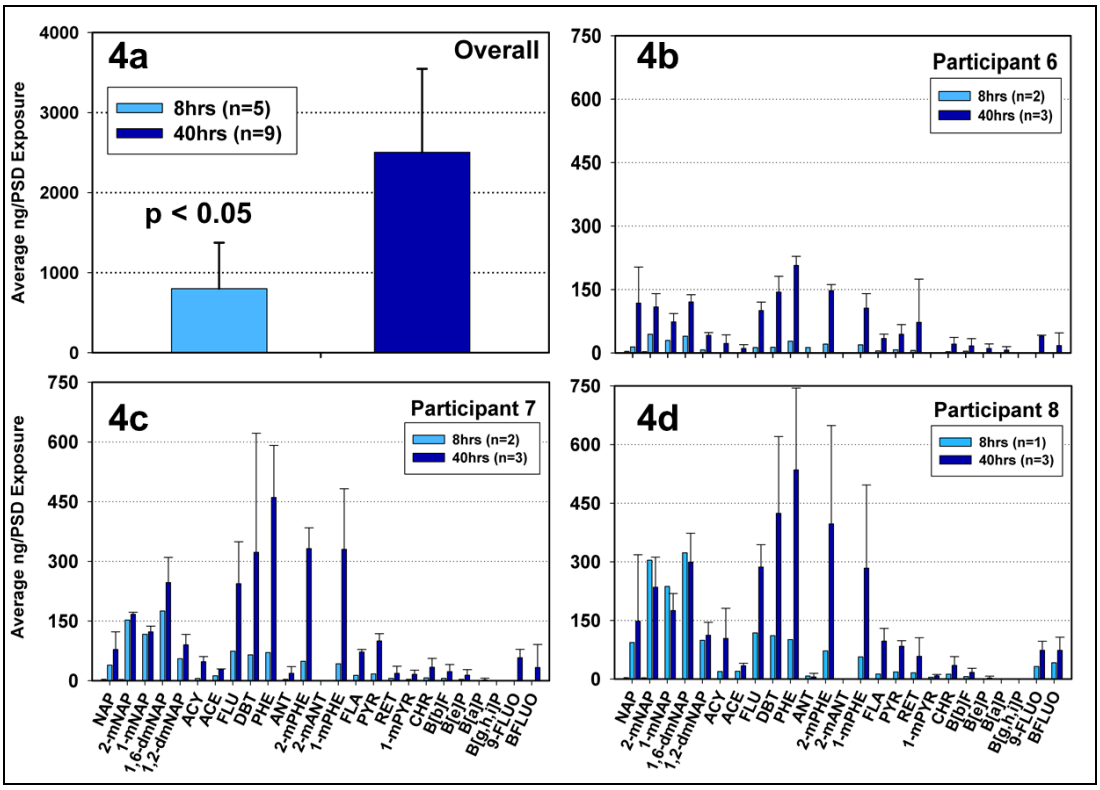

Figure 4.4 Worksite PSDs (all configurations) with sum (4a) and individual PAH exposure (4b-d) for a single (8 hour) or multi-day period (40 hour). Individual profiles are scaled equally to observe differences in magnitude and profile between silicone samplers. Standard deviations are the result of PSDs pooled together from each participant. PAH abbreviations: naphthalene (NAP), 2-methylnaphthalene (2-mNAP), 1-methylnaphthalene (1-mNAP), 1,6-dimethylnaphthalene(1,6-dmNAP), 1,2-dimethylnaphthalene (1,2-dmNAP), acenaphthylene (ACY), acenaphthene (ACE), fluorine (FLU), dibenzothiophene (DBT), phenanthrene (PHE), anthracene (ANT), 2-methylphenanthrene (2-mPHE), 2-methylanthracene (2-mANT), 1-methylphenanthrene (1-mPHE), fluoranthene (FLA), pyrene (PYR), retene (RET), 1-methylpyrene (1-mPYR), chrysene (CHR), benzo[b]fluoranthene (B[b]F), benzo[e]pyrene (B[e]P), benzo[a]pyrene (B[a]P), and benzo[ghi]perylene (B[ghi]P). OPAH abbreviations: 9-fluorenone (9-FLUO), and benzofluorenone (BFLUO).

Chapter 5 - Exploring silicone implants as *in vivo* biomonitors of exposure and potential body burden sinks for lipophilic toxicants

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In preparation for submission

5.1 Abstract

Epidemiological evidence has found lower incidences of breast cancer in women with silicone implants. If silicone implants are sequestering toxicants throughout the duration of implantation, they may be good indicators of persistent organic pollutants (POP) exposure, and may even significantly reduce the overall body burden of toxicants in the body. Studies were conducted to address the utility of using *in vivo* silicone implants for chemical analysis and estimating potential body burden impact from implants. Human breast implants were extracted to determine if organic contaminants could be quantitated from *in vivo* silicone. Compounds found in human explants (p,p'-DDE and PCB 118) were then used in a model organism for a second study examining body burden in mice with silicone implants. Mice were dosed with p,p'-DDE and PCB 118 to compare the impact of silicone absorption to surrounding adipose concentrations, as well as to explore partitioning between the silicone and lipid-rich tissue. Partition values from the mouse study were utilized to predict human adipose concentrations, and were well within the range of those seen in the literature. Further testing is required to determine if silicone implants will reduce the concentration of compounds in surrounding adipose tissue.

5.2 Introduction

Recently, lower breast cancer occurrence was seen in women receiving silicone implants after either reduction mammoplasty or other breast reconstruction *(61,62)*. If breast tissue accumulation of organic pollutants is important to disease outcomes like breast cancer, then silicone may be an important sink to organic contamination, and may even have unintended health outcomes. Several reports of persistent organic pollutants within breast tissue have linked accumulation of organic pollutants with breast cancer development *(120,121)*, but epidemiological data suggest direct comparisons of several organic pollutants are at best presumptive *(122,123)*. Linking environmental exposures to internal dose and toxicological response are difficult challenges that are further magnified when inconsistent methods are used among studies. For example, limitations that explain why some epidemiological studies do not support toxicant data with breast cancer associations include: inconsistent tissue comparisons (blood, adipose, or plasma *(124)*), small study sizes *(125)*, or lack of pre-clinical markers among others *(122)*. In order to better understand the effects of silicone implants on complex toxicological outcomes like breast cancer, determining contaminant concentrations *in vivo* may help decipher the observed reductions in breast cancer from the epidemiological evidence.

Traditional i*n vivo* sampling has included biological tissues that may represent short-term (ex: blood and plasma), or potentially longer-term exposure (ex: adipose tissue). However, tissue samples can be difficult to analyze, and may involve multiple cleanup steps which are labor solvent and time intensive *(58)*. Extrapolating exposures from tissue samples can be complicated since known exposure durations, metabolic activity, and phenotypic differences can complicate contaminant exposure estimations. Recently, researchers have employed passive sampling techniques *in vivo* with fish that resulted in time-weighted averages of contaminants useful for environmental

monitoring or ecological bioaccumulation studies *(55-58)*. Passive samplers can sequester compounds from the environment through passive diffusion, and can be easily and quickly extracted for chemical analyses or biological assays *(36,37,79,126)*. Since passive samplers take up compounds in the freely dissolved phase *(39)*, much of the organic interferences are excluded, simplifying subsequent extractions for chemical analysis *(59)*. One popular passive sampling material is silicone, which has been used in several environmental applications in aqueous and atmospheric environments as well as in initial *in vivo* studies *(35,44,48,82,91)*.

Breast implants are constructed from similar silicone material as used in previous passive sampling studies, and human implants accumulate organic compounds through passive uptake kinetics similarly to those described in environmental deployments. Advantages of using silicone implants as passive samplers is that the application allows exploration of long-term contaminant exposures, estimates diffusion properties between tissue and silicone, applies a robust method to characterize contaminants, and finally, can explore why implants are associated with lower breast cancer. Over 23,000 implants were removed or replaced in 2013 within the United States alone *(127)*, and the discarded implants are typically incinerated as waste *(60)*. However, this waste may actually be an important resource for exposure monitoring and understanding the human body burden of organic pollutants. In fact, in a recent study several contaminants including chlorinated pesticides and biphenyls have been identified from human silicone implants *(60)*.

Therefore, our first aim was to demonstrate our ability to extract contaminants from human breast implants removed from human tissue (hereafter referred to as “explants”). In the second aim, silicone was implanted into murine models that were

dosed with compounds found from the first study and examined after 8 days. Silicone concentrations were compared with adipose tissue and the potential overall body burden reduction was estimated. Finally, silicone partitioning concentrations in mice were used to predict values in human adipose tissue and compared to literature values. If silicone can sequester *in vivo* compounds consistently in multiple organisms, this application of silicone passive sampling shows promise for future biomonitoring and body burden estimates, and may even elucidate unintended health outcomes from implant usage.

5.3 Methods

Solvents and chemicals

All chemicals (p,p'-DDE and PCB 118) were purchased from Accustandard (New Haven, CT). All solvents were Optima-grade or equivalent (Fisher Scientific, Pittsburg, PA), and laboratory water used in silicone or tissue processing was filtered through a Barnstead D7389 purifier (Dubuque, IA). Any glass or metal used for laboratory processes were solvent-rinsed, washed, and baked out at 500 °C.

Human explant collection

Explants were obtained from Oregon Health and Science University after approval from the Institutional Review Board at Oregon State University (OSU IRB#5851). All materials were numerically coded, and for the purpose of this study, no personal information was obtained or recorded during this project from any individual. In addition to human explants (**Figure 5.1A**), silicone implant "sizers" were analyzed as well to serve as negative controls for explant analysis. Sizers are similar to silicone implants in construction and used for temporary intraoperative purposes to determine necessary adjustments before the implants are inserted (**Figure 5.1B**). Sizers represent silicone

only briefly introduced to the human body during surgery, and thus provide potential background levels of target compounds from silicone manufacturing processes.

To examine intra-variability from each silicone sample, small sections (n ≥ 2) were removed from each explant or sizer for organic analyses (**Figure 5.1A-B**). Sub-sections were rinsed twice in purified water, and then briefly washed with isopropyl alcohol to remove biological material on the surface before storage at -20 °C. Extraction of each individual piece was similar to previously published extraction techniques with silicone *(128)*. Tetrachloro-m-xylene (TCMX) and decachlorobiphenyl were used as surrogates at 500 ng/mL. Silicone extraction employed two rounds of 100 mL ethyl acetate. Each round of extraction was on an orbital shaker set at 60 rotations per minute for at least 2 hours. Extracts were combined and blown down to 5 mL using closed-cell evaporators (TurboVaps®, Biotage, Charlotte, NC). Concentrated samples were transferred to centrifuge tubes and stored at -20 °C until analysis.

Mouse implant study: silicone and cocktail preparation

Silicone sheets were purchased from Stockwell Elastomerics Inc. (Philadelphia, PA), and discs of silicone (~0.5 cm^2) were cut with a punch hole. The average weight of each silicone disc was 0.023 +/- 0.001 g (n=25). Silicone was rinsed with water and cleaned with ethyl acetate, hexane, and methanol solvents as described previously *(128)*. Silicone discs were dried in a stainless steel drying keg (AEB Kegs, Delebio SO, Italy), and stored in polytetrafluoroethylene (PTFE) air-tight bags until surgery.

Neat compounds of p,p'-DDE and PCB 118 were chosen for the mouse study based on initial detection in breast explants *(60)*, potential linkages with human disease endpoints *(129)*, and resistance to metabolic processes which allow compounds to

accumulate in adipose tissues *(120,130)*. Neat compounds were dissolved in ethyl acetate and diluted to 0.212 mg/mL for p,p'-DDE, and 0.160 mg/mL for PCB 118 with filtered (0.4 µm) peanut oil. The cocktail given to mice was further diluted with peanut oil by 10-fold in order to reduce ethyl acetate to no more than 1% (v:v) for each dose. Mice received 0.131 ± 0.002, and 0.099 ± 0.001 mg/Kg for p,p'-DDE and PCB 118, respectively.

Animal care and surgery

Female ICR mice (Jackson Laboratory, Bar Harbor, ME) were maintained as an in-house breeding colony. All experimental procedures and treatments were approved by the Institutional Animal Care and Use Committee (permit #4389), and mice were held in the pathogen-free Laboratory Animals Resource Center (LARC) at OSU. Mice were given breeder food and water *ad libitum*. A total of 18 mice were used in this study, with 6 mice in each treatment group. One group received subcutaneous silicone discs and compounds (hereafter referred to as "SIL"), the next group received sham surgeries and compounds to control for any unintended effects of the procedure (SHAM), and the last group was given silicone and peanut oil vehicle (VEH).

On the day of surgery, animals were anesthetized with a mixture of isoflurane and oxygen. Prior to incision, the dorsal and abdominal regions were shaved, and treated with betadine and alcohol. Two incisions were made on each mouse: a dorsal midline incision between shoulder blades, and a second ventral midline incision in the abdominal area. Two pieces of silicone were placed subcutaneously to the left and right of the shoulder incision, and subsequently closed with sutures. Four additional pieces of silicone were placed to the left and right of the inguinal incision. In sum, six pieces of silicone were inserted subcutaneously per mouse (**Figure 5.2**). Total ratio of murine

body mass to silicone ranged from 332 to 494, and is within the range potential ratios of human body weight to silicone implants of 50 to 1500 (assuming an average body mass of 75.4 Kg *(131)*, and implant combined weights ranging from 0.05 – 1.5 Kg *(132)*). Surgical incisions were performed similarly on the SHAM group. Following surgery, mice received 1 ml/10 g body weight of subcutaneous fluids before receiving the contaminant cocktail. Intraperitoneal (IP) injection was chosen as the route of exposure for this initial test to reduce uncertainty in the given dose considering the novelty of the study, and to reduce animal suffering since it was administered while mice were still anesthetized. Mice were monitored during recovery and for 24 hours post-surgery by a veterinary technician. After nine days from surgery, mice were euthanized via CO_2 overdose and cervical dislocation. Previous research has suggested that week long exposures might be adequate to establish equilibrium between lipids and silicone *(59,133)*. No gross organ malformations or changes in body weight were observed in any treatment group. Silicone pieces and adipose samples were stored in amber glass jars at -20 °C until extraction.

Silicone and adipose extraction

After surgery, silicone pieces were rinsed with filtered water and isopropyl alcohol to remove surface material and extracted similarly to previous passive sampling methodology *(128)*. Briefly, silicone pieces were combined from each mouse (n=6, or ~0.12 g of total silicone), and placed into 7 mL amber vials for extraction. PCB 180 and PCB 100, each at 500 ng/mL, were used as laboratory surrogates for p,p'-DDE and PCB 118 respectively since they are physiochemically similar to the target compounds. Three rounds of 2 mL ethyl acetate extractions (≥ 2 hours), were combined and blown down to 0.5 mL extracts. All extractions were shaken on a Wrist Action® shaker (Burrell, Pittsburg, PA). Extracts were stored in amber chromatography vials at 4 °C until analysis.

Adipose tissue was extracted via a modified QuEChERS method previously reported *(134)*. First, samples were homogenized with liquid N_2 in a stainless steel mortar and pestle. PCB 180 and PCB 100 were used as laboratory surrogates for p,p'-DDE and PCB 118 respectively, and spiked with the adipose aliquots. Samples of homogenized tissue were transferred (0.02-1.2 g, depending on availability) into a plastic conical tube, along with 1 mL of water, 2 mL of solvents (2:1:1 by volume of ethyl acetate/acetone/isooctane, respectively), and 1.3 g of extraction salts ($MgSO_4$ and sodium acetate). Conical tubes were vortexed and hand-shaken between each addition for at least 60 seconds. Samples were centrifuged over 5 minutes at 3000 g, and the supernatant was removed. Supernatants were diluted with acetonitrile to 6 mL, and a 500 mg C-18 cartridge (Agilent) in a RapidTrace workstation (Biotage AB, Uppsala, Sweden) was used to remove excess lipids in the sample. Finally, clean extracts in acetonitrile were solvent exchanged to hexane, blown down to 0.25 mL, and stored at 4 °C in chromatography vials until chemical analyses.

Instrumental analyses

Specific method details can be found for the pesticide analytical method published previously *(90)*. Briefly, 4,4'-dibromooctafluorobiphenyl was used as an internal standard and was spiked into all extract aliquots just prior to instrumental analyses at 100 ng/mL. A gas chromatograph (GC) with an Agilent DB-XLB (30m, 0.25mm, 0.25 µm) and a DB-17MS (30m, 0.25mm, 0.25 µm) was used for dual column confirmation coupled with dual electron capture detection (model 6890N, Agilent). All compounds were calibrated with calibration curves of five points or more, and had correlations of 0.99 or better. Any concentrations calculated from less than 15% recovery were removed from consideration, as well as any concentrations below adequate signal to noise ratios of 3:1.

Initial contaminant screening for compounds was performed with GC/mass spectrometry (MS) retention time locking Automated Mass Deconvolution Identification Software (AMDIS) in conjunction with created and purchased libraries totaling 1,180 unique compounds. The column used was an Agilent DB-5 (30m, 0.25mm, 0.25 µm). Identification and confirmation criteria has been described previously *(91)*, but compounds had at least a 60% spectral match before additional confirmation criteria were used for each qualitative determination.

Quality control

Including laboratory, cleaning, and instrumental blanks, over 39% of the analyzed samples were for quality control (QC) purposes. In the mouse implant study, silicone was examined after cleaning and prior to surgery for analytically interfering background from oligomers. If the highest background peak on a full scan GC/MS analysis (range: 50-500 m/z) had an area less than 15 fold of a spiked internal standard of 500 ng/mL, then that background level was considered adequate for the study. The background criteria were shown to provide adequate cleaning to achieve the desired analytical sensitivity *(91)*. During post-surgery cleaning, non-deployed silicone was used to monitor any contamination prior to storage (-20 °C). Laboratory reagent blanks accompanied each batch of extraction for tissue or silicone samples. Before instrumental analyses could proceed, compounds were verified +/- 20 % of the true value by using verified standards. These standards were also run nominally every 10 samples, and/or at the end of the sample set. If a closing standard did not meet the above criteria, samples were re-run after the standard was verified. Finally, any background levels from QC samples were averaged, and the reporting limit was set as this average plus three times the standard deviation. Any sample concentrations below the reporting limit were not included in the results below.

5.4 Results and Discussion

Measuring organic contaminants in human explants

An initial analyses using AMDIS identified p,p'-DDE in explant sub-samples despite the rich silicone background. The quantitative pesticide method was then used for each sample. Recoveries of TCMX and decachlorobiphenyl surrogates ranged from 15-120%, averaging 62%. No detectable concentrations of p,p'-DDE or trans-nonachlor were seen in any sizer, reagent, or cleaning blank. Five explant samples contained measurable amounts of p,p'-DDE in both aliquots, while trans-nonachlor was only seen consistently in one explant (**Figure 5.3**). Sub-samples taken from the same explant had p,p'-DDE values within 17% of each other, suggesting adequate extraction methodology and homogeneous sequestering by the explant. Previous data on human explants reported variability typically below 30%, although in some cases, relative percent differences were as high as 160% *(60)*. One reason for slightly better variability observed in this study could be due to the solvent to silicone ratio during extraction. Ratios of 30- to 15-fold of solvent to silicone (v:v) were used in this work, while Allan et al. reported using ratios of only 3-5-fold *(60)*. If extractions are not consistently exhaustive, greater variability would likely result in replicate samples. As previous noted *(60)*, better cleanup of the extraction method is needed to reduce background levels of silioxanes for more sensitive analyses, and would likely increase surrogate recoveries. Overall, p,p'-DDE concentrations in many of the explants were above 15 ng/g silicone, and much higher than any other trans-nonachlor value (max: 1.2 ng/g, **Figure 5.3**). Interestingly, the concentration of p,p'-DDE was also the highest seen in the only other explant study reported, and the range of p,p'-DDE concentrations measured in this study (1.2-35 ng/g silicone) is similar to that previously observed (approximately 0.2-37 ng/g silicone) *(60)*. Trans-nonachor has previously been identified in human adipose tissue *(135)*, and was

also below that of p,p'-DDE concentrations by roughly 20-fold as seen in this silicone. The similarities between human tissue and explants suggest silicone may be a reliable surrogate of persistent toxicants in humans.

In vivo murine absorption: silicone concentrations and percent uptake

Silicone pieces (n=6) were extracted for each mouse, and examined to see if compounds from a single IP dose were absorbed *in vivo*. Excellent surrogate recoveries (66 to 96%) were seen in silicone from the mouse study, and detectable concentrations were found in all samples from the SIL group. No detectable levels of p,p'-DDE or PCB 118 were found in any reagent or cleaning blanks or the VEH silicone. Silicone p,p'-DDE concentrations ranged from 31-70 ng/g, averaging 49 ± 14 ng/g (**Figure 5.4**). PCB 118 concentrations ranged from 20-108 ng/g, averaging 57 ± 30 ng/g silicone (**Figure 5.4**). Although the amount introduced into each mouse varied less than 8%, the relative standard deviation (RSD) between silicone concentrations among mice were 34% for p,p'-DDE, and 54% for PCB 118. Higher silicone RSDs from mice are likely due to the increased complexity of inter-animal variability compared with sub-samples from human explants described in the previous section.

Within 10 days, the percent uptake from the organism into the silicone from the initial IP injection ranged from 0.05% to 0.33%, averaging 0.12% for p,p'-DDE, and 0.18% for PCB 118 (**Table 5.1**). This is some of the first data showing quantifiable concentrations of organic contaminants in silicone implants from a controlled dosing scheme. While the percent uptake represents a small percentage, there was still adequate detection sensitivity in each sample. Other silicone *in vivo* work generally shows uptake of compounds into silicone from environmental media, and is simply compared with whole homogenates of the organism, tissue samples, and/or the

surrounding aqueous environment *(56,136)*, so direct comparisons are not possible. Alternatively, some data are available for dissipation rates of compounds from silicone into ICR mice. Compounds with log K_{ow} values from 3.32-5.99 were dissolved into oil, injected into silicone tubing, and then the devices were sealed and implanted subcutaneously into ICR mice *(137,138)*. Most compounds were dissipated from the silicone by over 80% after 50 days *(137,138)*, and followed first-order kinetics over a range of initial concentrations *(138)*. Given this evidence of potentially longer times for equilibrium to occur between silicone and surrounding tissue, coupled with potentially longer equilibrium times for the compounds to distribute to fat tissue from an initial dose (up to 14 days for DDE in sheep *(139)*), it is possible that overall uptake amounts using the silicone discs in this study would increase if implant exposures are longer.

In vivo murine absorption: adipose tissue concentrations

Surrogate concentrations from tissue samples were more variable due to the more involved cleanup necessary for analysis. Slightly higher surrogate recovery was seen in abdominal samples over dorsal for PCB 180 (averages: 52% versus 31%), but was similar for PCB 100 in both adipose tissues (averages: 29% versus 24%). Some loss was expected since recoveries using similar methods with fatty samples ranged from 49 to 106 % over similar organic compounds *(134)*, but additional loss might have occurred during the extra C-18 cleanup step. Low recoveries and high variability are in fact the reason researchers are now using silicone to sample complex matrices such as animal tissue *(58,133,136)*.

Concentrations of p,p'-DDE and PCB 118 were able to be quantitated in all adipose samples in groups that received the cocktail. Tissue p,p'-DDE concentrations ranged from 210 to 1700 ng/g, and PCB 118 concentrations ranged from 450 to 1500

ng/g tissue. Dorsal tissue in SIL mice had an average p,p'-DDE concentration of 220 ± 11 ng/g, which was slightly lower than the SHAM group (230 ± 11 ng/g tissue, **Figure 5.4**). Abdominal concentrations showed a larger difference between SIL and SHAM, but were also not significant due to variability (1000 ± 200 ng/g tissue compared to 1100 ± 380 ng/g tissue, **Figure 5.4**). No difference in SIL over SHAM tissues were seen for PCB 118 concentrations, which showed higher or equal amounts for both dorsal and ventral tissue samples (600 vs 530 ng/g, and 1100 vs 1100 ng/g, respectively). Differences were seen between adipose samples, as abdominal concentrations for both compounds were higher than those from the dorsal adipose tissue (**Figure 5.4**). This observation was expected considering back adipose tissue is more vascularized brown fat, and likely has lower lipid content (example rodent data: 50-55% lipids in brown fat *(140)* vs 89-91% lipids found in abdominal white fat *(141)*). Brown fat also contains higher protein content *(140)*, which may have a higher affinity for multi-chlorinated compounds *(142)*, and explains higher PCB 118 concentrations compared with p,p'-DDE in dorsal tissue (**Figure 5.4**). Adipose tissue from either location was not lipid normalized since the purpose of this study was to directly compare adipose tissue concentrations and silicone implants, and not to model whole body distribution. Because the percent body fat of the breeder mice used in this study is unknown, it is difficult to accurately estimate percent uptake of target compounds into adipose tissue. However, if 15 % body fat is assumed from previous work *(143)*, and whole body adipose tissue concentration is assumed from the average of dorsal and ventral adipose samples, the percent uptake for p,p'-DDE ranged from 49-106 %, while PCB 118 ranged from 69-145 %. Overall, surrounding tissues did not significantly differ despite sequestration of target compounds from silicone, but longer exposure times might influence these results as mentioned above, and these data still provides useful information about *in vivo* silicone partitioning in murine and human models.

Human and murine predictive <u>in vivo</u> partitioning

Partition coefficients from other silicone and lipid work can be used to predict contaminants in surrounding tissues *(133)*, and then compared with actual concentrations measured in the tissue of individual mice (**Table 5.2**). Dorsal concentrations are quite similar to predicted values (within 2-fold), but measurements based on ventral estimates exceeded those expected values by roughly 10-fold (**Table 5.2**). Even if the tissues were lipid normalized, ventral values would likely be similar to current estimates, while dorsal values would become closer in agreement with ventral concentrations and further away from predicted values. Therefore, partition coefficients from oils and other inert lipids may not be representative of *in vivo* tissues due to a lack of metabolism and distribution. In addition, lipid type, capacity, and composition may differ between tissues *(144)*, and may not be represented from inert oils.

Despite the mentioned limitations, predicted concentrations for p,p'-DDE in adipose tissue from human explant data derived from either seal oil *(133)*, or estimates from the mice themselves (using either dorsal or ventral tissue), are consistent with adipose concentrations observed in several studies around the world (**Table 5.2,** *(145-147)*). Estimates using ventral mouse data are near the high end of observed concentration range, and those using seal oil or from dorsal mouse tissue data are near median levels of human cohorts. Clearly, more work would be necessary to be able to predict tissue concentrations with accuracy, but the observation that these values are within other observed amounts highlights the usefulness of using silicone implants for monitoring or body burden research. Future work would benefit from examining silicone equilibrium in different adipose tissues, using multiple time points of silicone

duration for better equilibrium estimates and partitioning, and more precise estimates of target analytes from fatty tissue.

5.5 Conclusion

Using silicone implants as passive samplers allows exploration of long-term organic contaminant exposures, may provide an adequate level of predictive tissue concentrations once adequate partitioning values are established, and eventually may be used to determine why implants are associated with lower breast cancer incidence. Explants represent long term monitoring devices of organic contaminants rather than blood (i.e. transient exposure) samples. If a monitoring bank is kept for silicone explants, it will be useful to characterize silicone absorption of pollutants *in vivo* to make pollutant monitoring data from the explants more meaningful. Silicone implants in murine models successfully sequestered both target analytes over a short duration, and these data encourages future work to investigate potential health impacts of silicone absorption *in vivo* as a potential sink for organic contaminants.

5.6 Acknowledgements

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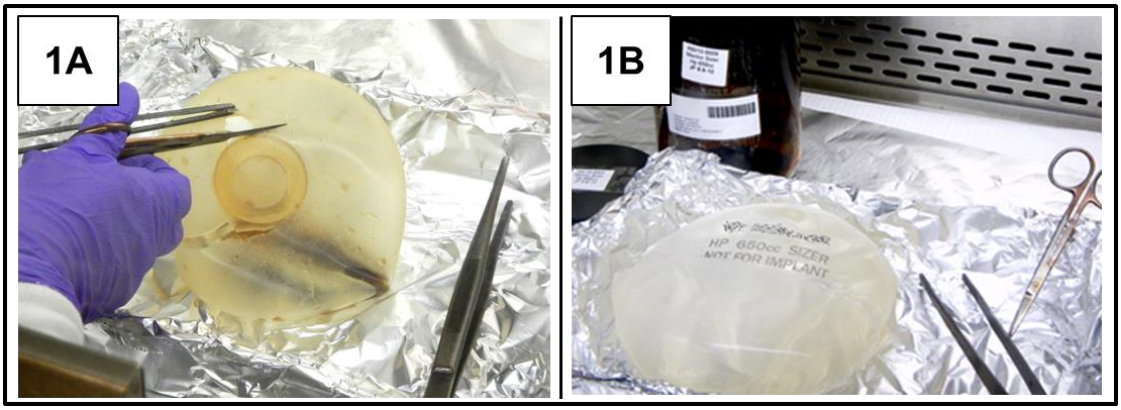

Figure 5.1 Silicone explant (A) and sizer (B). Sizers served as negative controls for organic compound background due to silicone manufacturing.

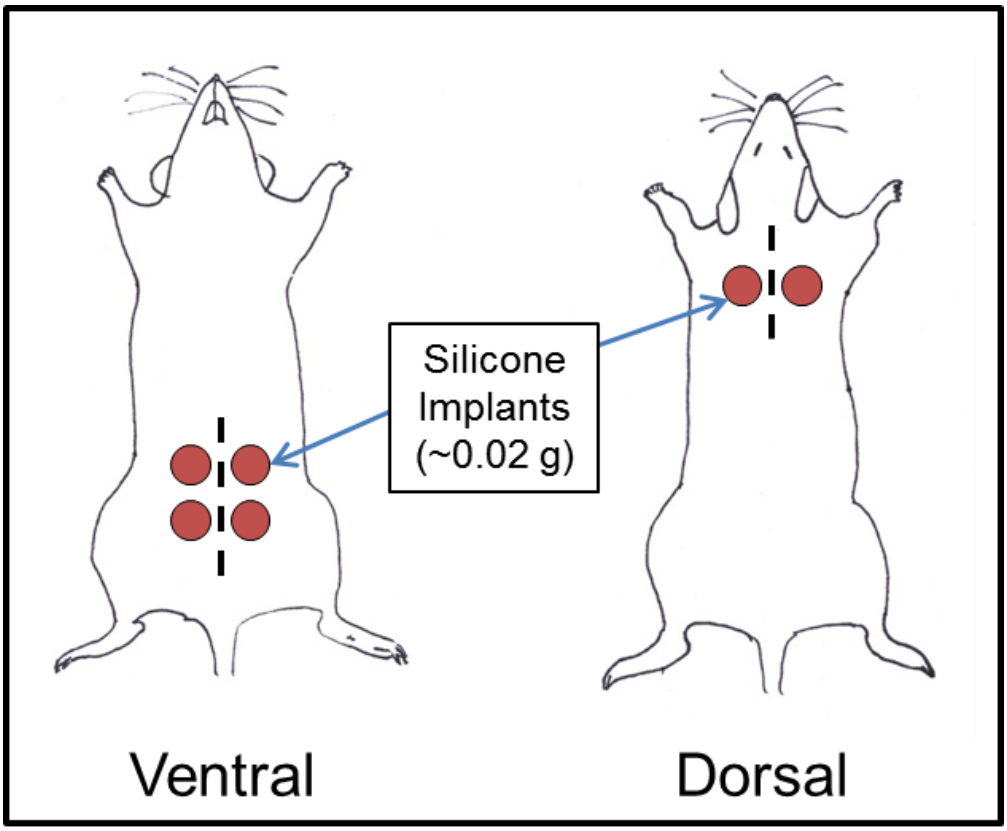

Figure 5.2 Silicone inserts in dorsal and ventral locations. All graphic representation is approximate and not to scale. Dashed lines represent approximate locations of incisions.

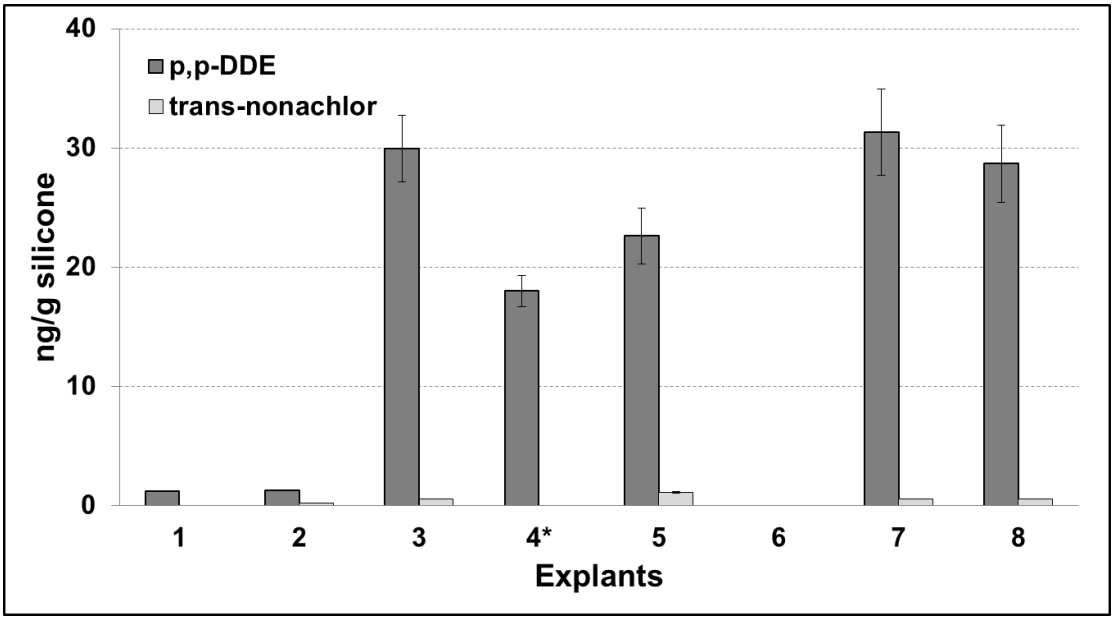

Figure 5.3 Normalized concentrations of contaminants found in eight explants (#1-8). Sub-sections (n=2) were extracted separately for each explant unless otherwise noted. One standard deviation is graphed for explants with at least two sub-sections with identified pollutants. *indicates n=3

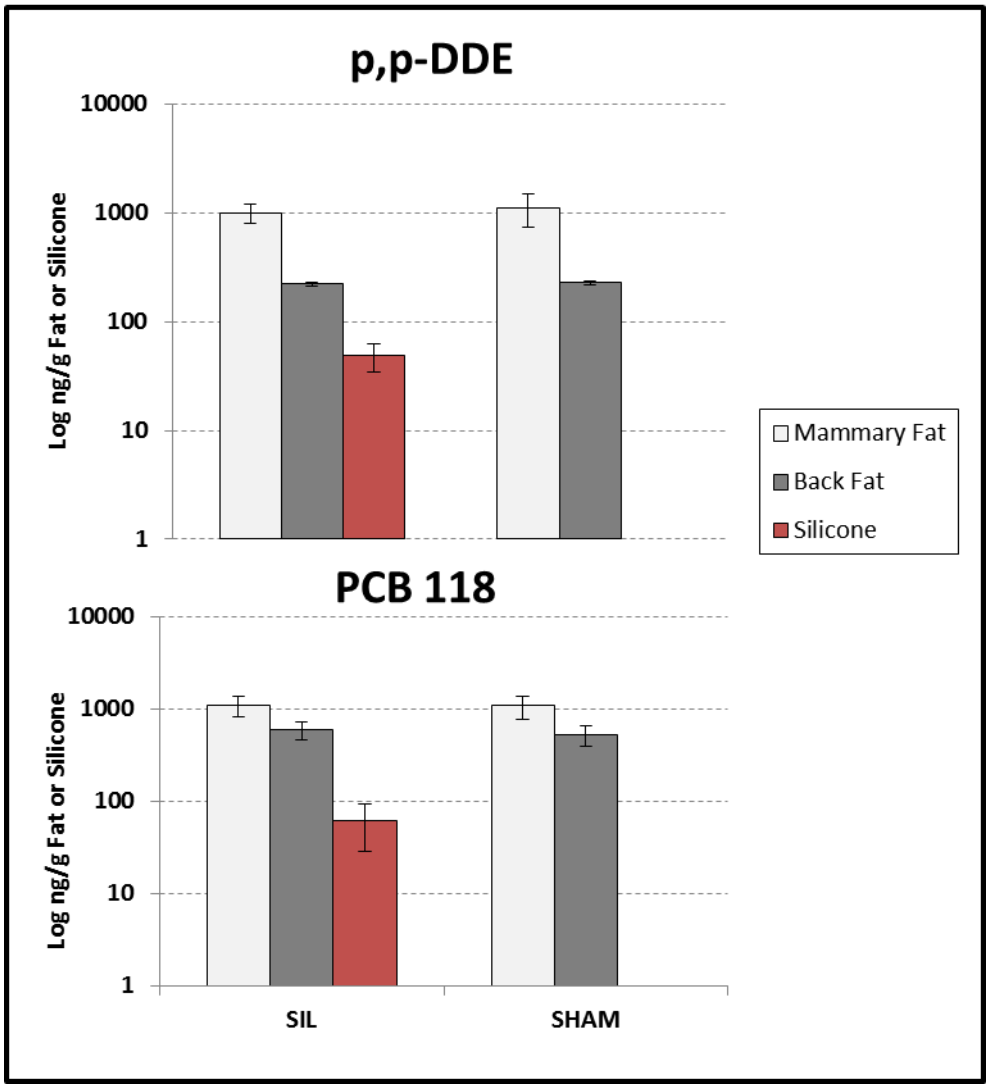

Figure 5.4 Concentrations of target compounds in silicone and surrounding tissues after one week from an IP injection. A) Relative log concentrations of p,p'-DDE in ICR mice given compounds either with or without silicone. No detectable concentrations of p,p'-DDE were observed in mice given vehicle. B) Relative log concentrations of PCB 118 in ICR mice given compounds either with or without silicone. No detectable concentrations of PCB 118 were observed in mice given vehicle.

Table 5.1 Percent uptake in one week into silicone from initial amounts injected into mice

Mouse #	p,p'-DDE			PCB 118		
	ng/Mouse	ng/Silicone	% uptake	ng/Mouse	ng/Silicone	% uptake
4	5936	4.7	0.08%	4480	5.3	0.12%
5	5830	8.3	0.14%	4400	6.4	0.15%
6	5936	6.5	0.11%	4480	8.8	0.20%
13	5936	9.5	0.16%	4480	15	0.33%
14	6572	4.2	0.06%	4960	2.7	0.05%
15	5300	7.8	0.15%	4000	9.0	0.23%
AVG	5918	6.8	0.12%	4467	7.9	0.18%

Table 5.2 Predicted adipose concentrations from *in vivo* silicone implants from mouse and human data

Murine	**p,p-DDE**				**PCB 118**			
Sample	ng/g Silicone	Predicted Adipose[a]	Dorsal Measured	Ventral Measured	ng/g Silicone	Predicted Adipose*	Dorsal Measured	Ventral Measured
M5	8.3	150	230	1020	6.4	230	680	1286
M6	6.5	120	N/A	1200	8.8	310		1337
M13	9.5	180	230	N/A	15	530	650	
M14	4.2	78	N/A	730	2.7	95		711
M15	7.8	140	210	1060	9.0	320	450	1072

Human	**p,p-DDE**					
				Median (range) ng/g adipose tissue in literature		
Samples	ng/g Silicone	Predicted Adipose[a]	Predicted Adipose (mouse data, dorsal - ventral)	Tabasco, Mexico[b] (n=150)	Granada Province, Spain[c] (n=387)	Antwerp, Belgium[d] (n=52)
E3-5, E7-8	18-32	330-580	470-4700	877 (50-5000)	93 (2.0-2300)	141 (15-8399)

[a]Based on silicone/mammalian oil partition coefficients (Jahnke et al., 2008 *(133)*)

[b]Waliszewski et al., 2012 *(146)*

[c]Arrebola et al., 2013 *(145)*

[d]Malarvannan et al., 2013 *(147)*

Chapter 6 – Discussion and Future Directions

In sum, this dissertation describes the successful use of silicone rubber as a passive sampling device for emerging compounds as exemplified by quantitative analysis of OPAHs, and through a variety of applications involving aqueous, atmospheric, and *in vivo* environments. Beginning with OPAH method development, improved sensitivities, optimization strategies, and the successful validation of two independent methods containing a large number of OPAHs were demonstrated. In total, 24 target OPAHs were able to be quantified, with the GC-MS method preferred over the LC-MS method. The GC-MS drastically reduces the likelihood of false positives that may be present in complex mixtures, and inter-day variability was also lower when compared to LC-MS methodology. Standard addition experiments helped to justify the choice of a labeled OPAH compound as an internal instrumental standard. Critical areas for future work can proceed by: 1) using additional labeled OPAHs for laboratory surrogate corrections based on empirical estimates; 2) adding more OPAHs to the target list based on recent environmental or toxicological data; and 3) exploring vicinal quinone behavior in other analytical systems. Adding more labeled surrogates will make either method more robust and accurate when analyzing complex matrices, and adding more relevant OPAHs to the target compound list aids in addressing one of the core questions of toxicology concerning the presence of chemicals in the environment. The final avenue is likely the most challenging, since vicinal quinones are difficult to consistently quantify in complex mixtures. Understanding the surface chemistry interactions that cause the variability will help other researchers add these compounds to their own OPAH methods.

Silicone passive samplers sequestered more OPAHs consistently than LDPE in Portland Harbor Superfund, and three pesticides predictably differed between the polymers based on log K_{ow}. Both polymers consistently sequestered 17 PAHs, and the ratio of individual analytes was conserved between polymers. Phenanthrene,

fluoranthene, and pyrene comprised the majority of ΣPAHs (56 to 84% in silicone, 67 to 81% in LDPE), and both polymers would have resulted in similar descriptions of field sites if used solely for Portland Harbor characterization for PAHs or OPAHs. However, silicone appears more appropriate for OPAHs especially for 9-fluorenone, benzanthrone, and 5,12-napthacenequinone. Overall differences were reconciled to average 3.5-fold or less (individual or ΣPAH) once both polymer extracts were calculated to water concentrations in ng/L, and likely will agree further once partition coefficients are determined for OPAHs. In total, this aqueous PSD work advances methods for using silicone passive samplers alone or in conjunction with LDPE. Future work should concentrate on two additional areas: 1) determine partition coefficients for a large range of semi-polar analytes for silicone, especially OPAHs, and 2) explore partitioning differences further between silicone and LDPE for predictive modeling. If certain physiochemical parameters can reliably predict partitioning of unknown chemicals, then researchers may take advantage of the most suitable polymer for recently discovered toxicants of concern prior to expensive field deployments.

Atmospheric sampling using silicone resulted in a wide range of chemicals sequestered, and represents a wide diversity of bioavailable compounds from unique participant exposures. In the ambient pilot study, 49 different compounds were identified, including PAHs, consumer products, personal care products, pesticides, phthalates, and other industrial compounds. Some compounds came as a surprise, and are hypothesized to have been sequestered from the skin and/or sweat. Silicone samplers from a focused occupational study with roofing professionals working with hot asphalt sequestered PAHs in every sampler, and two OPAHs (benzofluorenone and fluorenone) were able to be identified as well. The personal monitors were also found to be temporally sensitive between 8 and 40 hours ($p<0.05$, power = 0.85), and spatial

sensitivity was observed between two roofing study sites ($p<0.05$, power = 0.99). Silicone personal samplers present an innovative sampling technology platform producing relevant, quantifiable data that may be used in the future to assess occupational concerns about personal protection equipment, or compared with exposure limits and compliance measurements through *in situ* calibration. Several additional avenues of research are capable of progressing this platform of exposure sampling: 1) use isotope-labeled, or deuterated compounds to back calculate polymer concentrations to atmospheric values; 2) explore skin and sweat uptake into silicone wristbands for potential biomarker research or for compounds that are less volatile but of environmental concern; 3) expand analytical methods for sequestered analytes from qualitative wristband sampling data; and 4) compare silicone samplers to other passive or active devices to increase potential usage among other researchers. Ultimately, this application of silicone may become a valuable tool to address challenges of mixture toxicity unique to an individual.

In the final application of silicone, human implants and laboratory manufactured silicone implants in mice were able to sequester lipophilic compounds *in vivo*. Out of eight human implant samples, five contained measurable amounts of p,p-DDE ranging from 1.2-35 ng/g which is strikingly similar to the range in a separate study of silicone prostheses with a range of 0.2-37 ng/g *(60)*. Also in both studies, p,p-DDE was the highest contaminant observed out of multiple pesticides, PCBs, and brominated dioxins *(60)*. Using explant data, concentrations were observed in silicone implants inserted into mice after intraperitoneal injection of both p,p-DDE and PCB 118. Even though surrounding tissues did not significantly differ despite sequestration of target compounds silicone, *in vivo* absorption in murine models still provided interesting predictive values of adipose tissue concentrations in humans in line with observed

literature values. Overall, human explants may be able to be used as long term monitoring devices of organic contaminants rather than other transient or analytically difficult tissue samples. Future directions should focus on: 1) continuing to characterize silicone absorption of pollutants *in vivo*, specifically addressing equilibrium times of contaminants from tissue to silicone; 2) develop adequate siloxane cleanup from breast implants, and 3) ultimately use silicone *in vivo* sampling for investigating the role of environmental and dietary compounds with respect to toxicological outcomes.

In total, this dissertation addresses the simple question of "what chemicals are present" by creating new methods for an emerging compound class, and using silicone passive samplers to improve upon passive sampling technologies to monitor chemicals in aqueous, atmospheric, or *in vivo* environments.

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Appendices

Appendix 1 – Chapter 2: An Analytical Investigation of 24 Oxygenated-PAHs (OPAHs) using Liquid and Gas Chromatography-Mass Spectrometry

Figure A.1.1 is a representative OPAH calibration curve on the GC-EI/MS method. Concentration curves used in the study spanned 4 orders of magnitude using a 9-point curve, so all compounds were quantitated using quadratic models from ChemStation (Agilent) software. All OPAHs showed at least some non-linearity over the concentration range used in the study.

Figure A.1.2 shows average stability over 17 weeks for each individual OPAH on each method. Each time point consisted of three individual aliquots except for time point (0) for the LC-MS (n=1). Variability is represented by one standard deviation, and SigmaPlot™ (Systat Software, Inc., San Jose, CA) was used to model linear regressions for each stability series. Only 1,2-ANAPQ showed a significant decrease over the study period ($p<0.05$), but this result was later refuted by the data shown in **Figure 1.2d** and described in the main text.

Table A.1.1 lists quantitated values in mg Kg^{-1} of OPAHs for each method compared to NIST certificate of analysis values (COAs) [28] and to Layshock et al. 2009 [17]. Our purpose here is to show current individual quantitated values, comparisons made between the three bodies of work, and to demonstrate each method in our research as able to quantitate OPAHs in real matrices. Comparisons to NIST COAs have been originally documented in Layshock et al., 2009 [17], and extracts shown here were re-quantitated in 2012 from the original extraction date in 2009.

Figure A.1.3 represents additional evidence of suppression of GC-EI/MS data by silicone PSD background in an un-deployed sample. Standard addition values for all 5 OPAHs originally suppressed in **Figure 1.5** in the main text are all within 20% of the true value. However, IS quantitated values are all below 80% of the true value, and in the case of 1,4-B[c]PHEQ, an order of magnitude lower (IS-50 ng mL^{-1}; true value-500 ng mL^{-1}). Current work with silicone PSDs in our laboratory have shown more efficient cleaning processes as effective for removing silicone suppression of OPAHs (data not shown).

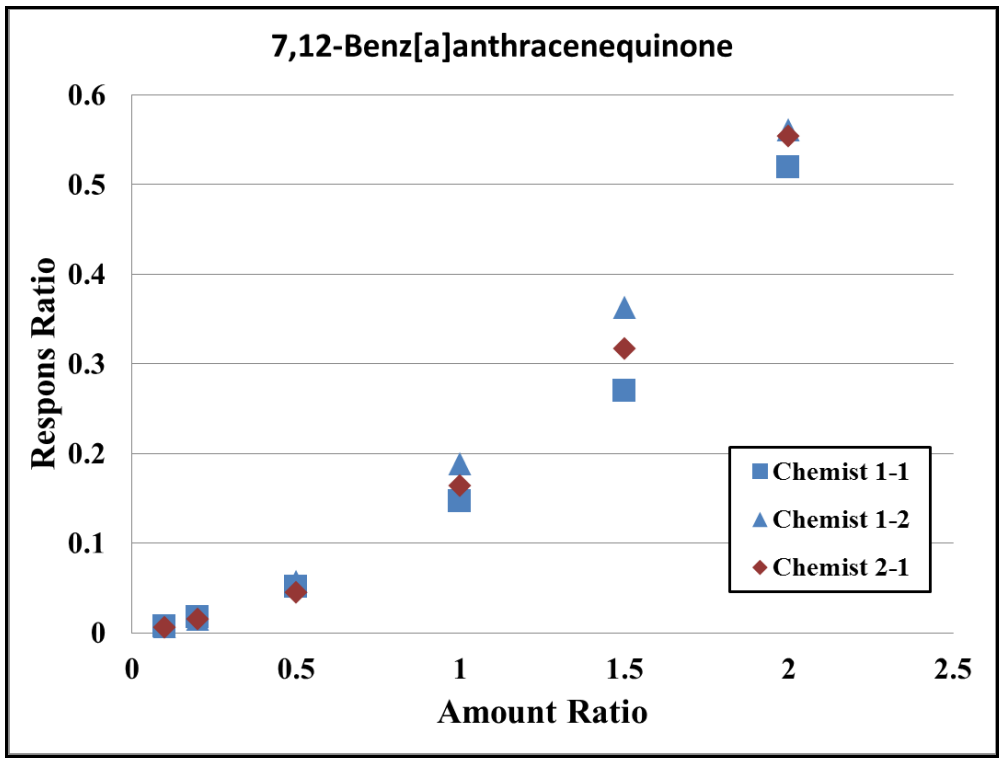

Figure A.1.1 Three independent calibration series from two chemists using the GC-MS. Colors correspond to the individual who made the calibration series, and each symbol represents a separately prepared series. The concentration range shown is 50-1000 ng mL^{-1} to show non-linearity within an order of magnitude.

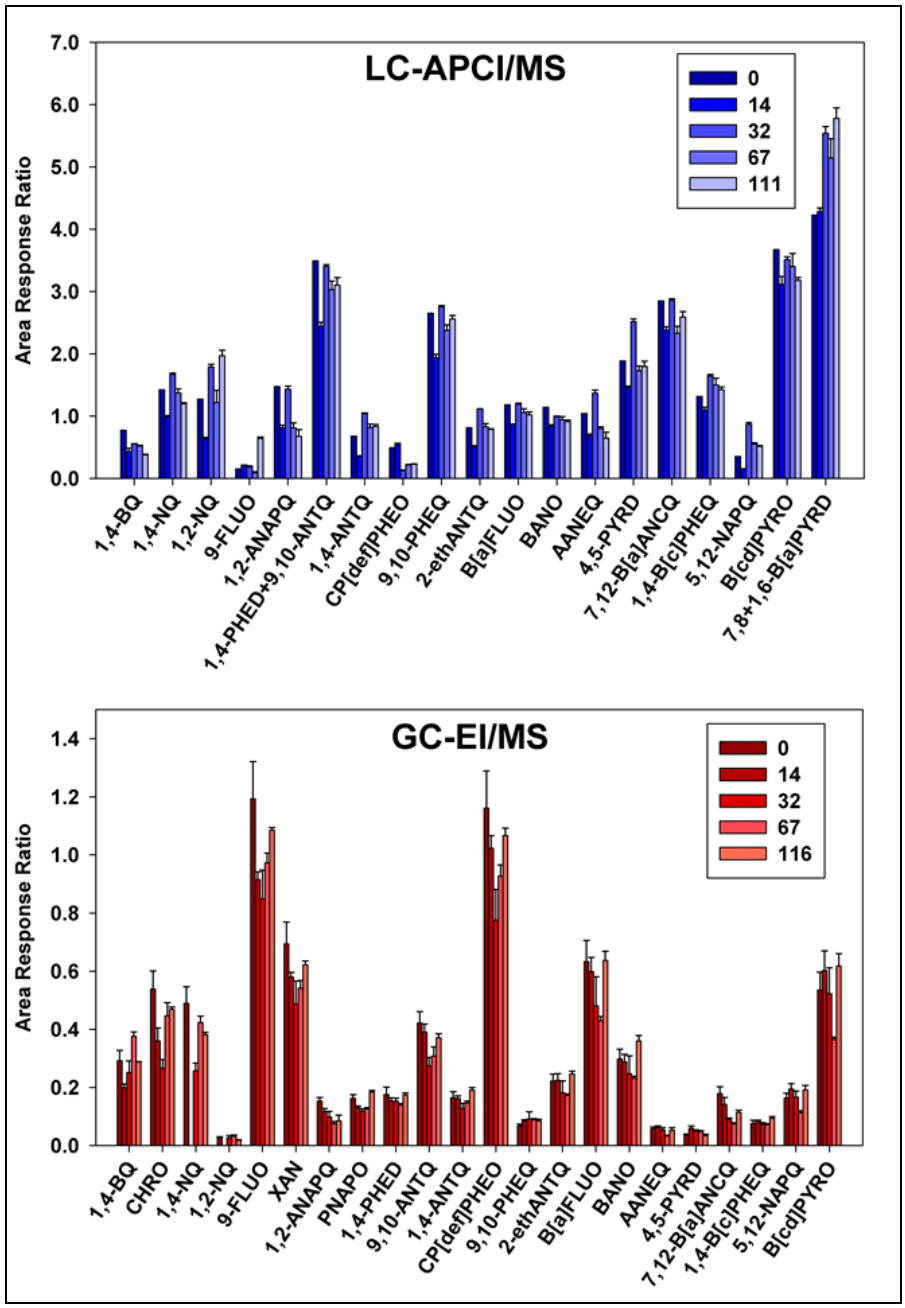

Figure A.1.2 Average stability (n=3) of OPAHs on the GC and LC-MS methods over 116 and 111 days respectively.

Table A.1.1 Comparison to Layshock et al., 2009 and NIST informational values in mg Kg^{-1} of sample. Compounds and values highlighted in green have not been published in either Layshock et al., 2009 or in NIST certificates of analysis (COA).

	Urban Dust SRM 1649b				Diesel Extract SRM 1975			Diesel Particulate Matter SRM 1650b		
OPAHs	This Study GCMS	This Study LCMS	Layshock et al. 2009	NIST	This Study GCMS	This Study LCMS	Layshock et al. 2009	This Study GCMS	This Study LCMS	Layshock et al. 2009
1,4 Benzoquinone	<LOQ	<LOQ	N/A	N/A	<LOQ	<LOQ	N/A	<LOQ	<LOQ	N/A
Chromone	<LOQ	N/A	N/A	N/A	<LOQ	<LOQ	N/A	<LOQ	<LOQ	N/A
1,4 Naphthoquinone	(0.25)	0.30	N/A	N/A	<LOQ	0.51	N/A	<LOQ	1.1	N/A
1,2-Naphthoquinone	(2.8)	<LOQ	N/A	N/A	(5.2)	<LOQ	N/A	<LOQ	<LOQ	N/A
9-Fluorenone	0.76	1.2	0.74	1.4	2.6	2.3	2.6	19	18	25
Xanthone	0.19	N/A	N/A	N/A	<LOQ	<LOQ	N/A	6.2	<LOQ	N/A
Acenapthenequinone	<LOQ	<LOQ	<LOQ	N/A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Perinaphthenone	<LOQ	N/A	N/A	N/A	<LOQ	<LOQ	N/A	150	<LOQ	N/A
Phenanthrene-1,4-dione	<LOQ	<LOQ	<LOQ	N/A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
9,10-Anthraquinone	2.6	1.3	1.5	1.8	8.1	2.2	5.4	64	21	54
1,4-Anthraquinone	<LOQ	<LOQ	<LOQ	N/A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
4H-cyclopenta[def]phenanthren-4-one	0.73	1.5	0.60	N/A	7.0	6.0	7.5	9.2	7.6	3.4
9,10-phenthrenequinone	<LOQ	<LOQ	<LOQ	N/A	<LOQ	<LOQ	<LOQ	<LOQ	2.6	<LOQ
2-Ethylanthraquinone	(0.51)	0.93	N/A	N/A	<LOQ	0.51	N/A	4.4	19	N/A
Benzofluorenone	0.78	0.97	1.6	N/A	1.8	1.7	3.2	18	17	16
Benzanthrone	1.18	0.62	3.9	1.6	1.5	0.62	4.8	23	13	39
Aceanthracenequinone	<LOQ	<LOQ	<LOQ	N/A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Pyrene 4,5 dione	<LOQ	<LOQ	N/A	N/A	<LOQ	<LOQ	N/A	<LOQ	<LOQ	N/A
7,12-benz[a]anthracenquinone	1.4	1.1	3.0	3.6	3.4	2.1	6.9	5.7	5.1	9.2
Benzo[c]phenanthrene-[1,4]quinone	0.20	0.32	<LOQ	N/A	<LOQ	<LOQ	<LOQ	<LOQ	27	<LOQ
5,12-Napthacenequinone	0.72	0.80	2.0	N/A	<LOQ	<LOQ	0.81	<LOQ	7.1	<LOQ
Benzo(cd)pyrenone	0.56	0.33	1.7	N/A	0.71	<LOQ	2.2	5.0	2.5	10.4
Benzo[a]pyrene-7,8+Benzo[a]pyrene-1,6	N/A	<LOQ	N/A	N/A	N/A	<LOQ	N/A	N/A	1.6	N/A
ΣOPAHs	**13**	**9.0**	**15**	**8.4**	**30**	**16**	**33**	**304**	**140**	**158**

Abbreviations: SRM – Standard reference material, <LOQ – below limit of quantitation for that study, N/A – Not applicable

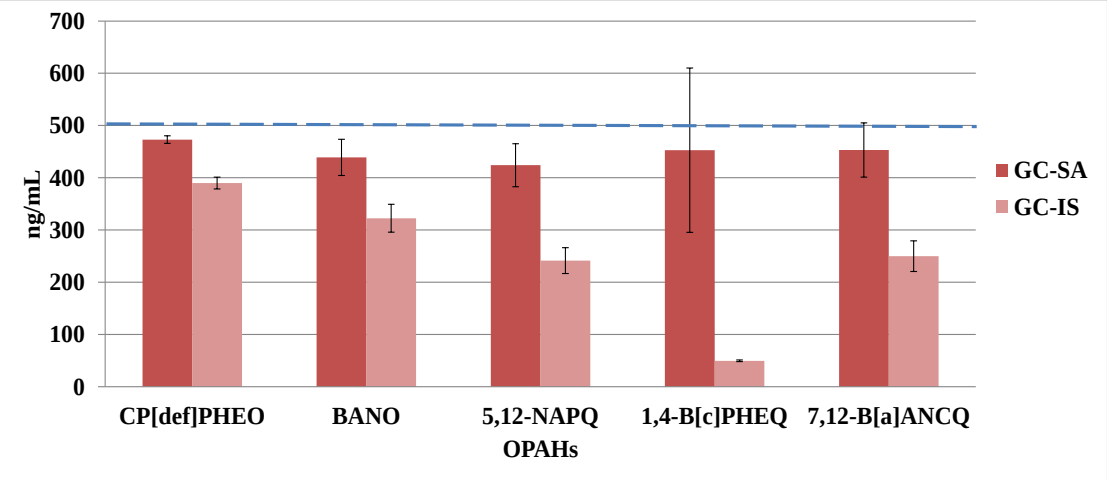

Figure A.1.3 Standard addition and internal standard quantitation on a non-deployed silicone sample using the GC-EI/MS method. Since the silicone had no background, the true value of the overspikes and addition should be 500 ng mL^{-1}, and is represented by the dashed blue line.

Appendix 2 – Chapter 3: Improvements in Pollutant Monitoring: Optimizing Silicone for Co-deployment with Polyethylene Passive Sampling Devices

Calculation Information

Compound uptake during deployment was not assumed to be in any particular phase (kinetic, linear, or equilibrium, and no assumptions are necessary with the following modified Huckins equation:

$$\boldsymbol{Eq\ 1.}\ C_w = \frac{N_{analyte}}{M_s K_{sw}\left(1 - \exp\left(-\frac{R_s t}{M_s K_{sw}}\right)\right)}$$

Where $N_{analyte}$ is the concentration of the analyte in the polymer, M_s is the mass of the polymer, K_{sw} is the partition coefficient, R_s is the sampling rate, and t is time (in days). In this modified equation from the Huckins' original, mass is exchanged with the volume of the polymer since the partition coefficient models were based on mass and not volume.

Additional Pre-cleaning Information

The initial cleaning method used five exchanges of ethyl acetate, and the pre-cleaning solvent was found to have fewer siloxane peaks on a full-scan LCMS method with each subsequent soaking period (**Figure A.2.1A**). However, the silicone itself was still found to have considerable chromatographic interference with OPAH analyses *(86)*. A successful cleaning process included hexanes in addition to ethyl acetate, and reduced siloxane background peaks with shorter exchange times than the original process (48h versus 120h; **Figure A.2.1B**).

AMDIS Qualitative Results Among Polymers

A total of 30 compounds were identified using AMDIS from the three remaining silicone polymers and LDPE. Overall, identified compounds represented a wide range of physicochemistry (log K_{ow} - 2.0, ethiolate to 9.5, di-n-nonyl phthalate). **Table A.2.2** lists several commercial compounds identified using the software, including pesticides such as N,N-diethyl-m-toluamide (DEET), propiconazole, and oxadiazon, as well as phthalates and phosphates. Silicones sequestered those compounds with lower octanol-water coefficients than LDPE with a marked difference at a log K_{ow} of 4.9 (**Table A.2.2**). This is consistent with another study which reported preferential sequestration of lower K_{ow} compounds for silicone when compared to LDPE, even when using the same solvent for extraction *(48)*. Allan has also reported differences between silicone and LDPE around a log K_{ow} of 4.5 *(47)*, although this comparison only included PAHs.

Polymer Modeling

In other work*(48)*, Allan et al., 2013 showed that the ratio of concentrations of LDPE and silicone was fairly predictable along a Kow axis. Specifically, the higher the Kow of an analyte, the more likely LDPE would have a higher concentration of analytes over that of silicone. Below is a similar investigation, but includes other parameters (polar and non-polar properties) that logically follow from the argument in the introduction that the chemical structure of each polymer helps explain passive uptake. Although the model is slightly better with these parameters, much of the variance is left to be explained, and could involve other physical properties like steric hindrance or planarity.

Table A.2.1 Target, surrogate, performance reference compounds (PRCs), and Internal Standard (IS) lists for quantitative analytical methods used throughout this study.

Method	Compound	Target, IS, PRC or Surrogate	Kow	CAS	2010 Samples
OPAHs	2-Fluoro-9-Fluorenone^{13}C*	IS	*3.8*	334973-78-3	
	9,10-Anthraquinone-d8	PRC	*3.4*	10439-39-1	
	2-Methyl-1,4-Naphthoquinone-d8*	Surrogate	*2.2*	478171-80-1	
	9-Fluorenone-d8	Surrogate	*3.6*	137219-34-2	
	Benzoquinone	Target	0.2	106-51-4	
	Chromone	Target	1.4	491-38-3	
	1,4-Naphthoquinone	Target	1.7	130-15-4	
	Acenaphthenequinone	Target	2.0	82-86-0	
	1,2-Naphthoquinone*	Target	2.1	524-42-5	
	9,10-Phenanthrenequinone	Target	2.5	84-11-7	
	1,4-Phenanthrenedione*	Target	2.8	569-15-3	
	1,4-Anthraquinone*	Target	2.8	635-12-1	
	Xanthone	Target	3.4	90-47-1	
	Perinaphthenone*	Target	3.4	548-39-0	
	9,10-Anthraquinone	Target	3.4	84-65-1	
	9-Fluorenone	Target	3.6	486-25-9	
	1,4-Benzo[c]phenanthrenequinone*	Target	3.6	109699-80-1	
	Cyclopenta[def]phenanthrenone	Target	4.1	5737-13-3	
	Aceanthracenequinone*	Target	4.2	6373-11-1	
	4,5-Pyrenedione*	Target	4.2	6217-22-7	
	2-Ethylanthraquinone	Target	4.4	84-51-5	
	7,12-benz[a]anthracenquinone	Target	4.4	2498-66-0	
	5,12-Naphthacenequinone*	Target	4.5	1090-13-7	
	Benzofluorenone*	Target	4.7	479-79-8	
	Benzanthrone	Target	4.8	82-05-3	
	Benzo[cd]pyrenone*	Target	5.3	3074-00-8	
Method	Compound	Target, IS, PRC or Surrogate?	Kow	CAS	2010 Samples
PAHs	Perylene-D12	IS	*6.3*	1520-96-3	x
	Fluorene-D10	PRC	*4.2*	81103-79-9	x
	Benzo[b]fluoranthene-D12	PRC	*6.0*	93951-98-5	x

	Naphthalene-D8 SS	Surrogate	*3.3*	1146-65-2	x
	Acenaphthylene-D8 SS	Surrogate	*4.1*	93951-97-4	x
	Phenanthrene-D10 SS	Surrogate	*4.5*	1517-22-2	x
	Fluoranthene-D10 SS	Surrogate	*4.9*	X1070248-2	x
	Chrysene-D12 SS	Surrogate	*5.2*	1719-03-5	x
	Benzo[a]pyrene-D12 SS	Surrogate	*6.1*	63466-71-7	x
	Benzo[ghi]perylene-D12 SS	Surrogate	*6.5*	X1070842-0	x
	Naphthalene	Target	3.3	91-20-3	x
	2-Methylnaphthalene	Target	3.9	91-57-6	x
	1-Methylnaphthalene	Target	3.9	90-12-0	x
	Acenaphthene	Target	4.0	83-32-9	x
	Acenaphthylene	Target	4.1	208-96-8	x
	Fluorene	Target	4.2	86-73-7	x
	1,6-Dimethylnaphthalene	Target	4.3	575-43-9	x
	1,8-Dimethylnaphthalene	Target	4.3	569-41-5	
	1,2-Dimethylnaphthalene	Target	4.3	573-98-8	x
	2,6-Dimethylnaphthalene	Target	4.3	581-42-0	
	1,4-Dimethylnaphthalene	Target	4.4	571-58-4	
	1,5-Dimethylnaphthalene	Target	4.4	571-61-9	
	2-Ethylnaphthalene	Target	4.4	939-27-5	
	Dibenzothiophene	Target	4.4	132-65-0	x
	Anthracene	Target	4.5	120-12-7	x
	Phenanthrene	Target	4.5	85-01-8	x
	2-Methylphenanthrene	Target	4.9	2531-84-2	x
	Pyrene	Target	4.9	129-00-0	x
	Fluoranthene	Target	4.9	206-44-0	x
	2-Methylanthracene	Target	5.0	613-12-7	x
	9-Methylanthracene	Target	5.1	779-02-2	x
	1-Methylphenanthrene	Target	5.1	832-69-9	x
	Chrysene	Target	5.2	218-01-9	x
	Benzo[c]fluorene	Target	5.2	205-12-9	
	2,6-Diethylnaphthalene	Target	5.3	59919-41-4	
	Benzo[a]fluorene	Target	5.4	238-84-6	
	2,3-Dimethylanthracene	Target	5.4	613-06-9	x
	3,6-Dimethylphenanthrene	Target	5.4	1576-67-6	x
	1-Methylpyrene	Target	5.5	2381-71-7	x
	Triphenylene	Target	5.5	217-59-4	

	Benz[a]anthracene	Target	5.6	56-55-3	x
	9,10-Dimethylanthracene	Target	5.7	781-43-1	x
	Cyclopenta[c,d]pyrene	Target	5.7	27208-37-3	
	Benzo[b]fluorene	Target	5.8	243-17-4	
	7,12-Dimethylbenz[a]anthracene	Target	5.8	57-97-6	
	Benzo[b]fluoranthene	Target	6.0	205-99-2	x
	Benzo[a]pyrene	Target	6.1	50-32-8	x
	Benzo[k]fluoranthene	Target	6.1	207-08-9	x
	5-Methylchrysene	Target	6.1	3697-24-3	
	6-Methylchrysene	Target	6.1	1705-85-7	x
	Benzo[j]fluoranthene	Target	6.1	205-82-3	
	Dibenzo[e,l]pyrene*	Target	6.2	192-51-8	
	Benz[j]aceanthrylene*	Target	6.3	202-33-5	
	Retene	Target	6.4	483-65-8	x
	Benzo[e]pyrene	Target	6.4	192-97-2	x
	Benzo[ghi]perylene	Target	6.5	191-24-2	x
	Indeno(1,2,3-c,d)pyrene	Target	6.6	193-39-5	x
	Dibenz[a,h]anthracene	Target	6.8	53-70-3	x
	Benzo[b]perylene*	Target	7.0	197-70-6	
	Anthanthrene	Target	7.0	191-26-4	
	Picene	Target	7.1	213-46-7	
	Dibenzo[a,e]fluoranthene	Target	7.3	5385-75-1	
	Dibenzo[a,e]pyrene	Target	7.3	192-65-4	
	Dibenzo[a,h]pyrene	Target	7.3	189-64-0	
	Dibenzo[a,i]pyrene	Target	7.3	189-55-9	
	Naphtho[1,2-b]fluoranthene*	Target	7.3	111189-32-3	
	Naphtho[2,3-a]pyrene*	Target	7.3	196-42-9	
	Naphtho[2,3-j]fluoranthene*	Target	7.3	205-83-4	
	Naphtho[2,3-k]fluoranthene*	Target	7.3	207-18-1	
	Naphtho[2,3-e]pyrene*	Target	7.3	193-09-9	
	Coronene	Target	7.6	191-07-1	
	Dibenzo[a,l]pyrene	Target	7.7	191-30-0	x

Method	Compound	Target, IS, PRC or Surrogate?	Kow	CAS	2010 Samples
Pesticides	4,4'-Dibromooctafluorobiphenyl*	IS	7.1	10386-84-2	
	p,p'-DDE D4	PRC	*6.5*	93952-19-3	

	Tetrachloro-m-xylene (TCMX)	Surrogate	5.7	877-09-8
	Decachlorobiphenyl	Surrogate	8.3	2051-24-3
	Dimethoate	Target	0.8	60-51-5
	Propachlor	Target	2.2	1918-16-7
	Captan	Target	2.8	133-06-2
	Chlorothalonil	Target	3.1	1897-45-6
	Metolachlor	Target	3.1	51218-45-2
	Terrazole	Target	3.4	2593-15-9
	Chloroneb	Target	3.4	2675-77-6
	Alachlor	Target	3.5	15972-60-8
	Prophos	Target	3.6	13194-48-4
	Endosulfan sulfate	Target	3.7	1031-07-8
	Lindane (g-BHC)	Target	3.7	58-89-9
	b-BHC	Target	3.8	319-85-7
	a-BHC	Target	3.8	319-84-6
	Captafol	Target	3.8	2425-06-1
	Endosulfan I	Target	3.8	959-98-8
	Endosulfan II	Target	3.8	33213-65-9
	Dacthal	Target	4.3	1861-32-1
	Diallate	Target	4.5	2303-16-4
	Chlorobenzilate	Target	4.7	510-15-6
	Endrin aldehyde	Target	4.8	7421-93-4
	Chlorpyrifos	Target	5.0	2921-88-2
	Heptachlor epoxide	Target	5.0	1024-57-3
	Endrin ketone*	Target	5.0	53494-70-5
	Methoxychlor	Target	5.1	72-43-5
	Endrin	Target	5.2	72-20-8
	Trifluralin	Target	5.3	1582-09-8
	Dieldrin	Target	5.4	60-57-1
	Hexachlorobenzene	Target	5.7	118-74-1
	p,p'-DDD	Target	6.0	72-54-8
	Heptachlor	Target	6.1	76-44-8
	a-Chlordane (cis)	Target	6.1	5103-71-9
	g-Chlordane (trans)	Target	6.2	5103-74-2
	Trans-Nonachlor	Target	6.4	39765-80-5
	Trans-permethrin*^	Target	6.5	51877-74-8
	Cis-permethrin*^	Target	6.5	54774-45-7

	Aldrin	Target	6.5	309-00-2
	p,p'-DDE	Target	6.5	72-55-9
	Isodrin	Target	6.8	465-73-6
	Mirex	Target	6.9	2385-85-5
	p,p'-DDT	Target	6.9	50-29-3
	Bifenthrin*	Target	8.2	82657-04-3

All compound information was obtained from the U.S. National Library of Medicine (http://toxnet.nlm.nih.gov/; accessed February, 2014) unless otherwise noted.

*Denotes that K_{ow} value for this compound are calculated from U.S. EPA EpiSuite software

*^U.S. EpiSuite software was used, but K_{ow} values are based on experimental data.

Italics are used when K_{ow} values were assumed to be similar to the non-labeled homologue.

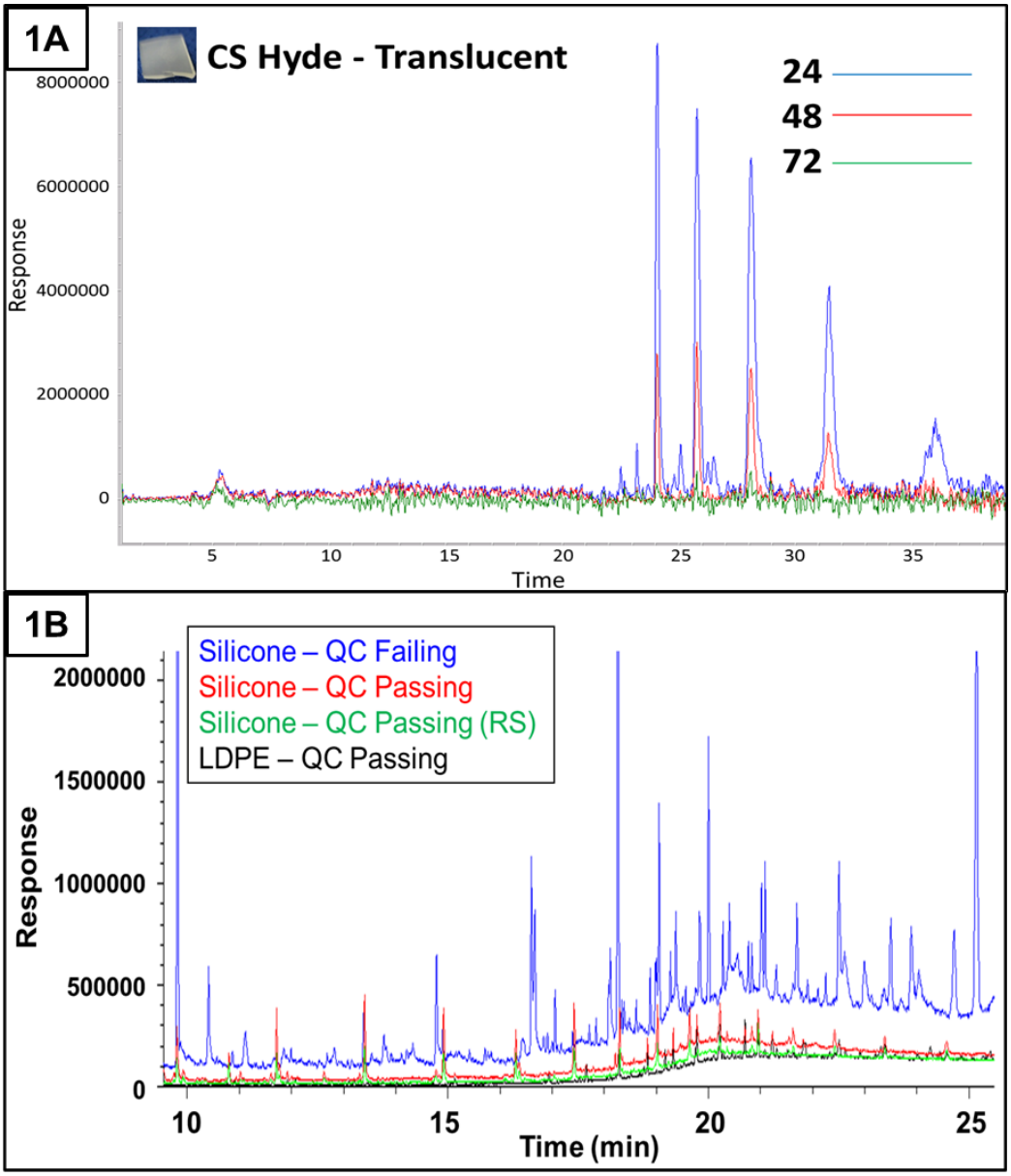

Figure A.2.1 Improvements in pre-cleaning among polymers. **1A** -reduction of impurities in solvents over three exchanges and 72 hours for CT silicone; **1B**-final optimization of pre-cleaning process using recycled solvents shows little difference in overall background between silicone (green chromatogram) and LDPE (black chromatogram). Failing QC was defined as having at least one peak with a 15-fold greater response (area count) over a 500 ng/mL standard. Abbreviations: QC – quality control sample, RS – recycled solvents used for background cleaning.

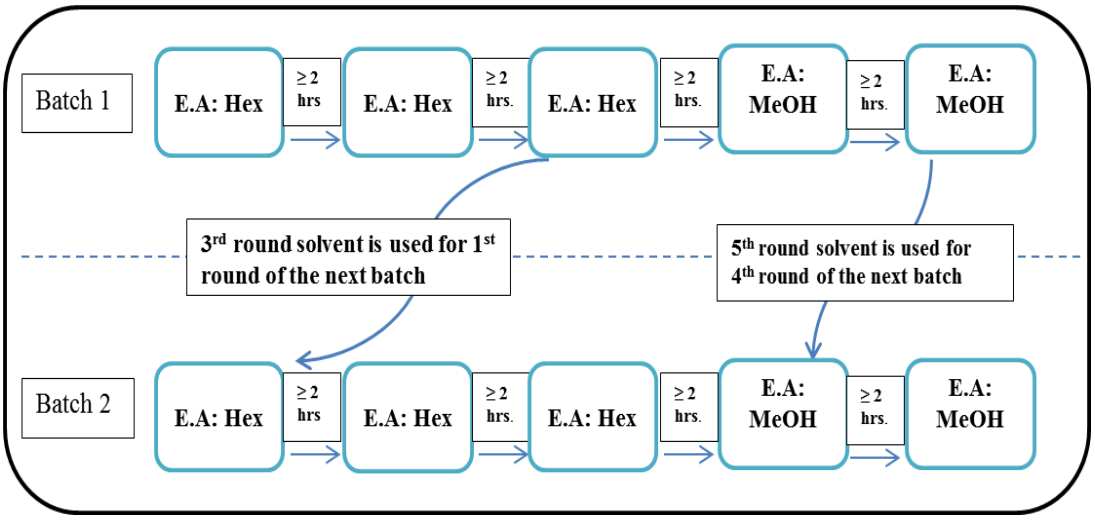

Figure A.2.2 Solvent recycling scheme to reduce 20% of total mixed solvents without losing cleaning effectiveness (see **Figure A.2.1**).

Table A.2.2 Comparison of polymers and analytes identified with the AMDIS method in Portland Harbor Superfund, OR. Compounds are listed in order of log K_{ow}.

Compounds	MW	log K_{ow}	CAS	CS[a]	ST[a]	SS[a]	LDPE[b]
Ethiolate	161	2.0	2941-55-1			X	
N,N-Diethyl-m-toluamide	191	2.2	134-62-3		X		
Propiconazole-I	342	3.7	60207-90-1	X	X		
Tris(2-butoxyethyl) phosphate	398	3.8	78-51-3	X	X	X	
Tributyl phosphate	266	4.0	126-73-8	X	X	X	
Diisobutyl phthalate	278	4.1	84-69-5		X		
Triphenyl phosphate	326	4.6	115-86-6	X	X		
Butyl benzyl phthalate	312	4.7	85-68-7		X		
Piperonyl butoxide	338	4.8	51-03-6		X	X	
Oxadiazon	345	4.8	19666-30-9		X	X	
Di-n-butylphthalate	278	4.9	84-74-2	X		X	X
Tonalide	258	5.7	21145-77-7	X	X	X	X
Hexachlorobenzene	284	5.7	118-74-1				X
Dicyclohexyl phthalate	330	6.2	84-61-7	X	X	X	X
Bis(2-ethylhexyl)phthalate	390	7.6	117-81-7	X	X	X	X
Di-n-nonyl phthalate	418	9.5	84-76-4	X	X	X	

[a]Denotes that one strip was used in each extract.
[b]Denotes five strips were used for each LDPE extract (this deployment only).

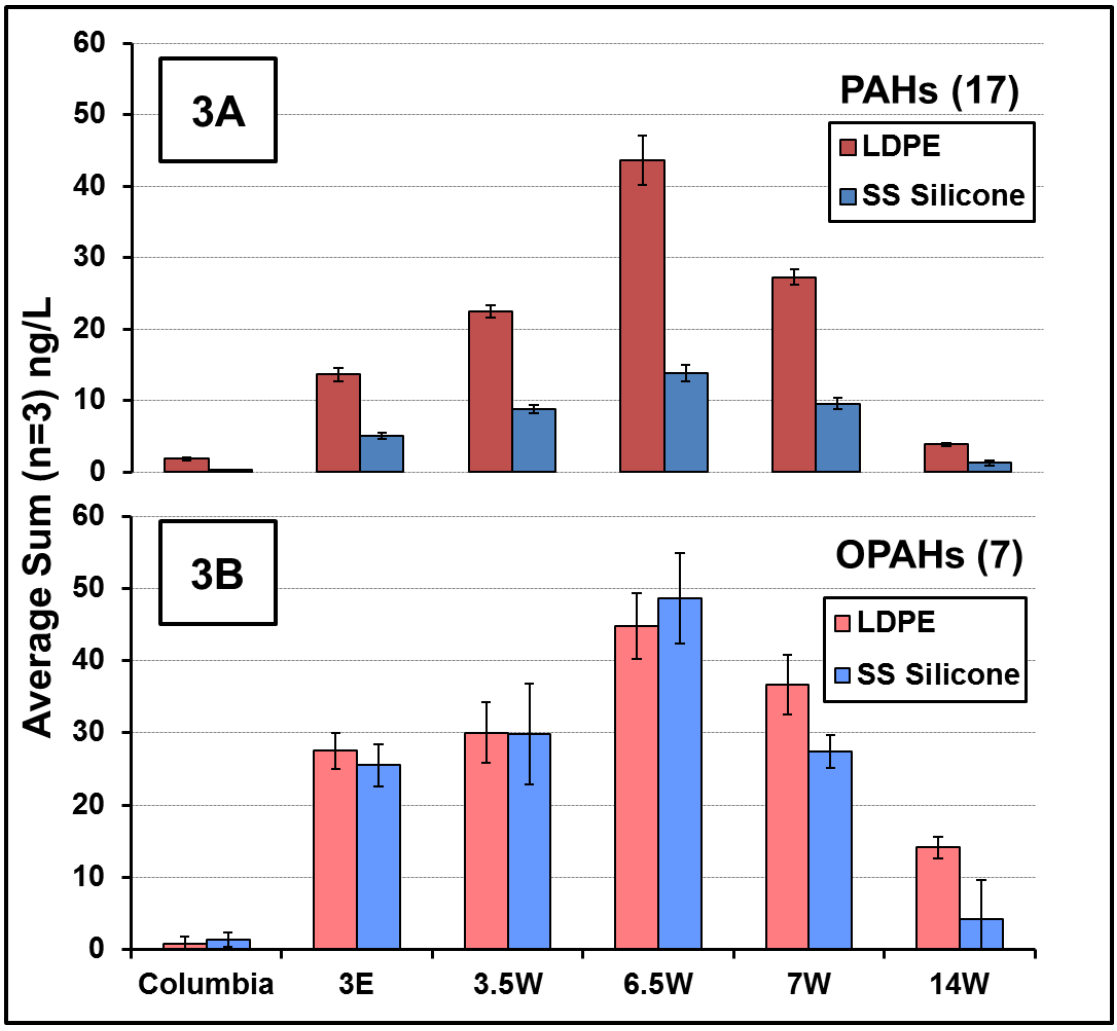

Figure A.2.3 Average (n=3) calculated water concentrations of PAHs (3A) and OPAHs (3B) for silicone and LDPE from a 22 day deployment in and near Portland Harbor Superfund, OR in 2011.

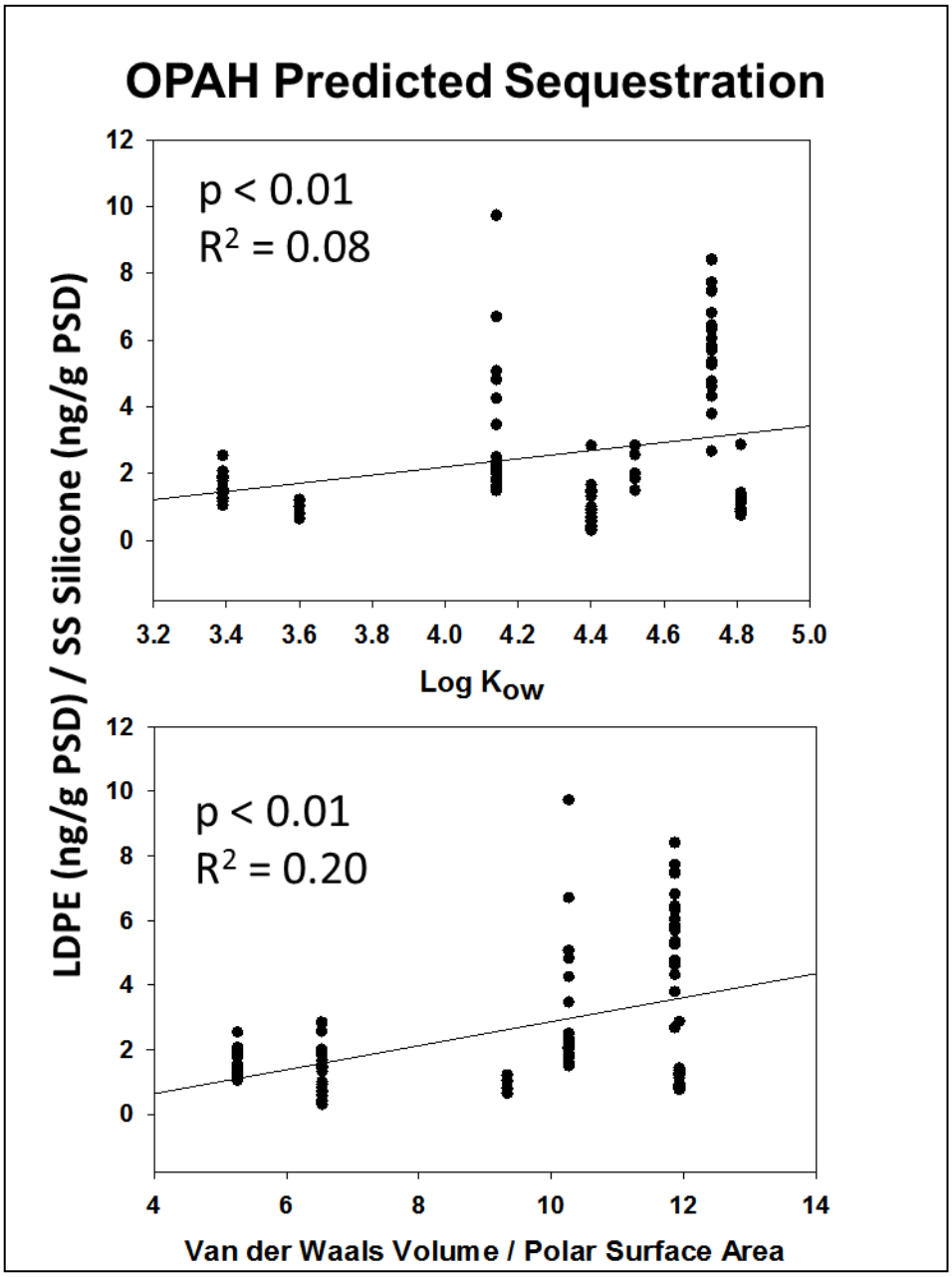

Figure A.2.4 Log K_{ow} does not fully predict sequestration of OPAHs between LDPE and SS silicone. The data is the a ratio of normalized polymer concentrations. Physiochemical parameters were obtained from www.chemicalize.org

Appendix 3 – Chapter 4: Silicone Wristbands as Personal Monitoring Devices

Addtitional Roofing Informtation

At the training facility, roofers spent 8 hours training and applying hot mopping-grade asphalt on a practice surface. On each workday at the rooftop, roofers spent four hours tearing down an old roof and four hours applying asphalt to create a new roof.

SI Figure Explanations

Extraction efficiency was determined by quantitatively analyzing subsequent rounds of extraction (100 mL ethyl acetate) from a wristband that had been infused with several compounds (four labeled PAHs) (**Figure A.3.1**). Differences in extracted amount did not differ between the two time treatments ($p=0.78$). Therefore, extractions for all silicone PSDs in this study were done with at least two rounds of extraction for at least two hours.

In **Figure A.3.2**, there is a pictorial demonstration of post-deployment cleaning. Surface particulates that are not sequestered as part of the vapor phase are removed prior to extraction with two rounds of ultra-pure water and one quick (less than 10 seconds) rinse with isopropyl alcohol which visibly removed much of the particulate matter. Each wristband was cleaned in a similar fashion including trip blanks. Water was not replaced during the cleaning of the wristbands since the fugacity of these hydrophobic compounds would not be primarily driven to the aqueous solution. However, the isopropyl alcohol was changed with each PSD. No carryover was observed during post-deployment cleaning.

Figure A.3.3 is a graphical representation of binary data from 49 compounds in the ambient demonstration. Positively identified compounds from AMDIS software were converted to binary identifiers and used in a non-metric multi-dimensional analysis to see if exposures appeared to be unique among individuals, or if all wristbands sequestered the same number and type of compounds. Several wristbands were worn by the same individual (optional choice by the participant), and are circled (graphical representation only) on **Figure A.3.3**. Although it is difficult to ascertain much from the data, it does appear that some exposures were more unique than others based on spatial differences on the graph (example: device #8 vs device #2). The total ion chromatogram of devices 13.1 and 13.2 had particularly high amounts of peaks that likely resulted from skin contact. Interferences from either month-long skin contact or an earlier cleaning process could at least partially explain poor resolution of partnered devices on the graph.

Figure A.3.4 shows the concentration remaining in wristbands (n=4) that were infused with 5 PAHs of varied physicochemistry and exposed for four hours in either direct sunlight or exposed in shade. The four hour exposure took place in September, 2013 between 11:30am - 3:30 pm on a clear day in Corvallis, Oregon. Silicone PSDs were placed on pre-rinsed foil on either an exposed aluminum 3 m rooftop, or underneath the roof between support beams. Distance between each set of samples was less than 2 meters, and shade exposed samples were protected from sunlight from above and below, but not from the sides so that air flow may be similar between each group. Data loggers (Onset Computer Corp., Bourne, MA) were used to log changes in temperature throughout the exposure. Temperature differences between each group were nearly 8 degrees centigrade (shade – 22.6 °C; sun – 30.4 °C), but no differences were observed between any group for any compound (**Figure A.3.4**).

Figure A.3.5 shows a comparison of three temperatures in simulated transport conditions. Silicone passive samplers were placed in PTFE bags for 72 hours, at -20 °C (walk-in freezer), ~23°C (ambient) and 35 °C (drying oven). Temperatures were monitored every 30 minutes from temperature loggers. The PSD had been infused with 5 PAHS as discussed above. Ratios of each analyte response divided by the internal standard response are reported in **Figure A.3.5** since an error with surrogate recovery artificially inflated variance across treatments. While fluorene does appear to have significantly different response ratios across all three temperature treatments (one-way ANOVA, p = 0.03), pairwise comparisons with the Holm-Sidak method does not report significant changes between any two treatment groups. Ultimately, the largest change among all analytes was still less than 13 % (fluorine-d10 – freezer and ambient treatments), so any changes in PAH concentrations were deemed negligible. Furthermore, potential changes in concentration would have been conserved since transportation was similar among PSDs within each pilot study or demonstration.

Silicone PSDs from the training exercise were pooled among each configuration (**Figure A.3.6**) to test whether devices differed from participant exposure (p=0.09). Although there are not any significant differences between devices, much of the variance listed here might stem from partially covered PSDs from protective clothing as described in the manuscript. Further evidence is needed to ascertain whether clothing does reduce air flow, and therefore changes the micro-environment of exposure around the silicone PSD.

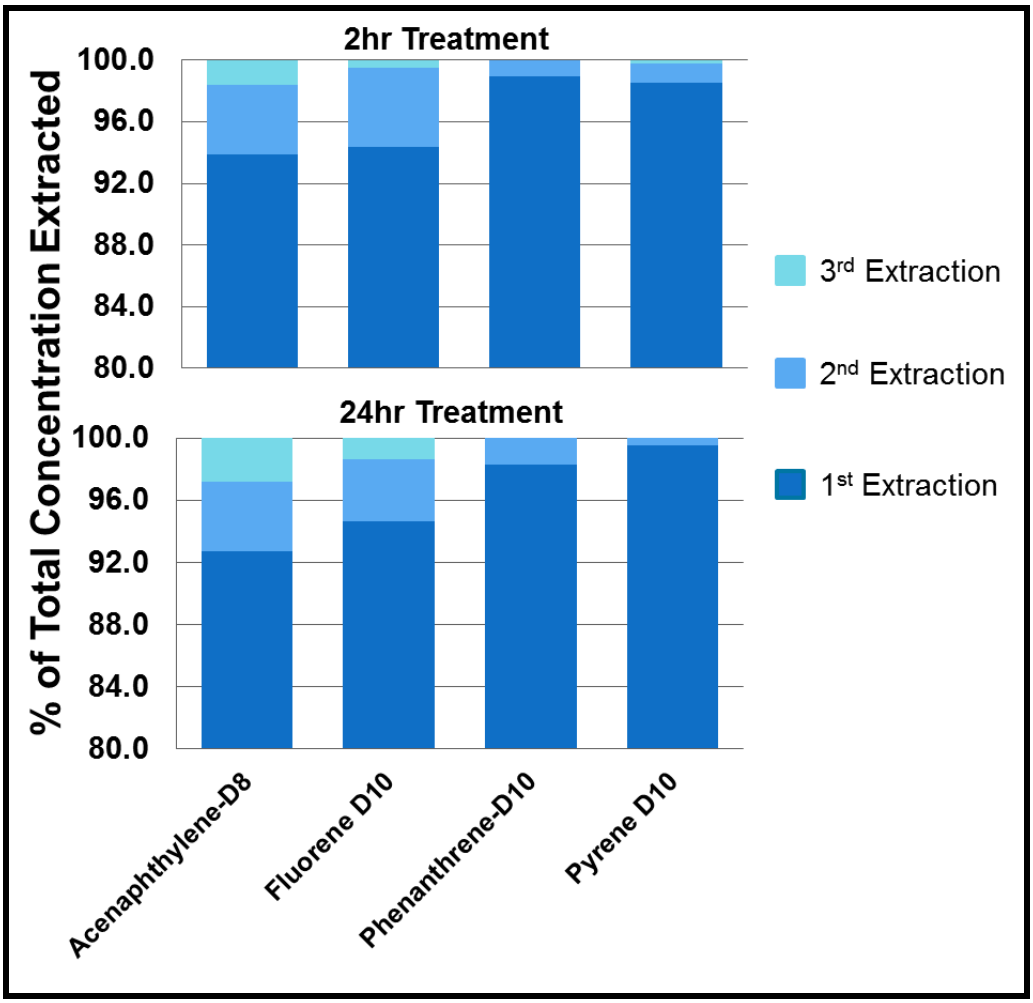

Figure A.3.1 Average (n=3) relative contributions of subsequent extractions under two extraction time treatments using ethyl acetate for four labeled PAHs. Each round consisted of either 2 or 24 hours for a total of 6 or 72 hours respectively.

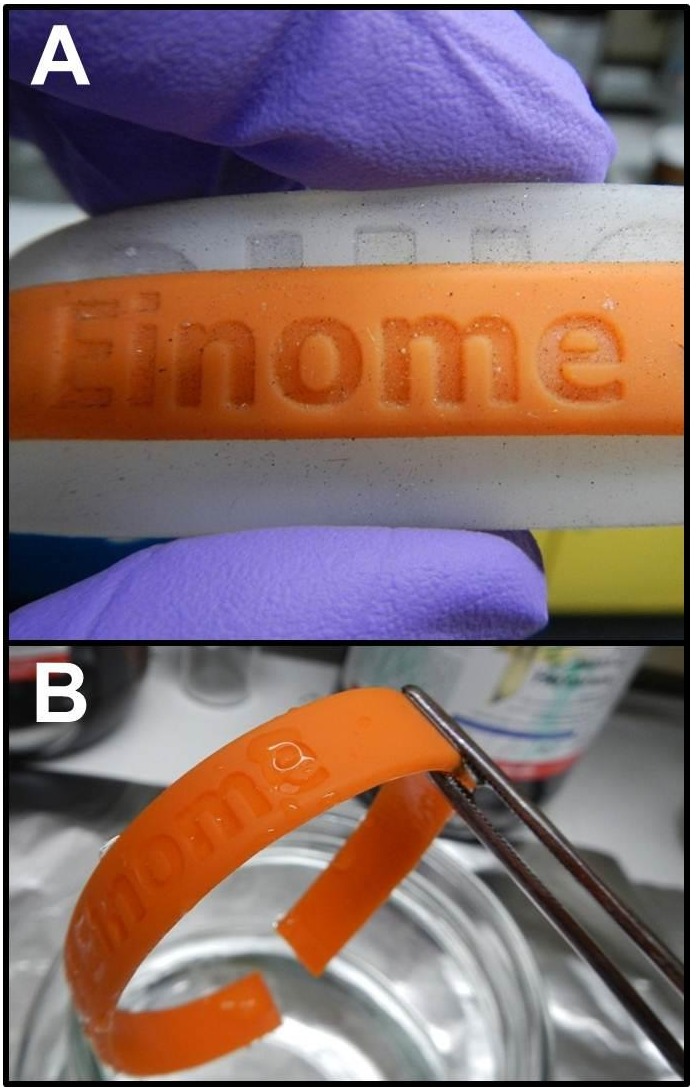

Figure A.3.2 Photographic demonstration of post-deployment cleaning before and afterwards. A) Stacked wristband configuration prior to surface cleaning. Particulates are present on the surface of the wristband. B) Top wristband after post-deployment cleaning. Surface particulates are no longer visible, and wristband is ready for extraction. The extra-wide bottom wristband was discarded.

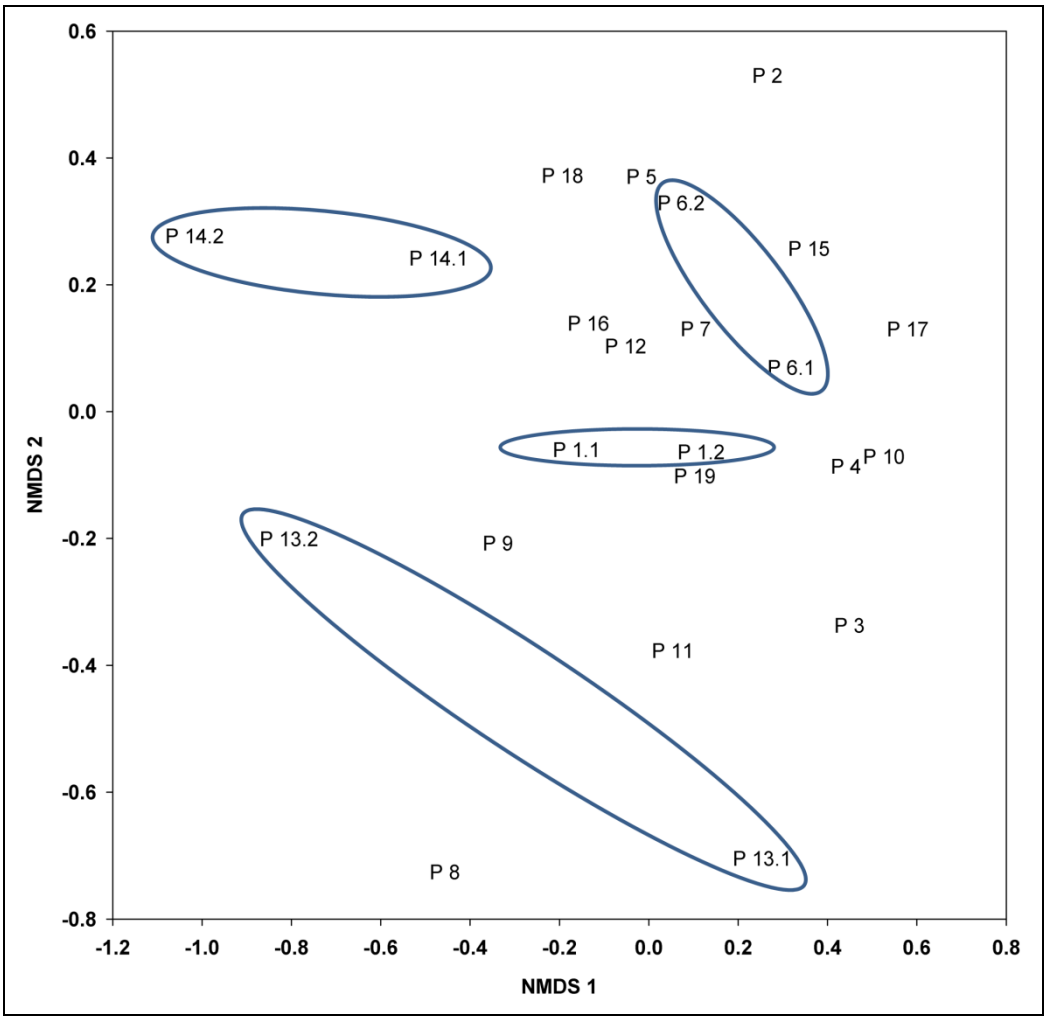

Figure A.3.3 A graphical representation of non-metric multidimensional scaling of binary ambient wristband data. Ellipses encompass wristbands obtained by the same participant.

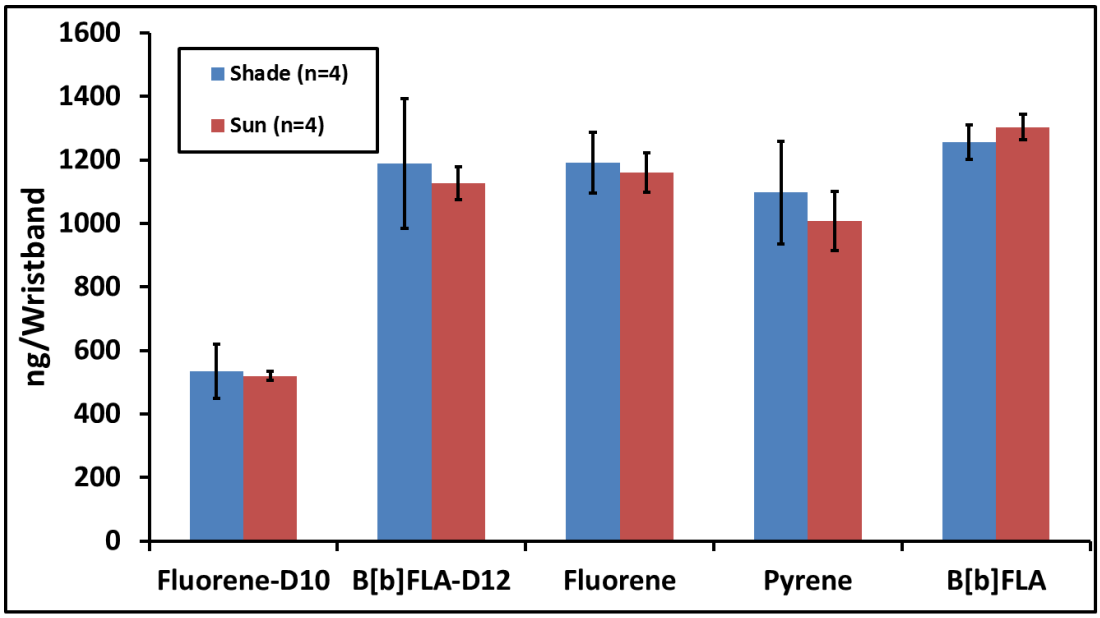

Figure A.3.4 Four hour exposure of PAH infused wristbands exposed in either full sunlight or in shade. No significant difference was seen between either exposure despite sunlight exposure and resulting temperature differences greater than 8°C. Standard deviations are the result of four replicates. Abbreviations: Benzo[b]fluoranthene (B[b]FLA).

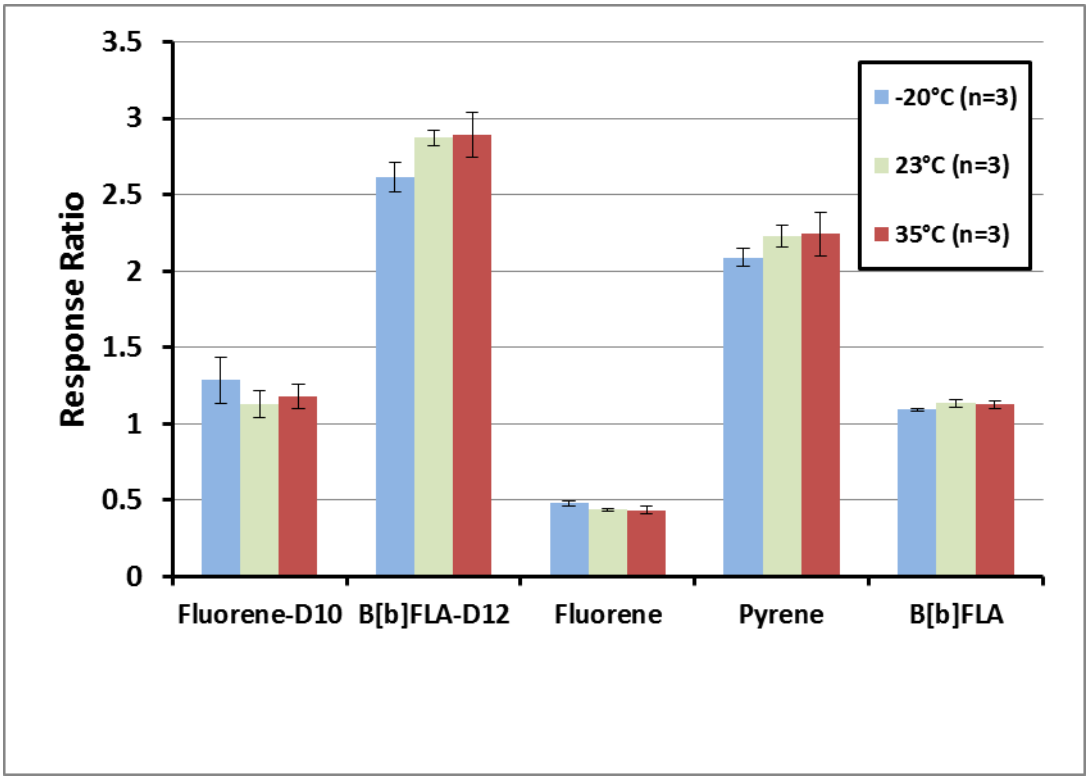

Figure A.3.5 A 72 hour simulated transport study PAH infused wristbands in PTFE bags stored at three different temperatures. No substantial losses of compounds were seen with higher transport temperatures. Amounts here are the response ratio of the analyte over the internal standard perylene-D12. Standard deviations are the result of three replicates. Abbreviations: Benzo[b]fluoranthene (B[b]FLA).

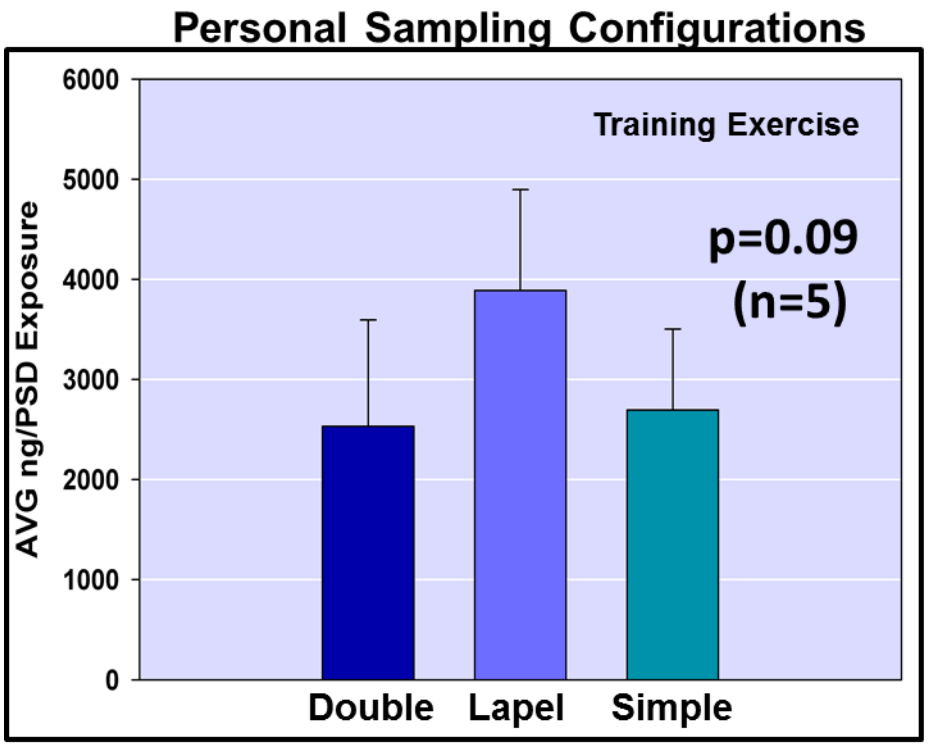

Figure A.3.6 Eight hour training PSD exposure. No significant difference was found between devices from occupational exposure.