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Kim A. Anderson

There are many links between exposure to environmental pollution and risks to human health. While advances in the fields of toxicology, exposure science, and environmental chemistry have shown light on many of these links, many more research challenges remain. One major challenge is how to accurately characterize the toxicity of a mixture of chemical pollutants. There is evidence to suggest that assuming the toxicities of chemicals in a mixture are additive is an oversimplification. Additionally, there are many chemicals that are commonly found in the environment or that are potentially toxic, but that remain under-studied. All of these caveats apply to polycyclic aromatic hydrocarbons (PAHs).

Another challenge is how to best quantify an individual's exposure to environmental pollutants. Traditionally this is done using a combination of self-reported exposure information from questionnaires, extrapolation of data from stationary monitors to the exposures of mobile individuals, and occasionally data from personal monitors. Passive sampling is an effective tool for measuring the fraction of chemical pollutants people are exposed to in the environment. The work presented in this dissertation uses passive sampling to measure PAH contamination in air, water, and the personal environment, and to predict PAH concentrations in crayfish. It also estimates carcinogenic human health risks associated with exposure to this PAH contamination.

Shellfish contamination data is needed for use in consumption advisories. However, existing methods of measuring this contamination are often prohibitively time and resource-intensive. It has been observed that passive samplers, coupled with predictive models, can accurately estimate PAH contamination in shellfish. In Chapter 2

we further validated the ability of passive water samplers and predictive models to predict PAH contamination in the resident signal crayfish, *Pacifastacus leniusculus*. This work was conducted within and outside of the Portland Harbor Superfund Megasite. We estimated PAH concentrations in crayfish from PAH concentrations measured by passive samplers in water, using a simple linear regression model that included 34 PAHs. The model predicted PAH concentrations in crayfish within an average factor of 2.4 of PAH concentrations that were measured in crayfish. Additionally, we observed substantially higher PAH levels, and carcinogenic PAH levels, in crayfish visceral tissue than in crayfish tails. This indicated that eating only the tail of a crayfish would drastically reduce a consumer's cancer risk compared to eating the whole crayfish. We also demonstrated the importance of appropriately characterizing the toxicity of chemical mixtures. For instance, benzo[c]fluorene was identified as the main contributor to carcinogenic potency in crayfish tissues. However, this PAH is not traditionally included in analyses of environmental samples. Additionally, we saw strikingly similar profiles of carcinogenic PAHs in crayfish tissues collected in this Superfund site in 2003 and 2013. This demonstrated that there are chemical mixtures that commonly occur at this Superfund site. Knowing this could enable researchers to drastically reduce the number of chemical mixtures to prioritize for toxicological study.

Natural gas extraction (NGE) activity has expanded rapidly in the U.S. in recent years. This rapid expansion has been met with minimal study of potential environmental or health effects, leading to concern among scientists and the public. In Chapters 3 and 4 we present two studies assessing the impact of NGE on environmental PAH levels, in a community heavily affected by the recent natural gas boom. In Chapter 3 we used passive air samplers to assess how proximity to the nearest active NGE well affected PAH concentrations in ambient air. In this study we saw decreasing PAH concentrations, as well as carcinogenic PAH concentrations, as air samplers moved farther from active NGE wells. We also saw predominantly petrogenic signatures of PAH mixtures measured closer to NGE wells. This suggested that measured PAH mixtures were impacted by

fugitive emissions of PAHs during NGE, and that this impact was stronger closer to active NGE wells.

In Chapter 4, we more thoroughly assessed spatial patterns of PAH concentrations in air using passive air samplers, at sites with and without active NGE wells. In this study we observed higher PAH concentrations in air at sites with NGE wells than at sites without. Again, we saw more petrogenic PAH mixtures at sites with active NGE wells than at sites without wells. This gave us further evidence that PAH mixtures in air near NGE wells are affected by direct releases from the earth during NGE. In this study we also assessed the impact of NGE on the personal PAH exposure of people living and working in this community. We did this using a novel personal passive sampler, the silicone wristband. PAH concentrations measured in wristbands demonstrated that living or working closer to an active NGE well was associated with increased PAH exposure. However, all carcinogenic PAH concentrations measured in air in both NGE studies were below the U.S. EPA's acceptable threshold. Thus, even the highest carcinogenic PAH concentrations measured in air closest to NGE wells would not be expected to increase lifetime cancer risk of people living or working nearby above background risk levels.

The work presented in this dissertation further validates the ability of passive samplers to predict PAH contamination in crayfish, provides evidence for PAH emissions coming from NGE, and comments on the estimated health risks associated with exposure to these PAH mixtures. Taken together these findings help solve the current challenges in environmental toxicology research, and provide ideas for solving the remaining challenges facing this field.

Human Exposure to PAHs in Air, Water, and Crayfish

by
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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.

L. Blair Paulik, Author

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CONTRIBUTION OF AUTHORS

Dr. Kim Anderson contributed to concept development, study design, data interpretation and writing in all chapters.

In Chapter 2, Dr. Brian Smith created the predictive model and performed additional data analyses. Alan Bergmann collected, extracted and analyzed the water samples from 2012 that were used for comparison, and helped prepare the manuscript. Dr. Greg Sower led the collection of the crayfish that were harvested in 2003 and used for temporal comparisons. Dr. Norman Forsberg performed the extraction of the 2003 crayfish tissues. Dr. Justin Teeguarden helped with data interpretation and with finalizing the manuscript.

In Chapter 3, Carey Donald helped select sampling sites, design the sampling plan, extract the samples, and prepare and finalize the manuscript. Dr. Brian Smith helped with data analysis and performed the PCA. Lane Tidwell helped with sample preparation and field sampling. Kevin Hobbie helped with field sampling and data interpretation.

In Chapter 4, Kevin Hobbie helped with field sampling and data interpretation, and made the concentration maps used in Figures 4.1 and 4.3. Dr. Diana Rohlman maintained communication with the volunteer participants before, during, and after the study, and helped with field sampling and data interpretation. Dr. Brian Smith helped with data analysis and interpretation.

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DEDICATION

This dissertation is dedicated to Dr. Steve J. Klaine. May the memory of his infectiously positive spirit remind us that there is always something to smile about.

Human Exposure to PAHs in Air, Water, and Crayfish

Chapter 1 – Considerations for Measuring Exposure to Chemical Mixtures

L. Blair Paulik and Kim A. Anderson

Chapter 3 in *Chemical Mixtures and Combined Chemical and Nonchemical Stressors: Exposure, Toxicity, Analysis and Risk*

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Abstract

Exposure to chemical mixtures contributes to human disease risk. While analytical capability is continuing to increase, many chemicals remain under-studied, both with regard to environmental occurrence and to toxicity. If a commercial chemical standard does not exist for a given chemical, then little to no quantitative data likely exist for that chemical. This chapter discusses exposure to organic chemical mixtures, lists necessary considerations for studying those exposures, and highlights research needs to continue to advance mixtures exposure science. When planning or reviewing studies that focus on exposure to chemical mixtures, important considerations include: spatial orientation of sampling, temporality of sampling, bioavailability of measured chemicals, measuring enough of the appropriate chemicals, potential for chemical transformations, and mixture effects. Importantly, relatively little is known about how exposure to mixtures of chemicals differs from exposure to individual chemicals. Given that the majority of toxicity studies are performed using single chemicals, characterizing the toxicity of chemical mixtures should be a priority for the scientific community.

Introduction to Measuring Chemical Stressors in the Environment

Non-scientists are often surprised to learn that science is incapable of measuring all of the chemicals people are exposed to on a daily basis. In reality, data about environmental chemical exposures only exist for chemicals for which detection methods have been developed. Those methods only exist for chemicals that a) have existing analytical standards, and b) are stable enough to reasonably be measured.[1] Additionally, the chemicals that meet these criteria are not always the ones that are the most relevant to environmental or human health, either with regard to toxicity or frequency of exposure.[2] In fact, we are exposed to many chemicals on a daily basis for which data about toxicity and/or environmental fate are sparse or nonexistent. To add another layer of complexity to this problem, people are constantly exposed to mixtures of chemicals. While we know that chemicals in mixtures do not necessarily behave the same as they do individually, we have limited information about what those differences

are.[3] Regardless, researchers, risk assessors, and other decision-makers must regularly determine whether exposures to measured levels of pollutants are concerning or not. In the face of these uncertainties and data gaps, these can be difficult decisions to make.

This chapter includes considerations for assessing exposure to mixtures of organic chemicals in the environment. These considerations should be addressed both when designing studies to answer questions about chemical exposures, and when interpreting results of such studies. Specifically, characterization of exposures to common chemical mixtures, considerations for assessing exposure to these mixtures, case studies to illustrate these considerations, and priority research needs to improve the assessment of exposure to chemical mixtures are discussed.

Exposure to Common Chemical Mixtures

Why Characterize Exposure to Chemical Mixtures?

Accurately assessing a person's chemical exposures is essential for determining the impact of environmental exposures on human health. It is well-established that exposure to many environmental pollutants is linked with disease. [4] While the science of externally measuring chemical exposures has come a long way, there is still much work to be done. The main principle of toxicology is that the dose determines the effects. It follows that a toxic substance does not increase disease risk if no exposure occurs. Consequently, there can be no useful assessment of toxicological risks without appropriately and quantitatively assessing chemical exposure. Therefore, we need to continue to learn both about what chemicals should be prioritized for study, and about how, where and when chemical exposures happen. The importance of this area of study was emphasized in the new Strategic Plan from the U.S. National Institute of Environmental Health Sciences (NIEHS). This plan aims to characterize human exposure to chemical mixtures over a lifetime, and to learn how those exposures may affect health and disease risk.[5]

Although the risk of developing disease is attributed to both genetic and environmental factors, it has been suggested that differences in environmental factors may substantially affect disease risk [6-8]. While the exact proportion of disease risk that can be attributed to environmental factors is under debate [8, 9], it is established that environmental factors, including environmental pollutants, play an important role in disease risk [8, 10]. Although recent definitions of a person's "environment" have encompassed the internal, external and psychological environment, and it has been suggested many of these varied factors may contribute to disease risk [4], the relationship between exposure to environmental pollutants and health remains a key area of study. Additionally, there are many chemical exposures that are still under-characterized [4]. To move exposure science forward, the scientific community needs more accurate and comprehensive assessments of chemical exposure. It is also important that these studies take into account how exposures vary with space and time.

How to Characterize Chemical Mixtures in the Environment: What are the Options?

Existing Technologies for Measuring Chemical Exposures in the Field: Traditional Methods

Epidemiologists frequently evaluate the effect of environmental exposures on disease using data from questionnaires. Inferences gleaned from these questionnaires often require extrapolating from a participants' response to a chemical exposure, and can therefore be uncertain.[11] For instance, a questionnaire may ask participants whether they grilled meat during the course of a study, and a "yes" response may be interpreted as exposure to carcinogenic chemicals that are products of incomplete combustion. However, it would be difficult to glean from this response which chemicals participants were exposed to, what concentrations they were exposed to, or how long or how often this exposure occurred. All of this extra information may affect how this exposure is interpreted by researchers, but none of it would be captured by the questionnaire. Situations like this would likely increase uncertainty in data from questionnaires. Thus, much care should be taken when either developing exposure assessment

questionnaires, or interpreting data from such questionnaires.[12] Additionally, epidemiologists rarely have access to baseline or historical exposure data. The lack of low cost, easy-to-use sampling technology for directly measuring chemicals in the environment, or for directly measuring exposure to those chemicals, hinders epidemiological studies. In rare cases when exposure assessments are based on data from environmental sampling, they often are limited in sampling time points or sampling locations, are limited to readily- accessible tissues, and are limited in subject size due to challenges of cost and compliance.

Exposures to chemical mixtures can be measured either internally (e.g., with biomonitoring), or externally. Biomonitoring has both substantial strengths and limitations. It has the advantage of directly measuring concentrations in participants' bodies. When a sufficiently specific and sensitive biomarker exists for a contaminant of concern, the direct nature of this tool can greatly reduce uncertainty about whether participants were exposed to that contaminant. However, biomonitoring studies can be hindered by the lack of specificity and sensitivity of many biomarkers, temporal challenges related to timing of exposure, and a relatively short list of measureable exposures. Another challenge is that biomonitoring estimates are transient snapshots of exposure, which can be difficult to interpret.[13] Collecting biological samples from study participants also presents additional challenges for participants, and so can have a negative impact on participant compliance compared to less invasive methods.

Exposure to environmental pollutants happens via three main exposure routes: inhalation, ingestion, and dermal. Additionally, characterizing chemical pollution for health assessments may entail measuring chemical mixtures in four broad types of media: sediment/soil, water, air, and food products. It is important to keep in mind that measuring chemicals in these media is only useful where it addresses exposures that may occur through at least one of the three main exposure routes (inhalation, ingestion, and dermal). While the last 50-60 years saw significant advances in analytical chemistry (e.g., enabling chemists to detect as little as a few femtograms of thousands of chemicals), much less progress has been made in how we collect samples from the

environment. Additionally, while it is encouraging that analytical chemists can now detect a few thousand chemicals, this does not mean that methods exist to detect all of these chemicals in all types of samples (e.g., soil, water, food, air). And there are still numerous chemicals present in the environment for which no analytical methods exist at all.

Existing Methodologies for Externally Measuring Chemicals in the Environment

There are a number of existing technologies for measuring exposures externally. Some traditional measurement techniques are unique to one of the three main exposure routes, while others bridge multiple pathways. In general, people can be exposed to chemicals measured in the sediment or soil through dermal exposure, and potentially through ingestion (if a child ingests the contaminated soil or sediment directly, or if that chemical moves from the soil or sediment into a plant or shellfish that develops in the soil or sediment). People are generally exposed to chemicals measured in water dermally, through ingestion if a chemical moves from the water into a fish that a person eats, or through ingestion if that chemical is not filtered out before the water is used as drinking water or through inhalation of volatiles/semi-volatiles vaporized from water. Exposures to chemicals measured in the air happen predominantly through inhalation. Finally, exposures to chemicals measured in foods occur through ingestion.

There are multiple techniques for measuring chemicals in each of the media listed above. To measure chemicals in the sediment/soil, traditional methods include taking what is known as a “grab sample” of sediment/soil, extracting the whole sediment/soil, and reporting the chemicals measured in the whole sediment/soil. Traditional methods of measuring chemicals in water include taking grab samples of water, extracting chemicals from that water, and reporting the totality of what is measured. Traditional methods of measuring chemicals in air involve setting up an active air sampler, which pumps air over a filter or other sorbent, and then extracting that filter. To measure exposure via ingestion of food, for instance in shellfish at a contaminated site, traditional methods include directly sampling the shellfish of

concern, and then performing an extraction and measuring chemicals in that shellfish. Extraction techniques are also often specific to the type of sample (e.g., air or sediment), and to a certain chemical or class of chemicals (e.g., only for mercury, or only for hydrocarbons). The near-infinite list of combinations of chemicals and sample types necessitates developing many different extraction methods, and this further complicates the task of efficiently measuring chemicals in the environment.

When coupled with appropriate analytical techniques, each of the traditional methods described above yields chemical concentrations that are representative of the specific location and time of sampling. Given that exposures to pollutants in the environment are dynamic, it is necessary to extrapolate from environmental concentrations to estimate human exposure. However, data from traditional sampling methods are commonly used to assess pollution levels that an individual, or a population, is exposed to. This is done using exposure factors to approximate how much of a chemical a person might be exposed to on a regular basis. Depending on the exposure route of interest, different exposure factors are required to make this extrapolation. Exposures factors range from estimating the number of hours individuals in a population spend in their houses each day, to the average number of grams of crayfish individuals eat each day, to the number of years individuals live adjacent to a source of pollution, and everything in between. Specific examples of many of these exposure factors that are commonly used in US Environmental Protection Agency (US EPA) risk assessment are given in the US EPA's 2011 Exposure Factors Handbook.[14]

Other chemical sampling techniques aim to measure chemical exposures in an individual's personal environment, by placing samplers directly on individuals. These methods are attractive because they eliminate (or greatly reduce) the need to extrapolate between a measured chemical concentration and an external exposure. Traditionally, techniques for measuring personal exposure to organic contaminants have included putting air sampling backpacks on study participants[15] or putting active samplers on participants' lapels.[16] However, these tend to be costly, monitor relatively few environmental contaminants, and require committed participants because the

devices can be bulky or noisy during use. Monitors exist to measure other types of contaminants, such as particulate matter in the air, in real-time.[17] Real-time monitors for freely dissolved organic contaminants, however, are still in development. While not a focus of this chapter, other techniques exist for measuring personal exposure to inorganic contaminants, such as metals. One such technique uses an acidic hand wipe to assess personal exposure to metals.[18] Existing tools for measuring personal organic chemical exposure all require some in-lab analysis after deployment. Sampling devices for individuals should ideally be: easily worn, adaptable, capable of measuring many chemicals, easy to use by research staff, integrated with many simultaneous measures (e.g., location, chemical exposures, health outcomes like lung function), rugged, non-invasive, and have the capacity to store and transmit data.

Passive Sampling

Thinking outside the "sampling jar" is required to accurately characterize chemical exposures. Since the early 1990s, passive sampling has been gaining momentum as an effective tool for measuring trace levels of contaminants of concern.[19, 20] Passive samplers measure time-integrated concentrations of the freely-dissolved concentration (C_{free}) of contaminants. Passive samplers are relatively low-cost, and they do not require energy or maintenance while deployed. The ability to infuse passive samplers with performance reference compounds (PRCs) before deployment further improves this tool's ability to accurately assess contaminant levels.[21]

Numerous polymer materials and membrane technologies for passive sampling of water, air and the personal environment have been explored. Low-density polyethylene (LDPE) and silicone are two widely-used materials to make passive samplers for measuring organic contaminants.[20, 22] When deployed in air, water, or sediment porewater, these polymers absorb hydrophobic organic contaminants via simple diffusion from the environment into the hydrophobic membrane. This process is analogous to uptake across a phospholipid membrane into an organism, making passive sampling well-suited to serve as surrogates for contamination in organisms.[23-27] Other

samplers, such as Diffusive Gradient Thin Films, or DGT, passively absorb inorganic contaminants.[28-30]

Another attractive aspect of passive sampling is that some passive samplers can be paired with high-throughput bioassay systems, such as the embryonic zebrafish assay. This allows chemical mixtures measured in the environment (by a passive sampler) to be tested in toxicity bioassays.[31] The zebrafish bioassay is also attractive because it requires relatively small amounts of sample. Traditional animal bioassays are much more expensive and time-consuming, and require much larger amounts of sample. By pairing a passive sampler extract with the zebrafish assay, the bioassay helps reveal the toxicity of the whole mixture. More can be learned when bioassays are combined with fractionation techniques to separate components of the mixture based on physical or chemical parameters (e.g., polarity, size).[32] This will be discussed further below. While there has been significant progress with passive technologies, the nexus of the passive sampling platform technology is yet to be fully exploited.

Recently, new technology has emerged which passively samples individuals' chemical exposures. The passive wristband sampler is a new, wearable passive sampler that directly measures the chemicals a person is exposed to.[33] These wristbands accumulate thousands of common environmental chemicals,[33] including many used in commerce and organic compounds formed during natural and industrial processes. It has been demonstrated that the wristband can also capture emerging chemicals of concern. For example, it quantifies the concentrations of oxygenated polycyclic aromatic hydrocarbons (OPAHs), toxic compounds in asphalt fumes.[33] Another promising aspect of the wristband sampler is that it is easily used by citizen scientists making it easier to engage community members in research studies.

Limitations of Passive Sampling

As with any technology, passive sampling has its limitations. One challenge is accurately calculating an environmental (or a personal exposure) concentration of a chemical from what is measured in a passive sampling device. This requires adding labeled chemicals to

the passive sampler before deployment to act as PRCs, and measuring the loss of those PRCs during the deployment. The amount of PRC lost is used to estimate whether the sampler had reached equilibrium with the system for that chemical. This information is then used to correct the concentration measured in the sampler, to more accurately reflect the concentration in the environment. While the scientific community accepts this approach,[21] it does introduce some uncertainty into reported concentrations of environmental chemicals that are measured using passive samplers.[34, 35] Another aspect to consider is that passive samplers produce time-integrated concentrations of chemicals.[36] This is a strength or weakness of the technology depending on the goals of the study. It is a strength if the goal is to assess a person's total individual chemical exposure over a period of time, or to measure an average concentration of a chemical over a given time period.[34] It is a weakness, however, if the researcher wants to capture the elevated concentration at a specific moment during a pulse of contaminants (e.g., during a brief emission of air pollution).

Existing Technologies for Measuring Chemical Exposures in the *Lab*: Which Chemicals are Traditionally Measured?

Traditionally, analytical methods focus on relatively few chemicals, which are only those that are feasible to measure. This means that many analytical methods focus on a relatively limited number of chemicals. These are the chemicals for which analytical standards exist to develop analytical methods. These are not necessarily the chemicals that should be prioritized due to being frequently encountered in the environment, or due to being the most toxic.[2] In some cases, chemicals are prioritized for study based solely on availability of analytical standards.[1] Additionally, if a standard is mistakenly misidentified, this can lead to years of incorrect conclusions about that chemical.[1] There are many chemicals, therefore, for which environmental occurrence data, toxicity data, or both, are sparse. There are other chemicals for which these data do not exist at all. While this chapter focuses on human exposures to chemical mixtures, the contaminants we discuss also affect the environment as a whole.

For many chemicals found in the environment, toxicity information is limited or regulatory limits do not exist. Therefore, even when these chemicals are included in analytical methods and detected in the environment, they are often excluded from risk assessments. If there is no toxicity information for a measured chemical, it is difficult to include it in a risk assessment. Several approaches exist to close some of these toxicity data gaps. Many of these approaches involve predicting toxicity of under-studied chemicals (one such set of predictive tools is quantitative structure-activity relationships, or QSARs). The US EPA's ToxCast is a promising initiative, which aims to efficiently predict and characterize the toxicity of thousands of chemicals.[37] In this program, EPA scientists screen thousands of chemicals using high throughput toxicity bioassays. This enables researchers to prioritize which chemicals to study based on toxicity.

The gap between environmental measurements and toxicity is further widened as most dose-response studies are not conducted at environmentally-relevant doses, and are not performed with mixtures that are realistically found at contaminated sites. Conventional sampling methods may allow pollutants with unknown toxicological relevance to be overlooked, but passive samplers begin to address this problem. Although passive samplers are not exhaustive tools for chemical extraction from the environment, they can be designed to extract a wide range of contaminants. PAHs,[38, 39] polychlorinated biphenyls,[40, 41] pesticides,[41] flame retardants,[42] dioxins,[39] and metals[28, 43, 44] are all examples of contaminants that can be captured by passive samplers. Additionally, passive samplers provide real-world mixtures of chemicals that can be directly integrated with bioassays for toxicity testing. This is useful when studying contaminants for which toxicity data are sparse. Oxygenated PAHs (OPAHs) are an example of a group of chemicals that is often encountered in the environment, but for which relatively little toxicity information exists. OPAHs, also known as PAH ketones or quinones, have one or more oxygen-containing functional groups attached to the aromatic ring structure and may also contain other chemical groups.[45] It is possible to use passive samplers to simultaneously sample both PAHs (which are well-studied) and

OPAHs, allowing the researcher to answer multiple research questions with the same sampling campaign.

Un-monitored and Infrequently Monitored Chemicals: Approaches for Identification and Toxicity Exploration

Many commonly encountered chemicals (which may or may not contribute substantially to exposures affecting human health) are not currently included in analytical methods. In some cases, it is possible to glean more data from existing analytical techniques. For instance, it is possible to look more closely at a chromatogram obtained from high resolution GC-MS analysis of an environmental sample, and to identify unmonitored chemicals in that sample. Additional analytical approaches that can be used to identify non-target chemicals include nuclear magnetic resonance (NMR), two-dimensional gas chromatography (GC x GC), and others. If unmonitored chemicals are identified in this way, then toxicity of these chemicals can be explored. These approaches involve searching through analytical data, and trying to identify individual chemicals that are not traditionally monitored, using chemical parameters (e.g., structure, molecular weight, charge, etc.). Limitations of these approaches include: they are often very time intensive (requiring days, weeks, or even months), there are often no analytical standards available to confirm chemical identification, creating the potential to misidentify chemicals (potentially causing errors in databases) due to multiple chemicals having the same chemical formula, but differing in structure. Another challenge is that, at present, the methods and software for identifying unmonitored chemicals are often written by individual investigators in individual research labs. This can make it difficult to reproduce or compare data among different laboratories.

Another method for identifying non-target chemicals is known as "effects-directed analysis," or EDA. In this approach, environmental chemical mixtures are separated through a series of chemical and/or physical separations, creating different chemical fractions of the mixture[2, 32] (**Figure 1.1**). Fractionation techniques include normal phase chromatography, size exclusion chromatography, and many others. After fractionation, EDA can help researchers learn which fractions (and which chemicals) are causing

toxicity in the mixture. EDA is also useful to help researchers identify which chemicals in mixtures are not causing observed toxicity. Which fractionation techniques are most appropriate, and which fractions are of most interest, depends on the specific research question being asked. EDA enables the researcher to use these fractions in toxicity bioassays, to explore the toxicities of each fraction, and ultimately to elucidate which components of the chemical mixture are causing toxicity. This is a useful tool for identifying both environmental occurrence and toxicity of chemicals that are rarely or infrequently included in environmental monitoring or sampling. In a recent review, Brack suggested that EDA is especially useful for measuring non-target compounds at sites that are known to be heavily polluted.[2]

Considerations for Assessing Exposure to Chemical Mixtures

Field Methods: Appropriate Sampling Techniques

Spatial Considerations: Stationary (or Population-based) vs. Individual

In many cases, chemical levels measured at stationary sampling sites in air, water, sediment/soil, or food, are used to estimate exposure levels for people living nearby, or for larger populations (e.g., cities or regions). To use a concentration measured at a specific location in the environment to estimate a personal exposure level, researchers must make assumptions about how often, and for how long, individuals in a population are exposed to the contaminant in that environmental compartment through inhalation (if measured in air), dermally (if measured in water or sediment/soil), or through ingestion (if measured in food). A good example of this is when data from immobile air monitoring stations are used to estimate human health risks based on inhaling those contaminants. A few limitations with this approach are that fixed monitoring stations may be many miles away from the location(s) of exposure, they are not always continuously operated, they often only monitor a few chemicals, and they are likely not reflective of indoor air. Thus, there is a lot of uncertainty inherent in this approach and it

is often reported to generally be a poor surrogate for an individual's inhalation exposure.

Examples of typical exposures occurring through the three main exposure routes, in different locations, are shown in **Figure 1.2**. Each exposure route requires extrapolation with different exposure factors. For instance, to estimate inhalation exposure, the researcher must estimate how many hours per day, and days per year, individuals would be exposed to contaminants at the levels measured at the stationary air sampling site. For ingestion exposure, on the other hand, the researcher would need to estimate how often the consumer ate the food that was sampled, and how much of it they ate each time. These estimated exposure levels are then used in risk assessment, to determine whether the exposure exceeds acceptable regulatory levels of pollution. Estimating these exposure factors can increase uncertainty in exposure estimates, because not all members of a population have the same behaviors or the same exposures. The benefit of stationary or population-based measurements is that they can reduce costs, as a small number of samples can be used to estimate exposure levels for large numbers of people.

More recently, methods have been evolving to sample chemical exposures directly on individuals. One such tool is the passive wristband sampler, introduced in Section 2 above. When used in risk assessment, personal sampling techniques require much less extrapolation than stationary environmental sampling techniques. When the sampler is on a participant constantly, there is no need to make assumptions regarding the frequency of exposure, duration of exposure, or other factors that are required when making exposure inferences based on chemical concentrations at stationary monitors. Therefore, using personal sampling tools may provide more accurate individual exposure information, reducing uncertainty in exposure estimates used in risk assessments. For example, if a personal sampler were put on the woman in **Figure 1.2**, it would accumulate exposure information from all four of those exposure scenarios, as well as others throughout her day. However, if the goal is to estimate exposure for a population, extrapolation would still be required from the exposures of the individuals

measured, to the larger population. Additionally, depending on which sampling techniques are used, it may be prohibitively costly to sample enough individuals to characterize a population.

Moving forward, successful strategies for assessing exposures to mixtures of environmental chemicals will likely incorporate both stationary/population-based and individual sampling techniques. When choosing a sampling technique for a study assessing chemical exposures, it is important to consider which of these techniques best addresses the question being asked. In some cases, the best option may be to use a combination of sampling tools.

Temporal Considerations: Temporality of Sampling Should Match Temporality of Exposure

Understanding how temporality of sampling compares to temporality of exposure is another challenging aspect of assessing exposure to chemical mixtures. These two should be as well matched as possible, and temporality should be considered both within the day and within or among the year(s). For instance, if the goal is to study an exposure to emissions from a factory, and those emissions vary throughout the day, it would be important to sample for at least one full day to account for that variability. At the same time, if exposures are most likely to occur at one time of the day, then it may be appropriate to emphasize this time in sampling. Examples of exposures occurring sequentially throughout a day are visualized in **Figure 1.2**. There is also seasonal variation in levels of some chemicals in the environment.[46, 47] This can be either due to differences in emissions based on differing practices in different seasons, or based on climactic conditions affecting available concentrations. If the goal is to assess an exposure that would occur over many years, best practice would be to sample at multiple points throughout the year, to gain as much information as possible about the potential exposures. If an exposure would only occur at one time of year, over a lifetime, best practice may be to sample in multiple years. Additionally, exposures at different stages of life may lead to different toxic responses. An exposure to a developing fetus may have a much more substantial impact on health risk than an

exposure to a healthy adult.[4, 48] It is therefore important to capture the effects of exposures at various stages of life, especially during those life stages where people may be more susceptible (e.g., during development, or during old age). The importance of assessing exposures at susceptible life stages, to more accurately assess exposure to chemical mixtures over the entire life span, was identified by the NIEHS in their most recent strategic plan.[5]

Time-integrated Concentration vs. Grab Sample

As mentioned above, traditional "grab samples," only allow assessment of contaminant levels in the environment at one moment in time. In contrast passive samplers sequester chemicals over time, yielding time-integrated concentrations. This is useful because passive samplers can capture less frequent, acute episodes of exposure. It is also useful because passive samplers take up contaminants over time, making them good surrogates for the fraction of contaminants sequestered by an organism over time in the same environment. However this makes it important to consider the length of deployment, especially if the goal is to sample a brief pulse of a contaminant, as in episodic or catastrophic events (e.g., after spills or hurricanes). It may be most appropriate in these cases to use short deployment times.

Bioavailability: Fraction of Chemicals Sampled Should Match the Fraction to which Individual is Exposed

It is generally accepted that measuring the total amount of an individual chemical concentration in the environment is not enough to predict biological effects. Conventional methods for human exposure assessment involve measuring total contaminant concentrations in the ambient environment (e.g., water, sediment) and extrapolating to toxicological endpoints; however this approach has proven ineffective.[49, 50] Measuring total ambient contaminant concentrations yields at best rough estimates of exposure,[13] and in many cases does not reflect the fraction of contaminants to which people or organisms are actually exposed.[51] Instead, measuring the bioavailable fraction is thought to be most relevant to human and ecological health.

This is the contaminant portion to which an organism is directly exposed, or that a person is exposed to when eating a contaminated organism. Scientists call this the "freely dissolved fraction," or "C_{free}," in water or sediment porewater. Measuring the freely dissolved fraction of a contaminant is imperative when assessing chemical bioavailability, toxicity, mobility, and degradation.[50, 52] For most routes of exposure and health endpoints, it is the freely dissolved, or unassociated, form of hydrophobic contaminants that is transported across biological membranes of organisms and may exert toxic effects.[52] A decrease in freely dissolved contaminants directly reduces bioavailability and vice versa. It is therefore this bioavailable fraction that is thought to be the most relevant fraction to measure to understand exposures.

The bioavailable fraction of contaminants can be quantified using various analytical approaches. Passive sampling is well-suited to measure the bioavailable fraction of chemicals in water and sediment/soil, as passive samplers mimic the uptake of a cell or organism via both chemical and physical processes. Passive samplers may be used to assess contaminant levels, and subsequently exposure, in water,[53, 54] air,[55] and soils.[39, 56] A major advantage of passive samplers is the ability to distinguish between dissolved and bound molecules, rather than assessing the mere presence or absence of chemicals.[57] One important consideration, however, is that when passive samplers are used to measure contaminants in air, they only absorb the fraction of contaminants in the vapor phase. Given that both the vapor phase and particulate-bound phase of contaminants may be inhaled, this means that passive sampler-generated assessments of air contamination may be under-representative of total contaminants available for inhalation exposure. One definition of the bioavailable fraction (when measuring mixtures of chemicals in water) is the portion of contaminants that can be taken up by an organism. It has been observed that this fraction can be accurately measured using passive samplers.[21, 23, 25, 27]

Measuring the External Aspect of Chemical Exposure

Timely, high-quality data are needed to bridge the gap between environmental monitoring data and quantitative data about individual chemical exposures, and to

identify which of these exposures are most relevant to human health. In recent years there has been a movement toward measuring complete, lifelong exposures.[4, 48, 58] The goal of this work is to learn as much as possible about the relationship between environmental exposures and disease risks, and to use these findings to improve public health decision-making.[4] Similarly, one of the goals of the NIEHS' recent Strategic Plan is to "transform exposure science by enabling consideration of the totality of human exposures,".[5] There is evidence that environmental factors contribute heavily to disease risk, and that environmental chemical exposures are an important piece of the environment that may affect that risk.[4, 48] It has been suggested that interdisciplinary teams of scientists should work together to tackle this challenge with innovative technologies.[48] Effectively assessing cumulative chemical exposure will likely require collaboration among chemists, toxicologists, immunologists, public health specialists, epidemiologists, and others. From the perspective of measuring external chemical exposures, it will be important to choose environmental sampling techniques that measure as much of a person's chemical exposures as possible, as accurately as possible. Personal sampling devices, such as the passive wristband sampler, may be some of the best existing tools to address this piece of the exposure assessment puzzle.

Lab Methods: Appropriate Techniques

Which Chemicals Should be Measured?

The chemicals that are most heavily studied are not necessarily those that people are most commonly exposed to, or that are the most toxic, but rather are those that existing methods can detect. While the goal is to measure chemicals that are the most relevant to environmental and human health, there is also preference toward measuring chemicals for which regulatory guidelines exist.[1, 11] In many cases this is appropriate (i.e. a potentially hazardous chemical has been previously identified and regulated, and so it receives attention). The challenge is that this can make it difficult to study certain chemicals (e.g., a new chemical that does not fall into an existing regulatory category). Developing methods to detect chemicals requires analytical

standards, and each analytical standard must be created in response to demand for that specific standard. So, there are many chemicals for which no standards exist, and thus for which there are little to no data about their environmental occurrence or toxicity.

Most analytical methods are only used to quantify a few dozen chemicals. However, in most cases, there is additional information that could be harnessed from existing chromatographic analyses. Hundreds of additional chemicals could be quantified from many existing chromatographic methods, if the methods were further developed. By the time the sample is ready for analysis, much of the expense of the sample collection and processing has already been incurred. Often, precious research funds could produce more results if methods were further optimized. Generally only a few chemicals are quantified, when the same sample could be used to quantify hundreds or thousands of additional chemicals in the mixture.

Chemicals that share certain properties are often lumped into a group, or "class," as if all chemicals in a class have the same mode of action, and toxicity. In reality, not all chemicals in a class behave the same. Chemicals within a given class can follow wildly different environmental pathways after emission, and also can have different toxic modes of action and potencies.

Example Chemical Class: Polycyclic Aromatic Hydrocarbons (PAHs)

One class of environmental pollutants that has diverse physicochemical properties and modes of toxic action is polycyclic aromatic hydrocarbons, or PAHs. PAHs are pervasive environmental pollutants of concern, known to be associated with both hydrocarbon extraction and negative health impacts.[59, 60] The main categories of health concerns associated with exposure to PAH mixtures are cancer risk and respiratory distress. Some PAHs are pro-carcinogens, meaning they can be metabolically activated, creating biologically active intermediates which can form DNA adducts.[61] Thus, research has focused primarily on PAHs' carcinogenic risk.[62, 63] PAH-related cancer risk has been studied in relation to oil spills, traffic exhaust, wood smoke, and cooking. However, exposure to PAHs also increases the risk of cardiovascular disease[64] and the risk of

mortality from heart attack.[64, 65] Animal studies indicate that PAHs can increase blood pressure and heart rate and accelerate the progression of atherosclerosis.[66-69] Mechanistic evidence and epidemiological evidence associate PAHs with airway inflammation and asthma,[70, 71] and exposures are associated with developmental and behavioral deficits.[72-77] There are also multiple biological effects and targets for PAHs that remain unknown or under-studied. Additional toxic endpoints that have been studied in relation to PAHs include adverse developmental, reproductive, respiratory, and neurological effects.[77-81] All of this evidence suggests that assuming the potencies and modes of action of all PAHs are the same is an oversimplification.

Benzo[a]pyrene, or BaP, has been extensively studied in relation to its carcinogenicity. It is used as a model carcinogenic PAH in many studies and in regulatory guidance.[59] While there is substantial evidence that BaP is indeed a carcinogenic PAH, it is often incorrectly assumed that it is so widely studied because it is *the most* carcinogenic PAH. However, it is known to PAH experts that other PAHs are as or more carcinogenic than BaP. Analytical methods are lacking for some PAHs. For others, measurement methods are available, but they are rarely if ever encountered in the environment, and so they are not useful to study with regard to human exposures. However, there are some highly carcinogenic PAHs that are both measurable and found in the environment, and that receive much less research attention than BaP. One such PAH is dibenzo[a,l]pyrene (also known as dibenzo[def,p]chrysene, or DBC).[1] DBC was included in the US EPA's 2010 list of carcinogenic PAHs.[59] In this document, relative potency factors (RPFs) were assigned to 26 unsubstituted PAHs, to scale their carcinogenic potencies relative to that of BaP. BaP was assigned an RPF of 1, and the rest of the compounds' RPFs were scaled relative to BaP. DBC was given an RPF of 30, suggesting that it is 30 times as carcinogenic as BaP at the same concentration. While there are uncertainties inherent in this assessment, it is worth noting that DBC is still not included in regular environmental monitoring regimes. This may simply be the natural progression of the identification of hazardous chemicals. Momentum must gain behind a chemical before it can truly be well-characterized. For comparison, the study of the

carcinogenicity of BaP began in the late 18th century, when English surgeon Percivall Pott observed that chimney sweeps in London had higher rates of scrotal cancer. In the 1930s, what is now known as BaP was directly isolated from two tons of coal tar, and it was identified as a cancer-causing agent.[1] Since that time, the carcinogenicity of BaP has been demonstrated in numerous studies.[59] Given the advances that science has made since the initial identification of BaP as a carcinogen, identifying and prioritizing toxic chemicals should be much more efficient now.

It is similarly incorrect to assume that all PAHs behave the same in the environment. For instance, four examples of PAHs commonly measured in the environment are naphthalene, phenanthrene, pyrene and benzo[e]pyrene. These four PAHs have pure water solubility values of 32, 1.0, 0.1 and 0.004 mg/L, respectively, ranging about five orders of magnitude. This means that the fate of each of these PAHs is very different once it is released into the environment. Additionally, while water solubility values are reported for pure water above, few environmental waters are even close to pure. As the amount of dissolved organic carbon (DOC) in water increases, the solubility of organic contaminants increases as well. For instance, Johnson-Logan *et al* demonstrated the solubility of the pesticide chlordane in groundwater with a mere 34 mg/L DOC increased 500%.[82] The enhanced solubility is due to partitioning of hydrophobic organic contaminants onto the dissolved organic carbon within the water column. An increase in DOC can increase solubility, but it may or may not increase bioavailability. However, it most certainly affects fate and transport of the chemical.

PAH exposures occur to complex mixtures of PAHs, and the composition of these mixtures can differ dramatically depending on the source(s).[24, 83] For instance, the vapor phase PAH profile from wood-burning is different from diesel exhaust, which is different from petroleum. Stout *et al* recently observed that relying only on the US EPA's 16 Priority Pollutant PAHs can inhibit the researchers' ability to determine the source of the PAHs.[84] Diagnostic isomer ratios and alkylation patterns of PAHs are therefore commonly used to identify sources of PAHs.[84-86] Different sources emit PAH mixtures with differing magnitudes of individual PAHs and with different ratios of PAH isomers.

Including more alkylated PAHs and more isomers in analysis allows for more robust source identification. It is also important to understand exposure to individual PAHs, because they have different modes of action. For instance, Jung *et al* found that childhood asthma was associated with pyrene but not as strongly associated with five other measured PAHs.[87]

Another interesting facet of PAHs is that exposures occur through all three of the main exposure routes (inhalation, ingestion and dermal contact, with inhalation and ingestion typically being the primary routes). Respiratory PAH burden includes exposure to both PAHs in the vapor phase and the particulate-bound fractions. Air sampling is often focused on determining the concentration of particulate bound chemicals, but exposure to the PAH vapor phase has also been shown to contribute to the cancer risk from inhalation exposure.[88-90] Hassan *et al* demonstrated that 67% of inhalable PAHs were in the vapor phase at a study site in Giza, Egypt.[91] Significant effort has been put toward clarifying the association between PAH inhalation and increased incidence of respiratory syndromes, especially asthma and lung cancer,[92, 93] so accurately understanding PAH levels in air is important.

Accounting for *Transformations* of Chemicals in the Environment between the Source and the Exposure

The importance of chemical transformations in the environment is often underrepresented in both study designs to measure chemicals in the environment, and interpretation of environmental chemical occurrence data. Chemical exposures estimates are often calculated based on emissions reports from point sources, large volume chemical use reports, or from environmental monitoring programs. However, many of these reporting techniques only focus on short lists of chemicals, and do not account for chemical transformations that occur in the environment. Degradation, adsorption, transport and other chemical fate processes can transform the chemical composition of a point source emission.

Chemical transformations may lead to less or more toxic chemical mixtures than what is measured at an emission source. In some cases, transformations that alter the parent compound after emission may make a product that is less toxic than the parent [1]. In other cases, transformative processes can create chemicals that are more toxic than what was in the original mixture. These transformation products are often not included in analytical methods, and so they often cannot be detected, and thus are entirely missed from the exposure discussion. For many degradation products, toxicity information is sparse. In some cases, specific transformation products that lead to increased toxicity after transformation may not have been identified. Interestingly, EDA has been identified as a useful tool for identifying specific transformation products that are causing toxicity in transformed mixtures.[2]

Regardless, transformations often make the exposure experienced by an individual different from the original source of emissions. The magnitude of this difference depends on the chemicals in the original mixture (and their potential for transformations), and on the time and distance from the emission source to the exposure. Exposure to sunlight, microbial activity, changing temperature, and precipitation are all factors that could transform chemicals in the environment, after they leave their sources and before exposure occurs.

Additionally, it is important to consider that PAH mixtures redistribute in the environment. For instance, if a certain mixture of PAHs is emitted from a source into the air, that mixture will be different at a sampling device 10 miles away, even without chemical transformations. This is partly due to differences in physical and chemical parameters of chemicals, which lead to differing environmental fate of different individual chemicals. For instance, if a mix of PAHs is emitted into the air, more of the higher molecular weight PAHs may partition preferentially into the soil, while more of the lower molecular weight PAHs may remain in the air. Thus, the mixture measured in those two matrices would not look the same as the mixture at the source.

One example of compounds that are commonly formed through environmental transformations, may have greater toxicity than their parent compounds, and are

relatively under-studied, are oxygenated PAHs, or OPAHs.[20, 94] OPAHs can be formed from parent PAHs in the environment, through chemical oxidation, photo-oxidation, or biological transformation.[94] There is evidence that some OPAH compounds are more toxic than the unsubstituted parent PAHs.[94-98] However, they are rarely included in environmental monitoring. When designing a study, it is important to consider how much transformation may have occurred in the environment for the chemicals of interest. If there is the potential for transformations, then both the degree of transformation of the parent compounds, and the creation of new transformation products should be considered. It is worth considering how these transformations might alter the toxicity of the mixture exposures compared with the original source. Exposure to mixtures near an emission source might be quite different from individuals that are distant from the source. This is an example why personal monitoring, as opposed to stationary monitoring, can provide more accurate estimates of exposure.

Transformation products that are formed through environmental degradation processes are sometimes the same products that are created during *in vivo* metabolism. This can mean that the same chemical could enter a person's body both exogenously (as from a source where it was used in the environment) and endogenously (through metabolism of a different chemical). This can lead to errors in assessment of chemical exposure. One example of this is that mammalian metabolism of organophosphate pesticides (OPs) results in formation of a series of dialkyl phosphates (DAPs) that are excreted in the urine. It has often been assumed that the concentration of DAPs in the urine is directly related to dietary exposure to parent OPs. Improved analytical ability to measure DAPs in urine has resulted in an increase in studies using this biomarker of OP exposure. However, the same enzyme-mediated oxidation and hydrolysis reactions that produce DAPs in humans are also responsible for the transformation of OPs in the environment. Thus, DAPs are formed in the environment, and are present on many foods before they are ingested. When DAPs are consumed they do not change in vivo and are excreted.[99] Traditionally, studies of the environmental fate of OPs have not analyzed for DAPs. This means that if all urinary DAPs are assumed to be from OP exposures, then this

is likely a significant overestimate of exposure. Given that OPs have known health effects, and DAPs are generally considered non-toxic, it is important to distinguish between exposure to OPs and exposure to DAPs. However, this has not traditionally always been done. This is an example of the importance of considering whether transformations in the environment are possible with the contaminants of interest, and how those transformations may impact the data.

Considerations for Assessing Exposures to *Mixtures* of Environmental Chemicals: Limited Data on Mixture Interactions

The health community has traditionally evaluated the toxicity of one compound at a time, identified its health effects, and determined an acceptable exposure level for regulatory use (e.g., a Permissible Exposure Limit in the case of occupational exposures regulated by OSHA in the US). However, we are constantly exposed to *mixtures* of chemicals in our daily lives. Limited data exist concerning how exposure to these mixtures may differ from exposure to the individual components of the mixture. In mixtures such as car exhaust fumes, the toxicity of PAHs may be additive, greater than additive, or less than additive.[100] Siddens *et al* demonstrated that PAH mixtures are more potent skin carcinogens compared to benzo[a]pyrene than the EPA's Relative Potency Factor approach would suggest.[100] Novel approaches to assess the health impacts of mixtures are needed.

Risk Assessment of Mixtures: Appropriateness of Assuming Additivity?

Current methods of estimating risk from exposure to chemical mixtures often assume that the toxicities of individual chemicals from the same class (e.g., PAHs) will be dose-additive. This assumption requires presuming that the mode of action of each PAH is the same. However, it has been observed that interactions between individual compounds in a mixture can lead to a greater or less than additive toxic responses.[3] One way to improve risk estimates would be to directly test the mixtures of chemicals measured in the environment in toxicity assays. While testing the endless list of potential mixtures would be prohibitively challenging, a starting place may be to assess the combined

toxicities of mixtures that commonly occur in the environment, such as the mixture of PAHs measured in water or an organism at a site contaminated by a common profile of pollution sources.[27] This will require close communication between exposure scientists and mixture toxicologists.

EDA is a promising framework within which the toxicity of chemical mixtures measured in the environment can be assessed. It is especially attractive because it allows the researcher to break down the chemical mixture piece by piece, and explore the toxicity of each of these fractions (see **Figure 1**).[2, 32] This technique has a lot of potential for helping researchers characterize which components of a chemical mixture are contributing which aspects to the observed toxicity. Importantly, EDA may also eliminate broad groups or classes of chemicals that are not contributing to toxicity. It can also help elucidate how various chemicals behave in the presence of the whole mixture, relative to just in the presence of a chemically similar fraction of that mixture, relative to individually. With new low-volume bioassays, the fractionation process can be scaled accordingly and this allows for rapid turnaround of fractionations and bioassay assessments on the order of days.

Historically EDA would employ, for example, Soxhlet extraction of samples before fractionation. However these extracts yield fractions that bear little connection to the chemical exposures. Soxhlet extraction involves using a strong solvent, high heat and/or elevated pressure to extract as much contaminant as possible from an environmental sample (e.g., sediment). Thus, the concentration of contaminants measured using Soxhlet extraction may be much higher than what would truly be bioavailable in the environment. Often chemicals may be toxic at high levels, but are not bioavailable *in situ* at concentrations sufficient to cause toxicity. EDA aims to characterize the toxicity of mixtures. If it is performed using chemical mixtures that are not representative of true environmental concentrations, this defeats the purpose. One way to avoid this problem is to use passive sampling to collect mixtures for use in EDA. Passive samplers absorb bioavailable fraction of contaminants when deployed in the environment. Passive samplers are extracted using simple solvent-extractions, without

elevating temperature or pressure. This yields more realistic estimates of the bioavailable fraction of contaminants in the environment. These estimates are more relevant for use in EDA than artificially heightened concentrations that may be obtained using other techniques. After extraction, EDA employs multiple rounds of fractionation using various techniques (e.g., normal phase chromatography, size exclusion chromatography, etc.) to separate the various chemicals within the mixture. These fractions can then be individually used in toxicity bioassays, allowing the researcher to further elucidate both which parts of the chemical mixture may cause toxicity, and which parts are not causing toxicity.

All of these tools and more need to be employed for exposure science to begin to address the substantial data gaps surrounding exposures to mixtures of chemicals in the environment.

Importance of Looking for Risk-driving Chemicals

In the absence of comprehensive data describing exposures to all chemical mixtures, the majority of chemical risk assessments assume that the toxicities of chemicals in a mixture are additive (i.e., conform to an assumption of either dose addition or independent action). This approach requires assuming that there are no pharmacokinetic or pharmaco-dynamic interactions among chemicals. As long as this is the paradigm, it will be especially important to measure the chemicals that are most impactful to health risks. The importance of measuring the more toxic PAHs in the mixture (or, at least, the PAHs with the highest known toxicities) for use in risk assessment was illustrated in a recent study measuring PAH mixtures in crayfish tissue.[27] In this work, it was observed that risk estimates were slightly higher than estimates presented in a previous public health assessment for the study area. It was suggested that one factor likely increasing the estimates in the study was that the study's analysis used the EPA's 2010 RPF approach to scale the carcinogenic potency of the PAH mixture relative to that of benzo[a]pyrene.[59] When this was combined with an analytical method that quantifies 23 of the 26 PAHs that were given RPFs by the EPA 2010[59] document, risk

estimates increased relative to previous methods. This increase is due both to quantifying more PAHs (relative to looking for the EPA's 16 priority pollutant PAHs, for example) and to using the extended list of potency values presented by the EPA in 2010 (compared to a shorter list, such as the EPA's 1993 list of 7 RPFs[101]). Additionally, the crayfish tissue contained a few of the PAHs from the EPA 2010 document that have RPFs higher than benzo[a]pyrene. Even a small quantity of a highly potent chemical can change the risk assessment picture. For instance, benzo[c]fluorene was measured in some of the crayfish tissues presented in this study.[27] This compound has an RPF of 20, suggesting that, if a sample had the same concentration of benzo[a]pyrene and of benzo[c]fluorene, benzo[c]fluorene would contribute *20 times* more carcinogenic risk. However, this compound is not included in traditional monitoring programs, and is not in the EPA's previous priority pollutant list.[102] Given that the EPA's 16 priority pollutant list is still often used as the standard PAHs to measure, benzo[c]fluorene, or other carcinogenic PAHs such as DBC (discussed above) could often be present in the environment without being detected. Assuming that these newer estimates are accurate would suggest that previous risk assessments, which only included the EPA's shorter list of priority pollutants, may have been inadvertently under-representing the potential for risk.

Summary of Considerations for Interpreting Data from Exposure Assessment Studies:

It is important to consider the factors mentioned in this section when interpreting data from studies assessing environmental exposures. **Below is a list summarizing the main considerations outlined above:**

- **Spatial orientation and choice of sampling technique:** Does the chosen sampling technique fit the research question? For instance, does the choice between a personal or stationary sampler make sense? If one was used and not the other, consider how the choice of sampling technique may affect the results. It is also important to consider sources of uncertainty related to each sampling technique.
- **Temporality of sampling technique:** Was the sampling method timed appropriately to capture the exposure concentration of interest? How does

temporality of the sampling event compare to expected temporality of exposure? If they are different, what impact might that have on the results?

- **Is the appropriate fraction of contaminants measured?:** Did the sampling strategy measure the fraction of contaminants that a person would be exposed to, via the exposure route of interest? Did the sampling design measure the bioavailable fraction of contaminants? If not, what impact could that have on results?
- **What chemicals are measured?** Are they the appropriate ones to answer the question, or enough of the appropriate ones to answer the question? Can the toxicity of the entire mixture be explored (e.g., through EDA)? Could more chemical data be gleaned from the analytical techniques that were used?
- **Transformations of contaminants in the environment:** Is there potential for transformations to have occurred between the chemicals' source and the sampling site, or the exposure site? If so, was this addressed? If not, what impact might that have on the results?
- **Mixture effects: Was the challenge of assessing exposure to mixtures addressed?** Was a form of dose-additivity assumed? If so, is that consistent with EPA guidance for mixtures risk assessment (e.g., EPA 2000[103])? How might any assumptions, or data gaps, about exposures to the mixtures being studied affect results?

Priority Research Needs for Assessing Exposure to Chemical Mixtures

Field

Develop sampling techniques that accurately assess exposures, while minimizing cost and maximizing compliance: Optimal sampling techniques for any study ideally consider spatial and temporal factors of exposure, and whether personal or stationary/population-based sampling is most appropriate. As identified by the NIEHS's strategic plan, this must include considering how exposures may differ at more susceptible life stages.[5] In many cases, a personal sampler may be the best tool for assessing exposures. To make this feasible, there is a need to develop more sampling tools that are cost effective, and have the ability to measure a wide range of chemicals to which a person is exposed. By continually measuring chemicals on an individual, these tools allow researchers to more accurately estimate what chemicals an individual is exposed to, over a longer period of time. These samplers can also be paired with

toxicity bioassays, and incorporated into pre-existing or new public health studies. The passive wristband sampler is an example of such a tool.

Move toward personal, *in situ* sampling – more representative of true exposure: It has been suggested that, to best assess total chemical exposures, we must develop interdisciplinary teams of scientists measuring many aspects of exposures.[48] From the environmental chemistry perspective, it seems appropriate to focus efforts on measuring personal chemical exposures, in situ.

Integrate personal sampling with other technologies to learn even more: For example, a recent study demonstrated the utility of combining a personal sampling device with a GPS-tracking device, questionnaires, and a spirometer to track lung function. [104] This is an excellent example of an interdisciplinary project, where multiple forms of data are used to assess the relationship between exposures and adverse health outcomes.

Sampling technologies that more accurately reflect temporal changes: Technology that continually samples chemical concentrations, as opposed to only measuring concentrations at distinct time points, enables estimations of chemical exposures to be normalized over time. This is more representative of the entirety of a person's exposure over a given unit of time. These technologies are therefore promising for use in epidemiological studies or for use in human health risk assessment

Lab

Which chemicals to measure? How to prioritize them?: The EPA's ToxCast work is one example of how chemicals are being prioritized for further study.[37] ToxCast rapidly screens and predicts the toxicity of thousands of chemicals, in order to prioritize which to study. Another useful tool is the EPA's ExpoCast.[105] ExpoCast uses high-throughput approaches to rapidly estimate exposure for thousands of chemicals. Taken together, ToxCast and ExpoCast should help exposure scientists prioritize which chemicals are most relevant for study, based both on toxicity and exposure. Additionally, EDA is another tool that can help identify non-target chemicals that are

eliciting adverse effects. One way to identify currently unmonitored chemicals is to look more closely at and reexamine existing analytical data. There is often additional information in analytical results that just needs to be identified. This is an argument for collecting and sharing all analytical data possible. Even if some of the data are outside the main goals of a study, sharing all of it allows other researchers to use it to make new discoveries, and to make observations and connections the primary researchers may not have had resources to exploit.

Assess risk of exposure to *chemical mixtures* using various approaches, (and importance of using the right metric to estimate risk):

Screen commonly-found mixtures: The exposure science community needs more studies directly measuring the bioavailable fraction of chemicals in the environment. This will add to our knowledge of the chemicals where human exposure is common or frequent, and therefore which to prioritize for study. This will also improve our ability to make public health decisions that have the greatest possible reduction in public health risks associated with environmental exposures. Additionally, mixtures that people are commonly exposed to should be prioritized. A recent study used ecological niche theory to identify that certain pesticides co-occur much more often than others in US child care centers.[106] Characterizing mixture effects in these commonly-occurring pesticide mixtures would be much more useful than in mixtures that rarely occur in the environment. Continuing to identify commonly occurring chemical mixtures would allow toxicologists and risk assessors to better prioritize which mixtures to study, and to more accurately assess risk associated with exposure to chemical mixtures.

Interface with bioassays: Rarely can currently-used technologies for measuring mixtures of chemicals in the environment integrate directly with in vitro or in vivo toxicity assays. The environmental health science community needs more comprehensive measures of exposure and how they vary as a function of space and time. An example of this is testing a chemical extract from an environmentally-deployed passive sampler, representing a mixture of chemicals from the environment, in a bioassay such as the embryonic zebrafish model. The utility of this technique has been

demonstrated previously.[31, 107] Combining techniques like these allows the researcher to directly observe health effects of real-world mixtures.

Holism vs. reductionism: Holism and reductionism represent two different approaches to revealing the links between chemicals and our health. Holism attempts to understand the properties of a whole system by studying all of its parts together. The basis of the approach is that some system properties cannot be found by studying the individual components separately due to the complexity and integration of the system. Reductionism attempts to reveal the properties of the system by separating the components (e.g., measuring the toxicity of chemicals individually). While this approach is the foundation of centuries of successful scientific exploration, the method has shortcomings when it comes to assessing the effects of chemical exposures on human health. In the same way that an organism would not be well represented by its isolated cells, a chemical mixture should not be described exclusively by the properties (environmental occurrence, toxicity, etc.) of its components. One could argue we need both holistic and reductionist approaches. Because it is much easier to apply the reductionist method to analytical methods, this has been the predominant approach for decades. However, the exposure science community should be striving to include more holistic approaches, both for analytical methods, and exposure estimation.

Effects-directed analysis (EDA): The goal of EDA is to determine which chemical (or chemicals) in a complex sample may be causing toxicity, by manipulating the sample to simplify the analysis. Equally important it may be possible to eliminate large numbers of chemicals that do not elicit toxicity. However, to conduct an accurate effects-directed analysis, it is necessary to consider bioavailability. An additional area of useful research would be developing other methods of extracting the bioavailable fraction of contaminants from the environment, for exploration through EDA.

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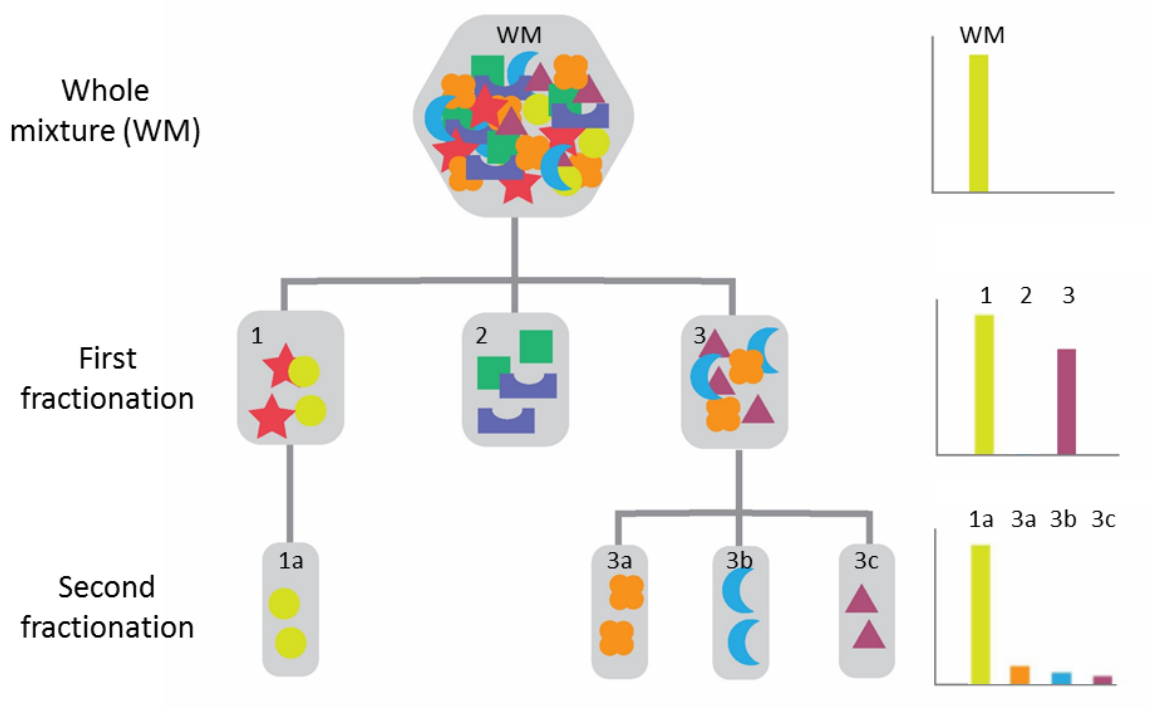

Figure 1.1. A whole chemical mixture is separated into fractions using effects-directed analysis (EDA). The toxicity in the whole mixture is depicted by the yellow bar in the top graph. In the first fractionation, the whole mixture was separated into three fractions (1-3). Both fraction 1 and 3 contained some toxicity, but no toxicity was observed in fraction 2. The toxicity of fraction 1 appears to be the same as the toxicity of the whole mixture. However, there is additional toxicity in fraction 3. This suggests that there was less than additive toxicity in the whole mixture before fractionation. This illustrates the power of EDA to help researchers identify both toxic and inert fractions within chemical mixtures. In the second level of fractionation, fractions 1 and 3 were separated into fractions 1a and 3a-c. In these fractions, we see that the toxicity of fraction 1a is the same as the toxicity of fraction 1, and of the whole mixture. This could be interpreted to mean that the majority of toxicities observed in the original mixture and in fraction 1 were coming from the yellow circles. When these circles were completely isolated in fraction 1a, this fraction retained the same level of toxicity as in the whole mixture and in fraction 1. However, in this final fractionation we also see that the sum of the toxicities of fractions 3a-c is less than the toxicity of fraction 3. This suggests that there was the potential for greater than additive toxicity in fraction 3 before the second fractionation. This example illustrates the utility of EDA to help researchers identify: components that are causing toxicity within a mixture, mixtures (and fractions of mixtures) where greater or less than additive toxicity is observed, and fractions of mixtures that do not appear to cause toxicity, but may influence the toxicity of the whole mixture.

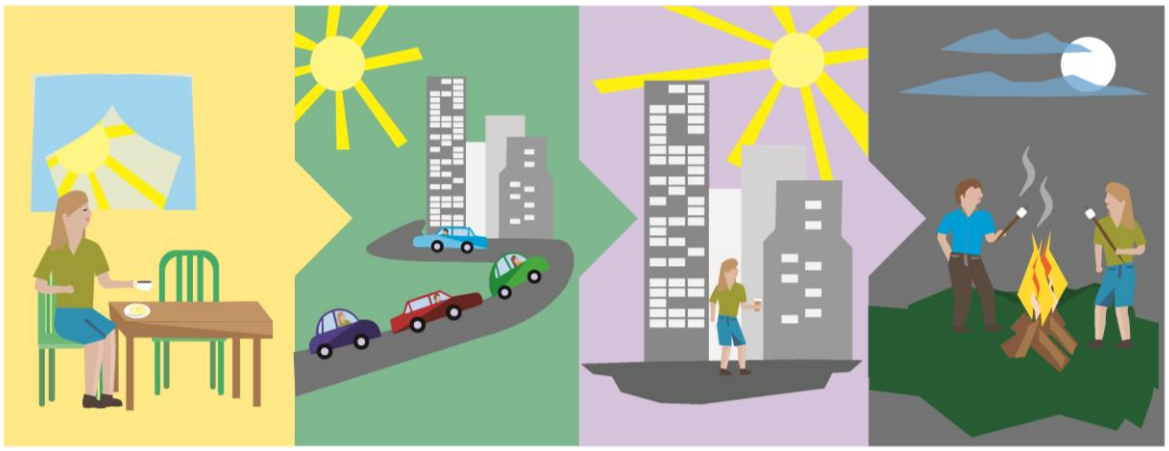

Figure 1.2. People are exposed to chemical mixtures, for example PAHs, through a variety of scenarios every day. Here, snapshots of a typical day depict four different exposure scenarios. This progression illustrates the spatial and temporal components of exposure, and provides examples of common exposures occurring through the three main exposure routes. On the left, an ingestion exposure is shown in the woman's kitchen. This could include ingesting chemicals like PAHs in cereals. Next, as the woman commutes to work in heavy traffic, an inhalation exposure is shown. She may be exposed to elevated levels of PAHs and other chemicals emitted from car exhaust while driving to work. Next, additional examples of inhalation and ingestion exposure are shown as she walks in the city. She may be exposed to PAHs and chemical mixtures by inhaling chemicals emitted from the myriad anthropogenic pollution sources present in a congested city. On the right, examples of inhalation, ingestion, and dermal exposure are shown in this recreational scene. While enjoying a campfire there is the potential to inhale PAHs and other chemical mixtures from smoke, to ingest chemicals present in food that has been roasted over fire, and to experience dermal exposure. Taken together, these vignettes represent a realistic suite of PAH and chemical exposures that could occur in the daily lives of many people. All of the exposure scenarios described, as well as others that are not pictured in these four snapshots, would combine to yield this person's cumulative daily PAH and chemical exposures.

Chapter 2 – Passive Samplers Accurately Predict PAH Levels in Resident Crayfish

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Abstract

Contamination of resident aquatic organisms is a major concern for environmental risk assessors. However, collecting organisms to estimate risk is often prohibitively time and resource-intensive. Passive sampling accurately estimates resident organism contamination, and it saves time and resources. This study used low density polyethylene (LDPE) passive water samplers to predict polycyclic aromatic hydrocarbon (PAH) levels in signal crayfish, *Pacifastacus leniusculus*. Resident crayfish were collected at 5 sites within and outside of the Portland Harbor Superfund Megasite (PHSM) in the Willamette River in Portland, Oregon. LDPE deployment was spatially and temporally paired with crayfish collection. Crayfish visceral and tail tissue, as well as water-deployed LDPE, were extracted and analyzed for 62 PAHs using GC-MS/MS. Freely-dissolved concentrations (C_{free}) of PAHs in water were calculated from concentrations in LDPE. Carcinogenic risks were estimated for all crayfish tissues, using benzo[a]pyrene equivalent concentrations (BaP_{eq}). ∑PAH were 5-20 times higher in viscera than in tails, and $\sum BaP_{eq}$ were 6-70 times higher in viscera than in tails. Eating only tail tissue of crayfish would therefore significantly reduce carcinogenic risk compared to also eating viscera. Additionally, PAH levels in crayfish were compared to levels in crayfish collected 10 years earlier. PAH levels in crayfish were higher upriver of the PHSM and unchanged within the PHSM after the 10-year period. Finally, a linear regression model predicted levels of 34 PAHs in crayfish viscera with an associated R-squared value of 0.52 (and a correlation coefficient of 0.72), using only the C_{free} PAHs in water. On average, the model predicted PAH concentrations in crayfish tissue within a factor of 2.4 ± 1.8 of measured concentrations. This affirms that passive water sampling accurately estimates PAH contamination in crayfish. Furthermore, the strong predictive ability of this simple model suggests that it could be easily adapted to predict contamination in other shellfish of concern.

Introduction

Resident aquatic organism contamination is often of concern at sites with environmental pollution. This is especially true when local communities rely on these organisms for food or income. However, characterizing the risk associated with consuming organisms can be challenging. Collecting enough organisms to assess contamination requires specific knowledge about the organism and the local ecosystem, and it is often prohibitively time and resource-intensive. Harvesting large numbers of organisms can also have adverse impacts on local ecosystems. Accurately assessing resident organism contamination is important for improving both human health risk assessments and ecological risk assessments.[108, 109] Using a predictive approach to assess organism concentrations is attractive because it requires substantially less time and resources than collecting organisms.

In the past 25 years, passive sampling has been gaining momentum as a useful tool for measuring trace levels of contaminants.[19, 20] Passive samplers measure time-integrated concentrations of the freely dissolved concentration (C_{free}) of contaminants in water. Passive samplers are relatively low-cost, and they do not require energy or maintenance while deployed. Infusing passive samplers with performance reference compounds (PRCs) before deployment further improves their ability to accurately assess contaminant levels.[21]

Low-density polyethylene (LDPE) is a widely-used material for making passive samplers.[22] When LDPE is deployed in water, hydrophobic organic contaminants (HOCs) diffuse into LDPE from the water into the hydrophobic polymer. This process is analogous to passive uptake by a phospholipid membrane into an organism's tissues, making LDPE well-suited to serve as a surrogate for contamination in organisms.[23, 24, 26]

Numerous studies have compared uptake of HOCs in passive samplers and aquatic organisms. Many of these investigated the potential for caged organisms to serve as sampling devices, or "biomonitoring organisms" (BMOs).[110] Many studies have used LDPE filled with triolein as a sampling device, known as semi-permeable

membrane devices (SPMDs). Anderson et al.[22] co-deployed SPMDs with triolein-free LDPE samplers and concluded that the two samplers behaved sufficiently similarly. Thus, the two will be directly compared in the present study.

Booij et al.[23] reviewed nine studies comparing SPMDs and BMO mussels, concluding that SMPDs yield less variable results, while identifying similar spatial trends. In the same year, Huckins et al.[21] reviewed over 30 studies comparing SPMDs and BMOs, concluding that there are substantial overarching similarities in HOC accumulation in aquatic organisms and SPMDs.

Recent research has continued to assess passive samplers as replacements for BMOs in assessing water quality[110-113] and as tools to estimate contaminant levels in resident organisms.[24-26] While some studies have highlighted key differences between contaminant accumulation in passive samplers and organisms,[108, 111] the majority report good agreement between contaminant accumulation in passive samplers and organisms.[24-26, 110, 112, 113]

Only a few studies have used predictive tools to assess human health risks associated with consuming resident organisms.[24, 25] Many studies have used predictive tools to assess the accumulation of HOCs in aquatic organisms. These predictive tools often require chemical or physical partitioning data such as bioaccumulation factors or partition coefficients between lipid and water, and including these can increase prediction variability.[21, 23, 114] Notably, Fernandez and Gschwend showed that using porewater C_{free} predicted more accurate and less variable tissue concentrations in clams than using the traditional biota-sediment accumulation factor [26]. However, even when predicting based on C_{free}, they suggested that using previously published values (to estimate lipid-water partitioning coefficients and the fraction of lipids in the clams) may have increased variability in their predictions.[26] Additionally, in their 2006 review, Huckins *et al* noted that lipid-normalizing tissue concentrations has been debated in the literature since the early 1980s.[21] It is therefore desirable to have a predictive tool that requires as few additional inputs as possible.

Forsberg et al.[25] demonstrated that mathematical models may work as well or better than physical or chemical partitioning data when predicting organism concentrations using passive samplers. If organismal concentrations could be reliably predicted using only passive sampler data and mathematical models, this would greatly reduce the time and information needed for a risk assessor to estimate contaminant levels in resident organisms.

Polycyclic aromatic hydrocarbons (PAHs) are pervasive environmental contaminants that pose risks to human health. Some PAHs are pro-carcinogens, that can be metabolically activated through oxidation by P450 enzymes, creating reactive intermediates which can form DNA adducts.[61] Diet is the main pathway by which nonsmokers are exposed to PAHs.[115, 116]

The Portland Harbor Superfund Megasite (PHSM) is located in the Willamette River in Portland, Oregon. Since the Industrial Revolution, Portland Harbor has been subjected to countless sources of pollution that left a legacy of pollutants, including PAHs.[31, 117] In 2000, the area between river mile (RM) 3.5 and 9.2 was designated the PHSM. The PHSM was later expanded, stretching from RM 2.0 to 11.8 as of 2013.[118] This area is home to many species that are harvested by local fishermen, including the native signal crayfish, *Pacifastacus leniusculus*.[117] Crayfish consumption was listed as a main route of exposure to pollutants in the Agency for Toxic Substances and Disease Registry (ATSDR)'s Public Health Assessment (PHA) for the PHSM.[117, 119]

It has been previously observed that PAHs accumulate in crayfish.[120] This is partly due to crayfish having less efficient cytochrome P450 systems than finfish.[121] Additionally, signal crayfish in the Willamette likely spend their whole lives in Portland Harbor due to their small home range. This means they are exposed for much more of their lifespans than organisms that only pass through the PHSM.[117] The combination of reduced metabolism and increased exposure duration may lead to greater bioaccumulation of pollutants in crayfish than in finfish. A similar dynamic would likely be observed in other shellfish that have similar behavioral and physiological patterns.

Thus, a model built using one shellfish species would likely also be easily transferred to predict contaminant levels in other shellfish of interest.

Forsberg et al.[25] observed that PAH accumulation in *P. leniusculus* correlates well with C_{free} measured by SPMDs. While this study successfully modeled PAH levels in crayfish, the model was limited to 15 PAHs, and crayfish and SPMDs were not directly spatially or temporally paired. These limitations may have necessitated adjustments in model development that reduced modelling accuracy. Thus, the ability of LDPE to predict PAH levels in crayfish and other resident shellfish warrants further investigation. The objective of this study was to use LDPE passive water samplers to predict PAH levels in crayfish, with sufficient accuracy to perform risk assessments.

Materials and Methods

Description of study site and sampling locations

The study was conducted in fall 2013, in the lower 18.5 miles of the Willamette River, within and outside of the PHSM. Samples were collected at five sites: upriver of the PHSM (RM 18.5 and 12E), within the PHSM (RM 11E and 3.5W) and downriver of the PHSM (RM 1NW). Although RM 12E is not in the PHSM, it is 0.22 miles upriver of the upper bound of the PHSM, as defined in 2013, and was considered within the "downtown reach" in the 2013 PHSM remedial investigation.[118] Site names in this study use approximate RM designations. Comparisons are made with crayfish and passive sampler data from previous sampling campaigns (in 2003 and 2012), which included different RMs, described below and in Appendix 1. A map of the study area is included in **Figure A1.1**, and GPS coordinates of sites are available in **Table A1.1**.

Crayfish sampling, dissection, and extraction

Resident signal crayfish, *P. leniusculus* were collected from the Willamette River, at the five sites listed above. A total of 130 crayfish were collected during September and October, 2013. Crayfish collection was performed in accordance with Oregon Department of Fish and Wildlife Scientific Taking Permit for Fish, number 18039. Frozen

tuna pieces and canned cat food were used as bait, and crayfish were caught in minnow traps that were retrieved within 24 hours. Upon trap retrieval, external crayfish surfaces were rinsed with ambient water, rinsed with 18 MΩ*cm water, and inspected for physical damage. Crayfish were euthanized on site using liquid nitrogen, and wrapped individually in aluminum foil. Crayfish were then transported in coolers with frozen ice packs back to the Food Safety and Environmental Stewardship (FSES) lab at Oregon State University (OSU) in Corvallis, Oregon. At the lab, crayfish were stored at -20°C until dissection.

Lab processing methods for crayfish are visualized in **Figure A1.2a**. Each crayfish was sexed, measured (full body length and carapace length), weighed, and then dissected. Of the 130 crayfish collected, 60 (12 from each of the 5 RMs) were dissected, with viscera and tail (abdominal muscle) tissue being composited separately. This yielded 3 composites of each tissue (viscera and tail) from 12 crayfish, for each RM (with each composite containing tissue from 4 crayfish). Composited tissues were stored at -20°C until homogenization. Each composited tissue sample was homogenized to a fine powder using liquid nitrogen and a metal mortar and pestle, as described previously.[25]

Homogenized tissue samples were extracted using a slightly modified QuEChERS method.[25, 122] Each tissue sample was spiked with surrogate standards (listed in **Table A1.2**) immediately before extraction, and 1.0 g (± 2%) wet weight of each composited tissue was extracted. Further extraction details are in Appendix 1.

Previous studies assessing the home range of *P. leniusculus*, in ecosystems comparable to the river system in the present study, showed that these crayfish typically migrate less than 225 m in a two-year period.[123, 124] Thus, PAH levels measured in crayfish in the present study are interpreted as site-specific, time-weighted average concentrations.

LDPE preparation, deployment, cleaning and extraction

Before deployment, LDPE was cleaned using hexanes and prepared as described previously.[22] Each LDPE strip was spiked with PRCs to enable calculation of *in situ*

sampling rates and time-integrated water concentrations.[21] PRCs used in this study were fluorene-d10, pyrene-d10 and benzo[b]fluoranthene-d12. Slightly different PRCs were used for the 2012 deployment, described in Appendix 1. Between 4 and 100 μg of each PRC was spiked into each LDPE strip. After PRC addition, each LDPE strip was heat-sealed at both ends.

Passive samplers were deployed in metal cages in the water column. Three PRC-infused LDPE strips were deployed in each cage. At RM 18.5 and 3.5W, three cages were deployed. At RM 12E, 11E and 1NW, one cage was deployed. For the 2012 deployment, three cages were deployed at RM 7E. Additional details and exact sampling dates are in Appendix 1. All samplers were rinsed in ambient water and transported back to the lab in amber glass jars. Samplers were stored at -20°C until cleaning.

LDPE processing that occurred in the lab after retrieval is visualized in **Figure A1.2b**. LDPE were cleaned in 1 N hydrochloric acid, 18 mΩ*cm water, and two isopropanol baths, stored in amber jars at -20°C, and extracted as described previously.[22] Briefly, extractions were performed using two dialyses of hexane. Immediately before extraction, surrogates standards were spiked onto LDPE to account for extraction efficiency. Extraction surrogates are listed in **Table A1.2**. Extracts were quantitatively concentrated to 1 mL using TurboVap closed cell evaporators, transferred to amber chromatography vials, and stored at -20°C. Details regarding chemicals and solvents are in Appendix 1.

Chemical Analysis

Crayfish and LDPE extracts were quantitatively analyzed for 62 PAHs using an Agilent 7890A gas chromatograph interfaced with a (modified) Agilent 7000 GC–MS/MS, as described elsewhere.[125] More information about the analytical method is in Appendix 1. Lists of PAHs, limits of detection (LODs) and limits of quantitation (LOQs) for the instrument method are included in **Table A1.2**. Lists of LODs for PAHs measured in crayfish tails, crayfish viscera, and water are included in **Table A1.3**. LODs for this study

ranged from 0.0003-0.03 ng/L in water, 0.03-0.78 ng/g in crayfish viscera, and 0.24-6.4 ng/g in crayfish tails (**Table A1.3**).

Water Concentration Calculation

Concentrations of PAHs freely dissolved in water (C_{free}) were calculated from concentrations in water-deployed LDPE using equations **A1.1-A1.6**, as described by Huckins et al.[21] Sampling rates (R_s) were derived by measuring loss of performance reference compounds (PRCs) during deployment. PRCs allow for accurate assessment of *in situ* uptake rates for a wide range of compounds in variable environmental conditions.[126, 127] These calculations are described in detail in Appendix 1. These C_{free} values will be referred to simply as "water" concentrations in the results and discussion.

Estimation of Carcinogenic Potency

The carcinogenic potency of the PAH mixture in each crayfish sample was estimated using the EPA's 2010 relative potency factor (RPF) approach.[59] This approach scales concentrations of PAHs based on their carcinogenic potency relative to benzo[a]pyrene, yielding a benzo[a]pyrene equivalent concentration, or BaP_{eq}. $\sum BaP_{eq}$ values were calculated using equation **A1.7**; a list of RPFs is in **Table A1.4**.

Predictive Modeling

Examining correlation plots of PAH concentrations in crayfish versus water suggested ordinary linear regression could be used to produce a predictive model with water concentrations predicting crayfish concentrations. Thus, a linear regression model was generated using the paired crayfish and water data for individual PAHs measured in the 2013 sampling campaign. All data were fourth-root transformed prior to modeling, to improve model performance. Other models which included factors such as individual PAH, site, and K_{ow} values were also explored. In the predictive model, any data that were below the LODs (BLOD) in either crayfish or water was excluded. The model was created using JMP Pro, version 11.2.0. Predicted PAH levels in crayfish were calculated using the model, with only the PAH levels measured in water as inputs. Model

performance was further investigated by calculating the factor differences between predicted and measured PAH values. To further validate the model, a separate linear regression was created using only 80% of the data. This model was then used to predict crayfish concentrations for the other 20% of the data. These subsets of the data were chosen randomly and non-overlapping. This is described further in Appendix 1. An additional model was made in which the LODs were substituted for all BLOD values. The performance of this model is discussed briefly as well.

Comparisons of PAHs in Crayfish Viscera: Measured in 2003 and Predicted in 2012

A subset of crayfish viscera collected 10 years earlier (fall of 2003) were analyzed for 62 PAHs for comparison with the 2013 crayfish viscera data. The 2003 sampling campaign has been previously described, so only pertinent details are included here.[25] In 2003, crayfish were collected at two sites upriver of the PHSM (RM 17 and 13) and three sites within the PHSM (RM 7W, 7E and 3E). Specific GPS coordinates for sampling locations are presented in **Table A1.1b**. Importantly, RM 7E is the site of the former McCormick and Baxter Creosoting Company, which was added to the US EPA's National Priorities List in 1994, independently of the PHSM.[128] Remedial actions were carried out by the EPA at this site, and were completed in 2005.[129]

Data were separated temporally by the sampling year and spatially relative to the PHSM. The PHSM was divided into RM 7E and the greater PHSM because of RM 7E's independent Superfund status and completed remediation. Additionally, the predictive model from the present study was used to estimate PAH levels in crayfish viscera at RM 7E in 2012, using C_{free} PAH concentrations from RM 7E in 2012 (these water data are presented in Appendix 1). These predicted values enabled comparisons between PAH levels in crayfish at RM 7E before and after remediation (in 2003 and 2012).

Quantitative Human Health Risk Assessment

Given that non-carcinogenic toxicity of PAHs remains understudied, and that carcinogenicity is typically the risk-driving endpoint for PAHs in human health risk

assessments,[130] this study focuses on estimating carcinogenic risk. **Equations A1.8 and A1.9**, described in Appendix 1, were used to estimate risks associated with consuming crayfish tissues containing the measured PAH mixtures. In data exploration, the crayfish ingestion rate was set at both 3.3 g/day and 18 g/day, which are the average and 95th percentile rates for adults in the United States.[117] Risk estimates generated using the 95th percentile ingestion rate are presented and discussed here, to estimate a worst case exposure scenario. All risk estimates would be ~6-fold smaller if the average ingestion rate were used. Excess lifetime cancer risk (ELCR) estimates were used to compare the potential cancer risks across samples. ELCRs exceeding one in a million are discussed, and presented graphically. This was the acceptable risk level used by ATSDR to screen contaminants in their PHA for the PHSM.[117] Values for all other exposure parameters for the risk assessment calculations were chosen in accordance with EPA or ATSDR guidance, and are available in Appendix 1.[117]

Measured $\sum BaP_{eq}$ values were used as the tissue concentrations to estimate ELCRs. The predictive model was used to predict $\sum BaP_{eq}$ in 2012 crayfish viscera from RM 7E. This predicted $\sum BaP_{eq}$ was used to estimate an ELCR for 2012 crayfish viscera from RM 7E. Additionally, where tail tissue data were not measured (in the 2003 dataset and in the predicted 2012 data), it was assumed that $\sum BaP_{eq}$ in tail tissue was 10% of $\sum BaP_{eq}$ in viscera. This is health-protective, as $\sum BaP_{eq}$ in tail tissue were as low as 1% of $\sum BaP_{eq}$ in viscera in some crayfish sampled in 2013. Finally, to estimate ELCRs associated with whole crayfish tissue, $\sum BaP_{eq}$ in tail and viscera tissue were summed, scaled relative to each tissue's average percent contribution to total wet weight of sampled tissues (45 and 55%, for tail and viscera, respectively). The resulting estimates are referred to as ELCRs for "whole tissue."

Statistical Methods

Statistical analyses of PAH data were performed using the statistical software R, version 2.15.3. Wilcoxon Signed-Rank tests were used to compare $\sum PAH$ and $\sum BaP_{eq}$ levels in viscera and tails of the same crayfish. Wilcoxon Rank-Sum tests were used to

compare ∑PAH levels in crayfish viscera collected in different locations in the same year, and to compare ∑PAH levels in crayfish viscera collected in similar locations in different years. Any PAH concentrations that were BLOD were treated as zeros. For all comparisons, significant differences were interpreted when $p < 0.05$.

Quality Control (QC)

Numerous quality control measures were taken. Crayfish extraction was duplicated for one sample each of tails and viscera. Additional QC steps taken for crayfish samples included homogenization blanks and extraction blanks for both viscera and tails, as well as an additional SPE blank for the viscera extraction. For the water-deployed LDPE, multiple QC steps were taken. The first was that one LDPE strip was hung in the lab for the entirety of the PRC-infusion process, to account for potential contamination. Samples (containing one LDPE strip each) were taken on each field sampling trip, where they were used as field blanks, to account for potential contamination during LDPE transport or while LDPE were being strung on water cages. LDPE sample deployment was replicated (n=3) at two of the five sampling sites. Before extraction, all samples were spiked with extraction surrogate standards ranging the span of $\log K_{ow}$s of target PAHs (surrogate standards are listed in **Table A1.2**). The analytical method was validated prior to use using its calibration, precision and accuracy, and detection limits. Before instrumental analysis, perylene-d12 was spiked into all sample extracts at 500 ng/mL to act as an internal standard. During instrument analysis, instrument blanks and continuing calibration verifications were run at the beginning and end of each set of samples. All laboratory and field procedures were performed according to FSES Standard Operating Procedures.

Quality Control Results

Carcinogenic PAHs were BLOD in all blank QC samples. Any PAH concentrations that were above the LODs in QC samples were subtracted before data were analyzed. Extraction surrogate recoveries in crayfish tails ranged from 78 ± 5% for chrysene-d12, to 88 ± 5% for naphthalene-d8. For crayfish viscera, extraction surrogate recoveries

ranged from 28 ± 11% for naphthalene-d8, to 79 ± 20% for benzo[a]pyrene-d12. The average differences between individual PAH concentrations for the two duplicate extractions were 0.9 ng/g and 2.5 ng/g for viscera and tails, respectively. For 2003 crayfish samples, quality control results have been previously reported [25]

For LDPE, extraction surrogate recoveries ranged from 48 ± 4% for naphthalene-d8, to 96 ± 7% for benzo[a]pyrene-d12. Averages of the few target compounds quantified in the field blanks were multiplied by 3 (to convert from one strip of LDPE used as a field blank to 3 strips used in a sample), and the resulting values were used to background-subtract target PAH concentrations in LDPE samples before data analysis. The average RSD among ∑PAH concentrations measured in LDPE replicates in water was 17.5% (n=3, at two replicate sites).

Results and Discussion

PAH Levels in Crayfish Viscera, Tails, and Water

Average ∑PAH in crayfish viscera, tails, and water are listed in **Table A1.5**. ∑PAH were significantly larger in crayfish viscera than in tails of the same organisms (Wilcoxon Signed-Rank Test, $p < 0.001$). ∑PAH in viscera were 5 to 20 times higher than in tails (**Figure 2.1A**). This is consistent with previous work, which observed that PAH levels are highest in the hepatopancreas and lowest in muscle tissue in crabs [131]. In crayfish, it has been suggested that oxidative metabolism of PAHs and other xenobiotics occurs in the hepatopancreas and in the green gland.[120, 132] It has also been observed that PAH uptake rates from water are 11-90 times higher in *P. leniusculus* hepatopancreases than in tail muscle tissue, while elimination rates are comparable or slightly higher in the muscle tissue.[133] Thus, elevated PAH concentrations in the viscera are not surprising, as the visceral tissue contains both the hepatopancreas and green gland.

Carcinogenic Potency of PAHs in Crayfish Viscera and Tails

$\sum BaP_{eq}$ were significantly higher in viscera than in tails (Wilcoxon Signed-Rank test, $p < 0.001$). Specifically, $\sum BaP_{eq}$ were 6 to 70 times higher in viscera than in tails

(**Figure 2.1B**). This is an even more dramatic difference than between viscera and tails for ∑PAH, indicating that the fraction of carcinogenic PAHs is higher in the viscera compared to the tails. This suggests that eating only the tail tissue of a crayfish would yield significantly less carcinogenic risk than eating the viscera, or than eating all of the tissue in the organism. This is important because ingestion is one of the main routes of PAH exposure in nonsmokers.[115, 134]

Predictive Modeling

Because the concentrations of ∑PAH were so low in tails, only viscera data were used in predictive modeling. The relationship between PAH levels in crayfish and the C_{free} in water (estimated using LDPE) from 2013 was explored extensively. Individual PAH levels in crayfish viscera and water correlated well across all sites, for both the PAH profile (**Figure A1.3**) and the magnitude of PAH concentration (**Figure A1.4**). This trend was not affected by sampling site or by $\log K_{ow}$ (**Figure A1.4**). Of the 62 PAHs in the analytical method, 18 were BLOD in both crayfish and water at all sites, and were thus not included in the predictive model. The majority of these PAHs were higher molecular weight ($\log K_{ow} \geq 6.75$), and thus would not be expected to be freely dissolved in water at high levels. It is interesting, however, that these higher molecular weight PAHs were BLOD not only in water, but also in crayfish tissues. This suggests that PAH profile is very similar between crayfish and water, providing further support for the use of passive water samplers to predict levels of PAHs, and likely other HOCs, in crayfish. There were instances where a PAH was detected in water and BLOD in crayfish, or vice versa. These PAHs were also excluded from the model. However, it is worth noting that, with the exception of 2,6-DMN (discussed in Appendix 1), the average value measured in one matrix but not the other was 0.09 ppb. Thus, if a PAH is BLOD in water, it is reasonable to predict that it will either be BLOD or very small in crayfish viscera (~1 ppb).

Previous studies have observed that the relationship between contaminant uptake in organisms and passive samplers is not one to one.[23] Thus, a linear regression model was generated to predict crayfish PAH levels using individual PAH levels

measured by LDPE in water (**Figure 2.2**). The r-squared value for the line of best fit was 0.52, which corresponds to a correlation coefficient of 0.72. The narrow confidence interval (darker shading) indicates that the average response is modeled well (**Figure 2.2**). The prediction interval (lighter shading) is wider, because it characterizes uncertainty associated with individual observations, rather than averages (**Figure 2.2**). The regression slope of 0.88 indicates that, in transformed units, a change of 1 in water corresponds to a change of 0.88 for fish in transformed units. The equation of the line of best fit is:

$$\boldsymbol{Eq\ 2.1.}\ [\mathrm{PAH}]_{\mathrm{crayfish}} = 0.88 \times [\mathrm{PAH}]_{\mathrm{water}} + 0.56$$

Equation **2.1** can be used to predict PAH concentrations in crayfish viscera, using only C_{free} PAH concentrations in water. The standard error for intercept is 0.054, and standard error for slope is 0.078. Additionally, the training model (built using 80% of the data) was almost identical to the model built using all of the data, and it accurately predicted crayfish concentrations for the other 20% of the data (**Figure A1.5**). Further details are provided in Appendix 1.

When LODs were substituted for BLOD data (instead of excluding them from the model as was done in the first model), the equation of the line of best fit was:

$$\boldsymbol{Eq\ 2.2.}\ [\mathrm{PAH}]_{\mathrm{crayfish}} = 1.0 \times [\mathrm{PAH}]_{\mathrm{water}} + 0.39$$

Comparison of Predictive Capability of Model to Previously Reported Predictive Tools

The model predicts PAH concentrations in crayfish using C_{free} concentrations of PAHs (measured using LDPE passive samplers) well (**Figure 2.3**). For the 34 PAHs included in the model, crayfish concentrations were predicted by the model within a factor of 2.4 ± 1.8 of measured concentrations (**Table A1.6**). The BLOD-substitution model (Equation **2.2**) predicted PAH levels in crayfish within a factor of 2.7 ± 2.4 of measured concentrations. This suggests that these two models are performing very similarly. The former model (Equation **2.1,** in which any data that were BLOD were excluded when creating the model) is used for the remainder of the analyses. Due to the

relatively small concentrations of many PAHs measured in crayfish, even seemingly larger predicted/measured ratios can be practically insignificant. The average difference between the predicted and measured concentration for the 34 PAHs included in the model at all sites was 1.2 ng/g. There were only 3 PAHs for which this difference was greater than 5.0 ng/g at any site. Measured concentrations, predicted concentrations, and the factor differences between predicted and measured concentrations for all PAHs are in **Table A1.6**.

Predicted/measured factor differences for individual PAHs are equal to or better than for previously published models, and the present model includes more PAHs than comparable models. In a recent study, Fernandez and Gschwend compared traditional methods of predicting PAH levels in clams (using biota-sediment accumulation factors (BSAF), the concentration in the sediment, and the fraction of organic carbon in the sediment) to predicting PAH levels in clams using LDPE passive pore-water samplers. The authors reported average predicted/measured ratios of 0.43, 3.7, and 1.1 for phenanthrene, pyrene and chrysene, respectively, with standard deviations that are close to an order of magnitude larger than the averages.[26] Forsberg at al.[25] reported a mathematical model predicting concentrations of 15 PAHs in crayfish viscera using C_{free} PAH concentrations in water (from SPMDs) within a factor of 3. The authors stated that this was an improvement over previous prediction techniques.[25] Thus, the average predicted/measured ratio of 2.4 ± 1.8 in the present study, from a model including 34 PAHs, is a substantial improvement over what has been previously observed.

Part of what is reducing variability in the present model may be that it does not require inputs for the physical or chemical parameters of the system (lipid-water partitioning coefficient, bioaccumulation factor, etc.). These have been observed to increase prediction variability, due to variability of factors including chemical bioaccumulation, organism behavior, and organism anatomy and physiology.[26, 135] An additional benefit of the model described here is that it includes 34 PAHs, some of which are both relevant to human health and rarely included in analyses.

10 Years Later: Comparison of PAH Levels in Crayfish Viscera in 2003 and 2013

Average ∑PAH in 2003 crayfish viscera are listed in **Table A1.5**. In 2003, upriver ∑PAH were significantly lower than at RM 7E, and significantly lower than in the PHSM (Wilcoxon Rank-Sum tests, p = 0.001 and 0.026). This is consistent with crayfish within the Superfund sites being exposed to higher contaminant levels than those upriver [25]. In 2013, the trend is reversed, with ∑PAH levels in 2013 crayfish viscera significantly higher *outside* the PHSM than inside (**Figure A1.6**). This was true when comparing both crayfish collected upriver and downriver to those within the PHSM (Wilcoxon Rank-Sum tests, p = 0.041 and 0.024). Additionally, ∑PAH in crayfish upriver of the PHSM were significantly higher in 2013 than in 2003, while ∑PAH in PHSM crayfish were not significantly different in the two years (Wilcoxon Rank-Sum tests, p = 0.002 and 0.700). The 2003 crayfish data also have much more variability than the 2013 data, both within and among sites. This is discussed further in Appendix 1.

The increased PAH levels in crayfish upriver of the PHSM, along with the shift from highest PAH levels being measured within to outside of the PHSM, shows that the distribution of pollution in the river has changed over this 10-year period. This could be due in part to remediation efforts within the PHSM, such as the early-action remediation at RM 7E and RM 7W. Outside the PHSM, storm-water runoff is the predominant source of PAHs.[46] Thus, the relative PAH increase upriver of the PHSM could be due in part to increasing inputs from nonpoint sources from the growing Portland metropolitan area. Differences between these two time periods may also have been affected by variation in the locations where crayfish were captured.

Average PAH levels in crayfish at RM 7E in 2003 were higher than at any other location in either year (**Figure A1.6**). RM 7E is unique because it was the site of the former McCormick and Baxter Creosoting Company. This was historically one of the most PAH-laden sites in this area of the river, but remediation was completed in 2005. Average predicted ∑PAH in RM 7E crayfish viscera in 2012 was 150 ng/g. This is 18-fold smaller than average ∑PAH measured at this site in 2003. This predicted decrease is

significant (Wilcoxon Rank-Sum test, p = 0.017). There were no significant differences between ∑PAH predicted in crayfish viscera at RM 7E in 2012 and ∑PAH measured in crayfish viscera in the PHSM in 2003 or in 2013 (Wilcoxon Rank-Sum tests, p = 0.714 and 0.714) (**Figure A1.6**). It is worth considering that 2012 samples were deployed during a rainier part of the year, when lower PAH levels have been observed in Willamette River.[46] However, even if predicted PAH levels were two-fold higher at RM 7E in 2012, this would still be a substantial reduction in pollutants compared to 2003. This suggests that remedial efforts at RM 7E substantially reduced the pollutant levels available for movement from the sediment into the overlying water, reducing PAH levels in crayfish.

PAH Profiling: ∑PAH vs. ∑BaP_{eq}

PAH profiling indicated that PAHs contributing most to ∑PAH are different from PAHs contributing most to ∑BaP_{eq} (**Figure 2.4**). In fact, fluoranthene is the only PAH in the top 10 PAHs for both ∑PAH and ∑BaP_{eq} in 2013 viscera (**Figure 2.4 B,D**), and only fluoranthene and chrysene are on both of these lists in 2003 viscera (**Figure 2.4 A,C**). This illustrates that ∑PAH can present a very different picture than ∑$BaP_{eq.}$ Therefore, measuring ∑PAH alone may not be a useful metric for comparing health risks among sites.

∑BaP_{eq} and ∑PAH also present different pictures of contamination levels at different sites throughout the river. For instance, in 2013, viscera from RM 3.5 had the highest average ∑BaP_{eq} (**Figure 2.1B**), while the same viscera only had the 3rd highest average ∑PAH of the 5 sites (**Figure 2.1A**). This is another demonstration of the importance of using the appropriate metric to estimate risk.

2,6-dimethylnaphthalene (2,6-DMN) was the most abundant PAH contributing to average ∑PAH in crayfish viscera (**Figure 2.4B**) and tails at all sites in 2013, but does not affect ∑BaP_{eq} because it does not have an RPF value. The prevalence of 2,6-DMN is discussed further in Appendix 1.

PAH Profiling: 2003 vs. 2013

PAH profiles are similar in crayfish viscera collected in the PHSM in 2003 and 2013 (**Figure 2.4**). Four of the top five PAHs contributing to ∑PAH in both 2003 and 2013 crayfish viscera collected in the PHSM are fluoranthene, phenanthrene, pyrene and retene (**Figure 2.4 A, B**). The profile of PAHs driving carcinogenic risk in crayfish viscera in the PHSM was well conserved after this 10-year period, despite crayfish being collected at different sites within the PHSM. While the magnitude of $\sum BaP_{eq}$ in viscera collected in the PHSM is much lower in 2013 than 2003, BaP_{eq} profiles in 2003 and 2013 are even more similar than PAH profiles. Nine of the top 10 PAHs contributing to $\sum BaP_{eq}$, are the same in 2003 and 2013 (**Figure 2.4 C, D**). Additionally, the percent contribution of each of these PAHs to $\sum BaP_{eq}$ is almost identical in 2003 and 2013 (**Figure 2.4 C, D**).

Human Health Risk Assessment Based on Crayfish Consumption

The trend for risk estimates associated with 2013 crayfish tissue is opposite of the trend for ∑PAH concentrations for the same samples. Estimated risk associated with consuming whole crayfish tissue is highest within the PHSM and lowest upriver of the PHSM, suggesting that risk-driving carcinogenic PAHs are relatively more abundant inside the PHSM (**Figure 2.5**, where ELCR estimates < 1 in a million are not shown). $\sum BaP_{eq}$ for crayfish viscera from the highest and lowest RMs are just shy of significantly different (Wilcoxon Rank-Sum test, $p = 0.10$). This illustrates that elevated ∑PAH does not necessarily indicate elevated carcinogenic risk, as individual PAHs have different mechanisms of toxicity and potencies.

Even at the 95th percentile ingestion rate, risk related to consuming whole 2013 crayfish is barely above the acceptable level of 1 in a million, with the highest ELCR at an individual site only being 4 in a million (at 3.5W, within the PHSM). All estimated risks are below 1 in 100,000, which is the acceptable risk level the Oregon Department of Environmental Quality applies when evaluating cumulative exposure to all carcinogenic chemicals.[136]

ELCR estimates were one to two orders of magnitude lower in 2013 crayfish tails than in viscera from the same crayfish. Thus, ELCRs estimated based on consuming

whole tissue are substantially higher than ELCRs estimated just based on consuming just tails (**Figure 2.5**). Average ELCR estimates for consuming tails were lowest within the PHSM and highest upriver. The highest ELCR for crayfish tails was found in crayfish collected slightly upstream of the PHSM, at RM 12E.[118] This site sits right next to a large storm-water effluent pipe, and beneath an overpass of a major interstate highway. This suggests that nonpoint sources (such as storm-water runoff) may be contributing risk-driving PAHs to crayfish tails. This deserves consideration, as point sources are often the focus of remediation efforts. However, these ELCRs estimate that risk associated with consuming crayfish tails is less than 1 in a million at all sites where crayfish were collected in 2013, even at the 95th percentile ingestion rate. Thus, risks associated with eating crayfish tails from sites within or near the PHSM appear to be minimal.

The substantially elevated ELCRs when viscera is included in consumption-based risk estimates show that eating only tails would significantly lower PAH-related carcinogenic risk (**Figure 2.5**). This has important implications for human health. The message to human health risk assessors and consumers is that even if crayfish are being collected from a contaminated area, eating only the tail meat offers a means of reducing personal exposure to carcinogenic PAHs. In the U.S., crayfish's whole bodies are often cooked in liquid along with other foods (such as a traditional crayfish "boil"). A common practice is to suck the fat and juice from the cephalothorax after a crayfish is boiled, before eating its tail.[137] These practices may inadvertently extract PAHs from the viscera, increasing the associated risk above that of eating just the tails.

Comparison of Estimated Risks in 2013 Crayfish, to Those for Previously Collected Crayfish

ELCR estimates for PAH mixtures in whole 2003 crayfish spanned three orders of magnitude among sites. ELCRs were substantially higher at RM 7E and within the PHSM than upriver (**Figure 2.5**, where ELCR estimates < 1 in a million are not shown). The highest ELCR from 2003 (RM 7E) was more than a factor of 30 larger than the highest ELCR from 2013 (RM 3.5W). However, if RM 7E is removed from the comparison, the highest ELCR from 2013 is the same as the highest ELCR from 2003. This suggests that

remedial action has been successfully removing some of the worst sources of contamination from the PHSM over the past 10 years. It also suggests background contamination has remained roughly the same within the PHSM.

ATSDR's PHA for the PHSM concluded that all crayfish contaminant levels produced potential carcinogenic risk estimates below 1 in 10,000, for both average and 95% ingestion rates, and thus that adverse health risks were not anticipated related to crayfish consumption.[117] This is slightly different from the conclusions reached in the present risk assessment. When the 95% ingestion rate is used, the present assessment estimated an ELCR slightly above 1 in 10,000 in whole tissue from RM 7E in 2003, and ELCR estimates above 1 in a million at RM 7W and 3E in whole crayfish from 2003, and at RM 3.5W in 2013.

The ATSDR PHA notes that there are limited data on crayfish consumption from the PHSM, either commercially or recreationally.[117] Crayfish ingestion rates may vary among different ethnic or cultural groups. Outside of the PHSM, crayfish consumption is traditionally higher among communities in the southeastern U.S., especially in Louisiana, than in most other groups in the U.S. Thus, higher ingestion rates may be needed to be protective of health risk if this were the population of concern.

One factor likely increasing the present estimates relative to those performed in the PHA is that the present analysis uses the EPA's 2010 RPF approach, combined with an analytical method that quantifies 23 of the 26 PAHs with RPFs. The PAH analysis in the PHA only included 5 PAHs, and it was published before the EPA 2010 RPF guidance document. Thus, the present study likely used higher carcinogenic potency values to estimate risk.

Another factor affecting comparisons of these risk estimates is that the PHA analyzed entire crayfish for their risk estimates, while the present study analyzed isolated viscera and tail tissue. Because PAH levels are so much higher in viscera than in tails, incorporating the entire body (including carapace) would dilute visceral PAH levels, reducing risk estimates. Additionally, viscera only contributed 13 ± 4% to total body

weight (when carapace is included in total weight) for crayfish used in the present analysis, suggesting that this dilution may be dramatic. In contrast, viscera contributed 55 ± 7% to the whole soft tissue (viscera and tail muscle) included in the present risk estimates. Thus, analyzing the whole organism would likely substantially reduce risk estimates, relative to analyzing the isolated viscera.

This illustrates that risk estimates, and therefore risk management strategies, may be affected heavily by which PAHs are included in analysis, by what toxicity information is available at the time of the assessment, and by which tissues are analyzed to estimate risk. There are many PAHs, and many contaminants, for which toxicity information remains sparse. There are also organisms of interest for which data on PAH levels in the environment are sparse. The more toxicity data risk assessors have, the better they will be able to make risk management decisions that are protective of public health.

Ability of the Model to Predict Risk Estimates

Using the 95th percentile ingestion rate, ELCR estimates predicted in whole crayfish were 9 in a million within the PHSM, and 1 in a million outside of the PHSM. When compared with ELCRs calculated using measured data (2 in a million within the PHSM and 1 in a million outside of the PHSM), the model predicts risk of crayfish consumption relatively well (predicting within a factor of 5 of measured concentrations within the PHSM, and within a factor of 1 outside of the PHSM).

Additionally, the model was used to predict PAH levels in crayfish in 2012, using only water data as inputs. These data were then used to estimate ELCRs associated with consuming crayfish from RM 7E in 2012 (**Figure 2.5**). The predicted reduction in risk estimates is consistent with crayfish posing less consumption risk post-remediation. These estimates seem reasonable, as they are similar to what was measured at other sites within the PHSM in the following year. This demonstrates that the model can be used to estimate tissue concentrations, which can be used to estimate risk associated

with crayfish consumption, based only on water concentrations derived from passive samplers.

Additional Considerations

Uptake and elimination of contaminants by organisms is a complex phenomenon, with various behaviors potentially altering organisms' contaminant levels.[109] Bayen et al.[109] proposed that for passive samplers to most accurately predict contaminant levels in organisms, information is needed about contaminant uptake and elimination in the species of interest, and with the proposed type of passive sampler. LDPE passive samplers are relatively well-studied, and the present model is already performing well, but continued study of crayfish uptake and elimination of PAHs could further improve the predictive capabilities.

Including EPA's 2010 extended list of PAHs with RPFs in human health risk assessments will increase estimates, compared to using EPA's previous 16 priority pollutants, where RPFs were only given for 7 PAHs.[101, 116] This is discussed further in Appendix 1. It is also important to consider that current methods of estimating risk associated with consuming a mixture of PAHs assume that the toxicities of each of the PAHs in the mixture are additive. However, it has been observed that interactions between individual compounds in a mixture can lead to a greater or less than additive toxic response.[3] One way to improve risk estimates would be to directly test the PAH mixtures that are repeatedly measured in the environment in toxicity assays. While testing the endless list of potential mixtures would be prohibitively challenging, a starting place may be to assess the combined toxicities of mixtures that commonly occur in the environment. For instance, in the present study, the profile of risk-driving carcinogenic PAHs in PHSM crayfish viscera was almost identical in 2003 and 2013. Characterizing the toxicity of this PAH mixture could provide useful information about a real-world exposure. Given that this mixture was conserved in these two sampling events 10 years apart, it may also be similar to the mixture in shellfish at other sites with similar sources of contamination.

The selection of an organism's tissues for analysis can be a major source of variation when directly measuring contaminants in organisms to assess risk. The present work illustrates this issue clearly, because crayfish viscera contained significantly higher $\sum BaP_{eq}$ than tails. Risk assessors have traditionally included different tissues in analyses to estimate risk. For instance, the ATSDR's PHA for Portland Harbor used whole bodies, while the EPA's guidance document for fish advisory data suggests only using tails.[117, 138] Given that this decision can substantially affect measured PAH levels, and subsequently risk estimates, care must be taken to ensure that the tissues being analyzed fit the question being asked.

Importantly, the agreement seen in the predictive model suggests that, with only a little more data, it could be used to predict contaminant levels (and subsequently to estimate associated consumption risk) in other shellfish, and perhaps even finfish. Additionally, assessing risks associated with dermal exposure requires only knowledge of water concentrations. Thus, the passive sampling approach described here provides risk assessors a way to estimate risks associated with multiple exposure routes, all from one set of data collected by passive samplers. Thus, only deploying passive samplers in the water would enable practitioners to estimate risks associated with multiple exposure pathways.

Conclusions

This work presents a model predicting levels of 34 PAHs in crayfish viscera, using only C_{free} PAH levels in water (measured by LDPE passive samplers). The model predicts PAH levels in crayfish within a factor of 2 of measured values, on average. Analyses revealed that $\sum$PAH are 5-20 times higher in crayfish viscera than in tails, and $\sum BaP_{eq}$ are 6 to 70 times higher in viscera than in tails. This suggests that eating viscera significantly increases cancer risk, compared to eating just tail meat. Additionally, PAH levels in crayfish were not significantly different after 10 years in the PHSM, at sites that have not been remediated, while a significant decrease in PAH levels is predicted in crayfish at a site within the PHSM that has been remediated. Finally, PAH levels in crayfish were

significantly higher after 10 years in a suburban area upriver of the PHSM. Additionally, the highest average $\sum BaP_{eq}$ in crayfish tails was measured just upriver of the PHSM, near a storm-water effluent pipe and under a highway overpass. This suggests that nonpoint sources in metropolitan areas, and not just point sources that typically receive remedial attention, may contribute substantially to PAH load in shellfish.

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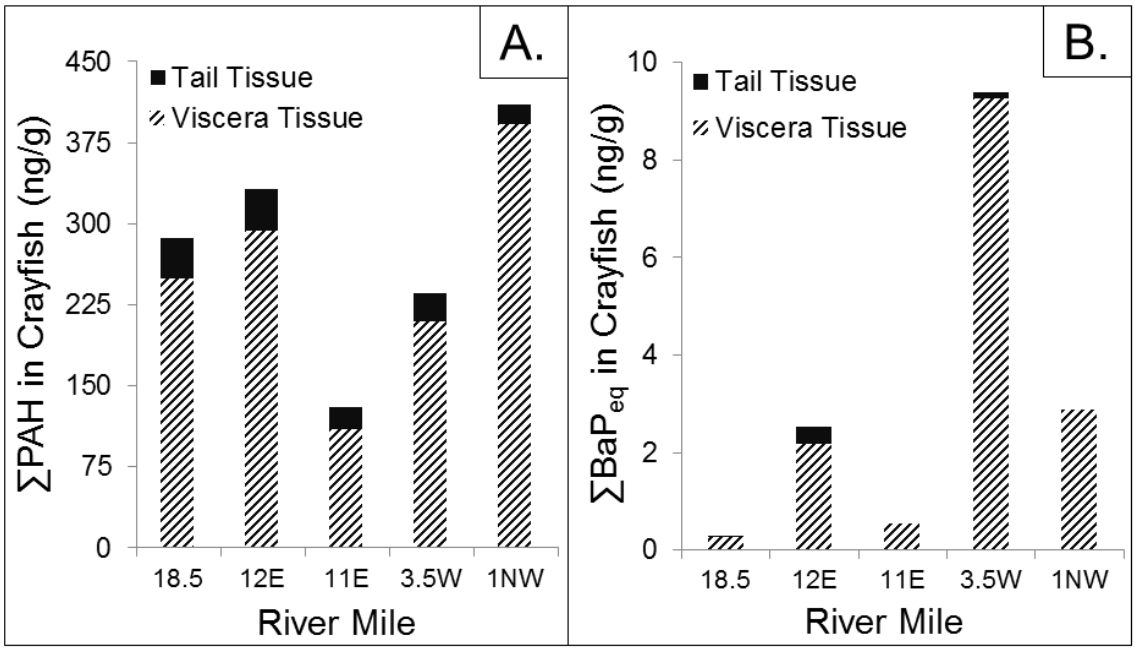

Figure 2.1 Average A. ∑PAH and B. ∑BaP$_{eq}$, in tail and viscera tissue from crayfish collected in the Willamette River in Portland, Oregon in 2013. Crayfish were collected upriver (RM 18.5), within (RM 12E, 11E, and 3.5W), and downriver (RM 1NW) of the Portland Harbor Superfund Megasite. Three composited samples of each tissue were analyzed from each river mile. All tissue concentrations are in ng/g wet weight.

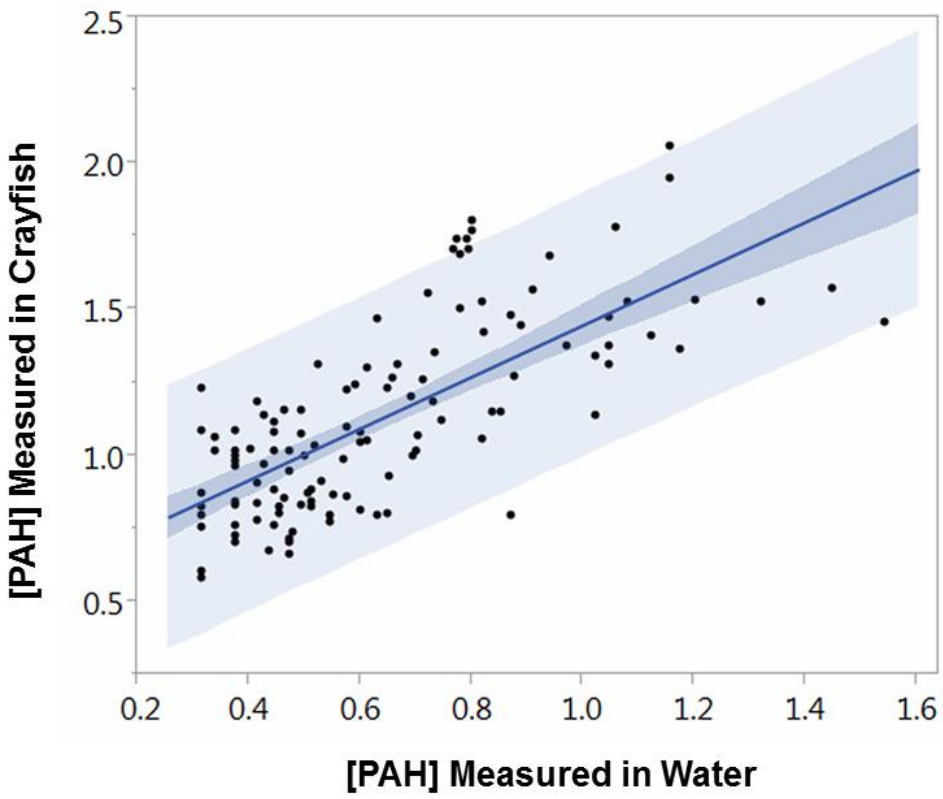

Figure 2.2. Linear regression of 34 individual [PAH], measured in spatially and temporally paired crayfish viscera vs. water. Crayfish concentrations are in ($\sqrt[4]{(ng/g)}$) and water concentrations are in ($\sqrt[4]{ng/L}$). Samples were collected upriver, within, and downriver of the Portland Harbor Superfund Megasite (PHSM) in the Willamette River in Portland, Oregon. R-squared = 0.52, correlation coefficient = 0.72. Dark shading represents confidence intervals and light shading represents prediction intervals. The slope of the line of best fit is 0.88 ± 0.078 and the intercept is 0.56 ± 0.054.

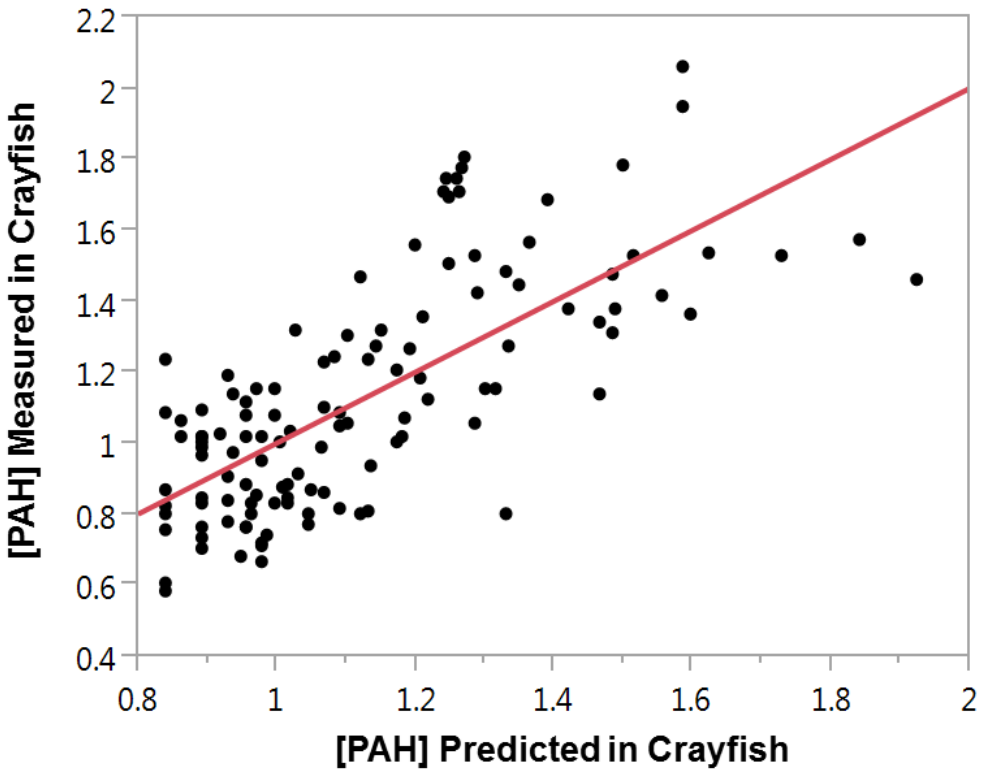

Figure 2.3. [PAH] measured in crayfish viscera, vs. [PAH] predicted in crayfish viscera from freely dissolved [PAH] in water. All concentrations are in ($\sqrt[4]{(ng/g)}$). [PAH] were predicted in crayfish viscera using Equation 1, from the linear regression model displayed in **Figure 2.2**. The diagonal reference line indicates where predicted and measured values are 1:1. The average un-transformed concentrations predicted by the model were within a factor of 2.4 ± 1.8 of measured concentrations.

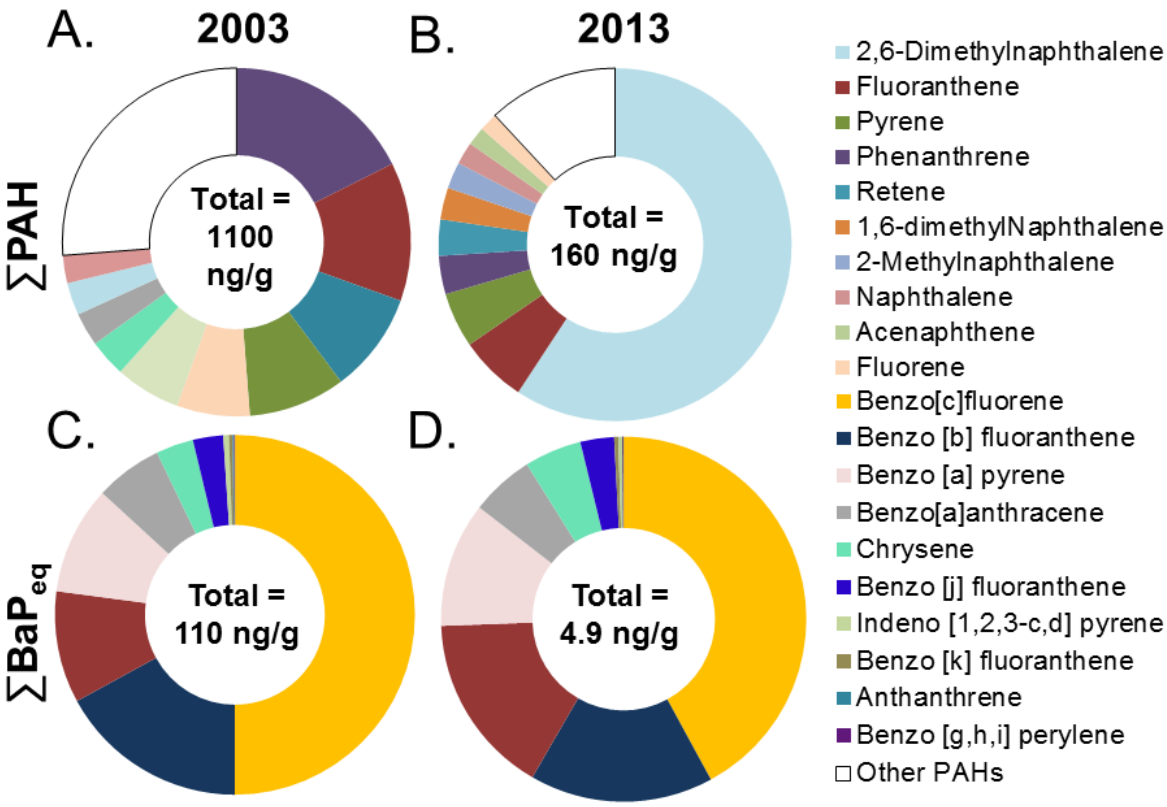

Figure 2.4. Percent contribution of the 10 most abundant PAHs to ∑PAH in 2003 (A.), ∑PAH in 2013 (B.), ∑BaP$_{eq}$ in 2003 (C.), and ∑BaP$_{eq}$ in 2013 (D.). PAHs were measured in crayfish viscera collected in the Portland Harbor Superfund Megasite (PHSM) in the Willamette River in Portland, Oregon. The top 10 PAHs contributing to ∑BaP$_{eq}$ in PHSM crayfish viscera are nearly identical in 2003 and 2013. The only PAHs that contribute substantially to both ∑PAH and ∑BaP$_{eq}$ are fluoranthene (2003 and 2013), and chrysene (2003). All tissue concentrations are in ng/g wet weight.

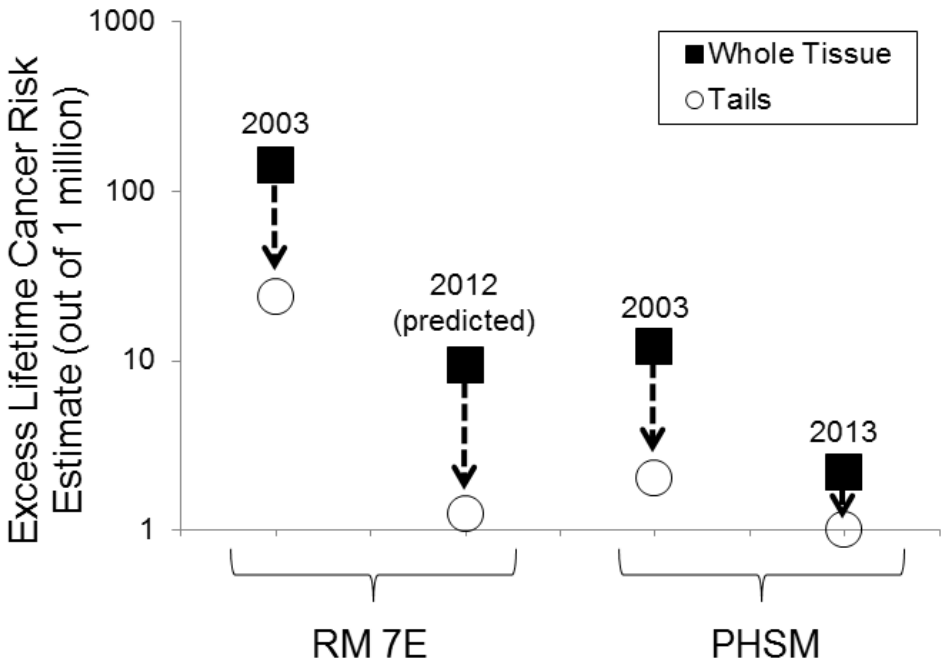

Figure 2.5. Average excess lifetime cancer risk (ELCR) estimates associated with eating whole tissue (solid squares) or just tail meat (open circles) of crayfish. ELCRs are presented for crayfish collected at RM 7E (the McCormick and Baxter Superfund site), and in the rest of the greater Portland Harbor Superfund Megasite (PHSM), in the Willamette River in Portland, Oregon. The 2012 ELCR was estimated from PAH concentrations predicted from water concentrations, using the predictive model described in this study and shown in **Figure 2.2**. ELCRs for "whole tissue" assume the consumer eats both the viscera and tail tissue. ELCRs less than 1 in a million are not shown.

Chapter 3 – Emissions of Polycyclic Aromatic Hydrocarbons from Natural Gas Extraction into Air

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Abstract

Natural gas extraction, often referred to as “fracking,” has increased rapidly in the U.S. in recent years. To address potential health impacts, passive air samplers were deployed in a rural community heavily affected by the natural gas boom. Samplers were analyzed for 62 polycyclic aromatic hydrocarbons (PAHs). Results were grouped based on distance from each sampler to the nearest active well. Levels of benzo[a]pyrene, phenanthrene, and carcinogenic potency of PAH mixtures were highest when samplers were closest to active wells. PAH levels closest to natural gas activity were comparable to levels previously reported in rural areas in winter. Sourcing ratios indicated that PAHs were predominantly petrogenic, suggesting that PAH levels were influenced by direct releases from the earth. Quantitative human health risk assessment estimated the excess lifetime cancer risks associated with exposure to the measured PAHs. Closest to active wells, the risk estimated for maximum residential exposure was 0.04 in a million, which is below the U.S. EPA’s acceptable risk level. Overall, risk estimates decreased 30% when comparing results from samplers closest to active wells to those farthest. This work suggests that natural gas extraction is contributing PAHs to the air, below levels that would increase cancer risk.

Introduction

Natural gas extraction from shale, colloquially known as “fracking,” has increased substantially in the United States in the past 15 years. U.S. shale gas production grew by 17% annually from 2000 to 2006, and then grew by 48% from 2006 to 2010.[139] This spike in activity has been driven predominantly by improvements to the technologies of horizontal drilling and hydraulic fracturing. Together, these processes enable companies to access gas reserves previously out of reach. As of 2011, the U.S. Energy Information Administration estimated that roughly 750 trillion cubic feet of natural gas were recoverable from shale reserves in the contiguous United States using these approaches.[140]

Despite this rapid expansion and implementation of technology, there has been relatively little research into the environmental and health impacts these processes may have. There has also been a lack of regulation, illustrated by the U.S. Energy Policy Act of 2005, which amended portions of the U.S. Safe Drinking Water Act and Clean Water Act to give gas-drilling companies more flexibility.[141] Concerns have arisen about the impacts that natural gas extraction (hereafter NGE) may have on environmental and human health, due in part to this historic lack of regulation.[142-145] In the past five years, studies have emerged assessing the impacts this activity may have on water quality, air quality, and human health.[146-158]

Many studies have acknowledged that impact to air quality may be the most significant risk to communities living near NGE.[146-153] Shonkoff et al. concluded that NGE has the potential to pose health risks through both air and water emissions, and urged that many important data gaps remain.[147]

Most of the air quality studies have focused on emissions of volatile organic compounds (VOCs). McKenzie et al. sampled air near NGE wells at different stages, measuring VOCs including BTEX (benzene, toluene, ethylbenzene and xylenes) and aliphatic hydrocarbons.[148] They performed a public health risk assessment and found an increased risk of cancer and non-cancer endpoints for people living within 0.5 miles of NGE well pads.[148] In a subsequent study, McKenzie et al. assessed the correlation between decreased birth outcomes and NGE. They found an increase in congenital heart defects and neural tube defects as mothers' residences got closer to NGE wells.[149] Roy et al. estimated emissions from NGE in the Marcellus Shale, and predicted that NGE contributes an average of 12% of all NO_x and VOC emissions to air in a given location.[150] Bunch et al. studied regional VOC levels in a part of Texas with NGE.[152] Contrary to the majority of the scientific literature, this study concluded that NGE is not polluting the air at concerning levels. However, Bunch et al. considered any risk estimates less than 1 in 10000 not to be concerning, which is the upper limit of risk that the EPA considers acceptable.[159]

Many studies have assessed the impacts of NGE on public health. [145, 147-149, 152, 154-158, 160] Colborn et al. performed a hazard assessment of the chemicals that are used during NGE, and concluded that over 70% of these chemicals are potentially risky to humans.[145] Other work has focused on the impacts of NGE on communities.[156, 157, 160] NGE often takes place in rural areas, where it may present a larger change to ambient pollutant levels than it would in urban areas.

Other studies have reviewed the state of the science surrounding NGE, emphasizing the need for more concerted field sampling and data generation. In one such review, Goldstein et al. called for toxicological studies to help characterize the potential risks of NGE activity.[155] Small et al. assessed the state of the science and regulation surrounding NGE in the U.S. They called for improved characterization of air pollutants emitted from NGE and their potential health impacts, and concluded that risks associated with NGE "remain under-analyzed."[144] Despite the recent surge of literature surrounding NGE, there are still many data gaps.

One data gap is the relationship between polycyclic aromatic hydrocarbons (PAHs) and NGE. PAHs are pervasive environmental pollutants of concern, known to be associated with both hydrocarbon extraction and negative health impacts.[59, 60] The main categories of health concerns associated with exposure to PAH mixtures are cancer risk and respiratory distress. PAH-related cancer risk has received a great deal of attention in relation to oil spills, traffic exhaust, wood smoke, and cooking. NGE involves extracting hydrocarbons from the earth, a process that is often associated with PAH emissions. NGE also brings large volumes of truck traffic into an area to move building materials, water, and product. Each of these stages could be sources of PAHs. Goldstein et al. and Adgate et al. both specifically include PAHs as a potential health concern at many or all stages of NGE.[146, 155] Colborn et al. sampled air near NGE well pads for 16 PAHs.[153] However, they ceased sampling PAHs after the drilling phase ended. They later concluded that PAH levels during drilling were of concern to human health, citing that these levels (ΣPAH_{16} ~15 ng/m^3) were comparable to those associated with small but

significant decreases in IQ at 5 years of age in children exposed *in utero.*[161] Colborn et al. conclude that the relationship between NGE and PAH emissions "deserves further investigation."[153]

Passive sampling could fill this data gap. Low-density polyethylene (LDPE) passive samplers sequester hydrophobic compounds through passive diffusion in a time-integrated manner, and are well-suited to passively sample vapor phase PAHs from air. Since this tool's development in the 1990s, many studies have demonstrated its ability to measure PAHs.[34, 55, 162, 163] The objective of this study was to use passive sampling to assess the impact of NGE on PAH levels in air in a rural community.

Correction to Original Article

This article is a corrected form of Paulik et al., which was originally published in 2015.[164] PAH air concentrations reported in the original manuscript were incorrect. Honest calculation errors resulted from using incorrect units of the ideal gas constant and improper cell linkages in the spreadsheet that was used to adjust air concentrations for sampling temperature. The original article was retracted by the authors.[165] This version of the article presents corrected versions of all of the original analyses and discussions.

Materials and Methods

Site Description and Sampling Design

This study took place predominantly in rural Carroll County, Ohio. As technology has made gas in the Utica shale more accessible in the last five years, NGE in eastern Ohio has increased. In 2011, Ohio had less than 50 horizontal natural gas drilling leases.[166] As of June 2014, that number had jumped to 1386, with 421 in Carroll County.[167]

Passive air samplers were deployed on the properties of 23 volunteers in February 2014. Average temperature during the deployment period was -1°C. Volunteer

landowners were identified by advertisement through a community meeting and word of mouth. Volunteers were excluded from the study if their properties were within a city limit, near an airport, or otherwise could have presented results that were difficult to interpret due to substantial background PAH levels. Each sampling site was located between 0.04 and 3.2 miles from an active NGE well pad. Oregon State University (OSU) researchers deployed one sampler on each volunteer's land. Each sampler consisted of three LDPE strips in a metal cage. Sampling was replicated in triplicate at one site. The OSU research team has over 10 years of field sampling experience collectively. The team took care to place samplers as far as possible from potentially confounding PAH sources such as chimneys or roads. Samplers were deployed for 3-4 weeks, and then trained landowners mailed them to the Food Safety and Environmental Stewardship (FSES) lab at OSU in Corvallis, OR. Volunteer training is described further in Appendix 2. Samplers were transported in airtight polytetrafluoroethylene bags with Clip N Seal assemblies (Welch Fluorocarbon). Landowners were provided individual results from air sampling on their property. To put results in context, individual results were compared to a summary of results from all sampling sites.

Passive Sampler Preparation, Cleaning and Extraction

Details about chemicals and solvents are in Appendix 2. Before deployment, LDPE was cleaned using hexanes as described previously.[22] Each LDPE strip was infused with performance reference compounds (PRCs) to enable calculation of *in situ* sampling rates and time-integrated air concentrations.[21] PRCs used in this study were fluorene-d10, pyrene-d10 and benzo[b]fluoranthene-d12. PRCs were spiked into LDPE at 2-20 µg per strip. Samplers were cleaned after deployment in two isopropanol baths, stored in amber jars at -20°C, and extracted as described elsewhere.[22] Briefly, extractions were performed using two dialyses with *n*-hexane. Prior to extraction, samples were spiked with deuterated PAHs to act as surrogate standards, allowing for quantification of extraction efficiency. Surrogate standards are specified in **Table A1.2**. Extracts were

quantitatively concentrated to 1 mL using TurboVap closed cell evaporators, transferred to amber chromatography vials, and stored at -20°C.

Chemical Analysis

LDPE extracts were quantitatively analyzed for 62 PAHs using an Agilent 7890A gas chromatograph interfaced with an Agilent 7000 GC/MS-MS.[125] An Agilent Select PAH column was used. Each PAH was calibrated with a curve of at least five points, with correlations ≥0.99. Limits of detection (LODs) range from 0.24 to 1.7 ng/mL, and limits of quantitation (LOQs) range from 1.0 to 7.1 ng/mL, with the exception of two compounds that have higher LODs and LOQs. A list of PAHs, LODs and LOQs is included in **Table A1.2.**

Air Concentration Calculation

Air concentrations (ng/m^3) of PAHs were calculated from instrument concentrations (ng/mL) using PRCs. *In situ* sampling rates (R_S) were generated using calculations described by Huckins et al.[21] These calculations estimate the R_S of each PRC by incorporating deployment time, initial amount and the temperature-corrected sampler-air partition coefficient ($K_{sa(T)}$). An R_S was calculated for each PAH, using the PAH's K_{OA} and the R_S of one of the PRCs. Temperature-corrected K_{sa} values were calculated using a modified van 't Hoff equation as described by Khairy et al.[34] Air concentration calculations are included as **Equations A2.1-A1.9**.

Data Analysis

Data were grouped by distance from each sampling site to the closest active well pad. Three distance groups were created, with the "close" group <0.1 mile from an active well, the "middle" group from 0.1 to 1.0 mile from an active well, and the "far" group >1.0 mile from an active well. The close, middle and far groups had 5, 12, and 6 samples each. All results are presented in these three distance groups. Distances were determined using Google Earth version 7.1.2.2041, and well status information was

taken from the Ohio Department of Natural Resource's Web site. A well was considered "active" if it was in the drilling, drilled, or producing stages at the time of sampling.

Parent PAH isomer ratios were used to determine sources of PAHs. Two PAH isomer pairs that are used to diagnose whether a PAH mixture is petrogenic or pyrogenic are phenanthrene and anthracene, and fluoranthene and pyrene.[85, 86, 168-172] Phenanthrene/anthracene ratios ≤10 indicate pyrogenic sources, while ratios ≥15 indicate petrogenic sources.[168-171] For this ratio, however, there is disagreement about interpretation of values between 10 and 15. Budzinski et al. state that values in this range suggest incomplete combustion of organic matter, and are thus pyrogenic.[168] Fluoranthene/pyrene ratios >1 indicate pyrogenic sources, while ratios <1.0 indicate petrogenic sources.[168, 170, 171] Ratios of one isomer to the sum of both isomers are also used in PAH sourcing. Fluoranthene/(fluoranthene+pyrene) ratios ≥0.5 indicate pyrogenic sources, and ratios ≤0.4 indicate petrogenic sources.[86, 169] Yunker et al. suggest that ratios between 0.4 and 0.5 indicate liquid fossil fuel combustion.[85] Anthracene/(anthracene+phenanthrene) ratios <0.1 indicate petrogenic sources and ratios >0.1 indicate pyrogenic sources.[85, 86, 172] A fifth ratio of two nonisomer parent PAHs, benzo[a]pyrene/benzo[g,h,i]perylene, was used to obtain sourcing information for the 5- and 6- ring PAHs measured in this study. For this ratio, values >0.6 are indicative of traffic emissions while values <0.6 indicate non-traffic emission sources.[86] There were samples in the middle and far groups for which benzo[a]pyrene, benzo[g,h,i]perylene, or both were below limits of detection (BLOD). So, the sample sizes for the close, middle, and far groups for this final ratio are 5, 9, and 4, respectively.

Risk Assessment

The carcinogenic potency of the PAH mixture at each site was calculated by multiplying the concentration of each PAH by the relative potency factor (RPF) it was given by the U.S. EPA.[59] A list of the RPFs is in **Table A1.4**. This estimate of carcinogenic potency is referred to as the benzo[a]pyrene equivalent concentration, or BaP_{eq}. These values were used in quantitative risk assessments to estimate cancer risks of exposure

to the measured PAHs through inhalation, using the EPA's framework.[173] Exposure parameters were modeled after the "residential" and "outdoor worker" examples presented to Superfund risk assessors by the EPA in 2014.[174] Specifically, the average lifetime was set at 70 years for all exposure scenarios. For the residential scenarios, exposure duration and exposure frequency were set at 26 years and 350 days/year, respectively. For the outdoor worker scenario, these parameters were set at 25 years and 225 days/year. The residential assessment was performed for a worst-case and best-case scenario, by adjusting the daily exposure time to 24 or 1 h, while the outdoor worker assessment was performed with daily exposure time set at 8 h. Risk assessment equations are included as **Equations A2.10-A2.11.**

Statistical Analysis

Welch's two sample *t* tests were performed on the data for ΣPAH_{62}, benzo[a]pyrene, phenanthrene, pyrene, and carcinogenic potency, between each pairwise combination of distance groups, using R version 2.15.3. It was assumed that variance between each two groups was unequal. Results were deemed significantly different when $\alpha < 0.05$. Exploratory principle components analysis is included as **Figure A2.1**.

Quality Control

During passive sampler preparation, one LDPE strip was hung in the room to account for potential contamination during PRC infusion. In the field, sampling was replicated at one site, $n=3$. A trip blank was taken to each sampling site to account for contamination during transport. One blank LDPE strip was included each day in the cleaning process after deployment, as a cleaning blank. This also doubled as a blank during sampler extraction. Perylene-d12 was spiked into all extracts at 500 ng/mL before instrumental analysis, to act as an internal standard. The analytical method was validated using its calibration, precision and accuracy, and detection limits prior to use. During instrument analysis, instrument blanks and continuing calibration verifications

were run at the beginning and end of each set of samples. All laboratory and field procedures were performed according to FSES Standard Operating Procedures.

Results and Discussion

PAH Levels and Trends

Many of the measured PAH levels decrease as samplers get farther from active NGE wells. Three distance groups were created, with the "close" group <0.1 mile from an active well, the "middle" group between 0.1 and 1.0 mile from an active well, and the "far" group >1.0 mile from an active well. Average levels of benzo[a]pyrene and phenanthrene decreased as samplers moved farther from NGE wells, while average ΣPAH_{62} levels did not show that trend (**Figure 3.1a-c**). Average ΣPAH_{62} were 7.4, 8.4, and 6.7 ng/m^3 ng/m^3 for the close, middle, and far groups. Naphthalene was the most abundant PAH in all samples, contributing 62% to average ΣPAH_{62} in all distance groups. The next most abundant PAHs were fluorene, 2-methylnaphthalene, 1-methylnaphthalene, acenaphthene and phenanthrene, collectively contributing more than an additional 30% to average ΣPAH_{62} in all distance groups. The other 56 PAHs made up the remaining ~8%.

The predominant health concerns associated with exposure to PAH mixtures are cancer and respiratory distress, so benzo[a]pyrene and phenanthrene were chosen as representative individual PAHs generally associated with each of these health endpoints. Benzo[a]pyrene has been extensively studied in relation to its carcinogenicity and phenanthrene has been associated with respiratory distress.[59, 175] Average benzo[a]pyrene levels were 14×10^{-6}, 7.1×10^{-6}, and 2.9×10^{-6} ng/$m^3$for the close, middle, and far groups. Average phenanthrene levels were 0.25, 0.18, and 0.17 ng/m^3 for the close, middle, and far groups. The close and far distance groups for phenanthrene were significantly different (Welch's two sample *t* test, p = 0.042). The close and far groups for ΣPAH_{62} and benzo[a]pyrene were above the $\alpha = 0.05$ significance level (Welch's two sample *t* tests, p = 0.76 and p = 0.13, respectively). Close and middle groups for phenanthrene were just above this significance level (Welch's two sample *t* test, p =

0.053).Additionally, all PAH concentrations that were significantly correlated with distance to active NGE wells were negatively correlated (Spearman's rho, $p < 0.05$). This supports the observation that PAH levels are elevated closer to active NGE wells.

Comparison to Literature Values

Results from the present study were directly compared to the sum of 14 PAHs in the air, reported in four previous studies (**Figure 3.2**). These 14 PAHs are listed in Appendix 2. Average ΣPAH_{14} for the present study were 1.2, 0.94, and 0.97 ng/m^3 for the close, middle, and far groups. Simcik et al. measured an average of 122 ng/m^3 ΣPAH_{20} in downtown Chicago, and an average of 21 ng/m^3 in a rural location in Michigan.[176] Ravindra et al. measured average ΣPAH_{14} levels of 90 ng/m^3 near a petroleum refinery in an industrial Belgian location, and 9.4 ng/m^3 in a rural Belgian location.[177] Khairy et al. used LDPE passive samplers to measure an average ΣPAH_{14} of 110 ng/m^3 in urban areas of Alexandria, Egypt during winter sampling campaigns.[34] Tidwell et al. used LDPE passive samplers to measure PAHs on the shore during the Deepwater Horizon Incident in the U.S. Gulf of Mexico. At the two shoreline sites closest to the incident (Louisiana and Mississippi), average ΣPAH_{14} were 4.8 ng/m^3 in observations immediately following the incident and 3.7 ng/m^3 in all subsequent observations over the following year.[83] All of these studies measured PAHs in the vapor phase, making results comparable. Simcik and Ravindra *et al.* used active sampling to measure vapor phase PAHs, while Khairy and Tidwell et al. used LDPE passive sampling to measure PAHs in the vapor phase.

Thus ΣPAH in the present study are lower or comparable to most reported in published literature. It is important to consider that sampling for the present study was conducted at an average temperature of -1°C. ΣPAH in the close group are in the same order of magnitude as levels previously measured in rural areas at freezing temperatures. Additionally, the PAH load in the present study is in the same order of magnitude as in a previous study in which PAHs were only sampled during the drilling phase.[153]

PAH Sourcing Techniques

Sourcing ratios indicate that measured PAH mixtures have predominantly petrogenic signatures (**Figure 3.3a-d**). Petrogenic signatures suggest that PAHs were released directly from the earth, while pyrogenic signatures suggest that PAHs came from combustion. For both fluoranthene/pyrene and phenanthrene/anthracene, average ratios were petrogenic for all distance groups (**Figure 3.3a,b**). Fluoranthene/(fluoranthene+pyrene) ratios were petrogenic in the close group, and gained more pyrogenic influence as samples moved farther from NGE activity (Figure 3.3c). Anthracene/(anthracene+phenanthrene) ratios were all petrogenic (Figure 3.3d). Fluoranthene/pyrene and fluoranthene/(fluoranthene+pyrene) ratios both indicated that PAHs moved from strongly petrogenic toward more mixed or slightly pyrogenic signatures as samplers moved farther from NGE activity (**Figure 3.3a,c**). Fluoranthene/(fluoranthene+pyrene) ratios between 0.4 and 0.5 are associated with liquid fossil fuel combustion.[85] This may suggest that PAH source becomes more affected by combustion as the sampler moves farther from active wells. Exploratory principle components analysis showed that the profile of the majority of PAH mixtures in the close group were similar, while the profile of PAH mixtures in the far group were also similar to each other, and different from the majority of PAH mixtures in the close group (**Figure A2.1**). Additionally, average pyrene levels were significantly higher in the close group than the far group, reinforcing the association between NGE activity and pyrene (Welch's two sample *t* test, $p < 0.05$).

Average values for the benzo[a]pyrene/benzo[g,h,i]perylene ratio in the close, middle and far groups were 0.94, 1.2, and 1.3. Given that values >0.6 suggest traffic emissions, these data suggest that higher molecular weight PAHs (≥5 rings) measured in this study may be influenced by traffic emissions at all sites. However, these 5- and 6-ring PAHs only contribute 0.001% on average to ΣPAH across all sites.

The predominant petrogenic signature suggests that PAH mixtures are heavily influenced by direct releases of hydrocarbons from NGE wells into the air, as opposed to other myriad anthropogenic processes which would produce pyrogenic signatures. It is

reasonable to expect PAH emissions alongside natural gas extraction. This association was substantiated by a hydraulic fracturing simulation study, which demonstrated that non-methane hydrocarbons, including aromatics, are emitted during natural gas extraction from shale.[178] Additionally, roughly half of the active wells were in the producing phase during the sampling period. This may further explain the predominant petrogenic signature, with PAHs mixtures being heavily influenced by direct release of hydrocarbons into the air, potentially as fugitive emissions during production. The petrogenic signature of measured PAHs and the increased levels closer to NGE wells suggest that NGE activity may be impacting ambient PAH levels in this rural area.

Wood burning is another common source of PAHs in air. Retene is a PAH that is commonly used as an indicator of biomass combustion, especially wood.[179, 180] Interestingly, average retene levels did not show the same trend as other individual PAHs across distance groups. Rather, average retene levels were comparable across distance groups. This suggests that wood burning had a similar impact on PAH levels in all distance groups, and adds weight to the conclusion that elevated PAH levels may be related to NGE activity, not to wood burning.

Carcinogenic Potency

Carcinogenic potency of PAH mixtures decreases significantly in the far group, compared to the close group (Welch's two sample *t* test, $p < 0.05$) (**Figure 3.4**). The average BaP_{eq} concentrations in the close, middle and far groups were 13×10^{-4}, 11×10^{-4}, and 8.6×10^{-4} ng/m^3. Benzo[c]fluorene, fluoranthene, and benzo[a]pyrene were the main contributors to carcinogenic potency, collectively contributing an average of 99% to the total potency in all groups.

Average BaP_{eq} concentrations in this study would likely not be concerning as chronic doses. While there are currently no regulatory levels for ambient PAH exposure in the U.S., the U.S. Clean Air Act specifies that a pollutant can be regulated when it is estimated to lead to more than 1 in a million excess cancers over the lifetimes of the most exposed individuals.[181] The World Health Organization suggested that 1.2×10^{-2} ng/m^3 BaP in ambient air would produce 1 excess cancer in a million exposed

individuals.[182] Additionally, Caldwell et al. proposed 0.48 ng/m^3 BaP as the benchmark concentration expected to cause excess cancer risk above 1 in a million.[181] No BaP_{eq} level measured in air in this study exceeds either of these concentrations. Both WHO and the European Union have suggested 1.0 ng/m^3 BaP as a guidance level for ambient air concentrations.[60, 183] If this guidance level were applied, ambient BaP_{eq} would not exceed this level at any sites in this study.

Quantitative Risk Assessment

Quantitative risk assessment indicates that carcinogenic risk associated with inhalation decreases as samplers move farther from active wells. For the maximum residential exposure scenario of 24 h/day, estimated excess lifetime cancer risk (ELCR) decreases from 0.040 to 0.027 in a million when moving from the close to far group. For the minimum residential exposure scenario of 1 h/day, estimated ELCR decreases from 0.0017 to 0.0011in a million when moving from the close to far group. The outdoor worker scenario was also calculated to approximate exposures working outside amidst NGE activity, such as farming or working on NGE wells. For this scenario, estimated ELCR decreases from 0.0082 to 0.0055 in a million when moving from the close to far group. These estimations depend heavily on exposure time, exposure frequency, and proximity to an active NGE well.

In all scenarios, the estimated ELCR decreases by about 30% when moving from the close group to far group, all other factors held constant. None of the estimated ELCRs were above 1 in a million, which is the conservative end of the range that the U.S. EPA considers acceptable. Thus, NGE in this study did not appear to emit PAH levels into air that would elevate carcinogenic risk associated with inhalation. PAHs are more likely to partition from the vapor phase onto particulate matter at colder temperatures, and this study was conducted at -1°C. Thus, these data may represent low estimates of carcinogenic risk associated with inhaling vapor phase PAHs at these locations.

Quality Control Results

Carcinogenic PAHs were BLOD in all quality control (QC) samples. Of the non-carcinogenic PAHs, any instrument concentrations above the LODs in QC samples translated to <2.0 ng/m^3 in air, on average. Any measurable levels in QC samples were averaged and subtracted from sample concentrations. Including field and laboratory blanks, >40% of analyzed samples were QC. PAH concentrations from the three co-deployed samplers at the replicate site were averaged, with an average standard deviation of 0.77 ng/m^3. Recoveries of laboratory surrogates ranged from 44 to 94%, averaging 76%. Instrument concentrations were surrogate-corrected. Instrument blanks were BLOD for all PAHs. Compounds were verified at ±20% of the true value for >80% of PAHs using verified standards before instrumental analyses of samples proceeded.

Additional Considerations

The LDPE passive samplers used in this study sample the vapor phase, but the particulate phase is typically enriched in carcinogenic PAHs. This is because the majority of carcinogenic PAHs are higher molecular weight, and the vapor phase typically contains a larger fraction of low molecular weight PAHs, while the particulate phase is typically enriched in high molecular weight PAHs.[184, 185] This may mean that the potency values and risk estimates presented here are under-representative of the actual carcinogenic risk associated with the air in the study area.

Sampling sites were on the private property of volunteer landowners. As a result, data do not represent a completely random sample of the population, and statistical inferences are only relevant to the portion of the population that was sampled.

As with any rapidly advancing technology, there are differences in the techniques used to perform NGE in different parts of the country and the world. It is possible that these differences could impact PAH emissions, and thus that these results may not be directly applicable to other regions. It has been observed, for instance, that NGE activities in different regions of the same state can have markedly different risks of leaks.[186] A recent commentary suggested that reasons for such differences may include

differing geology, rates of development, techniques or implementation.[187] All of these areas would be worth exploring in efforts to minimize emissions from NGE in the future.

Acknowledgements

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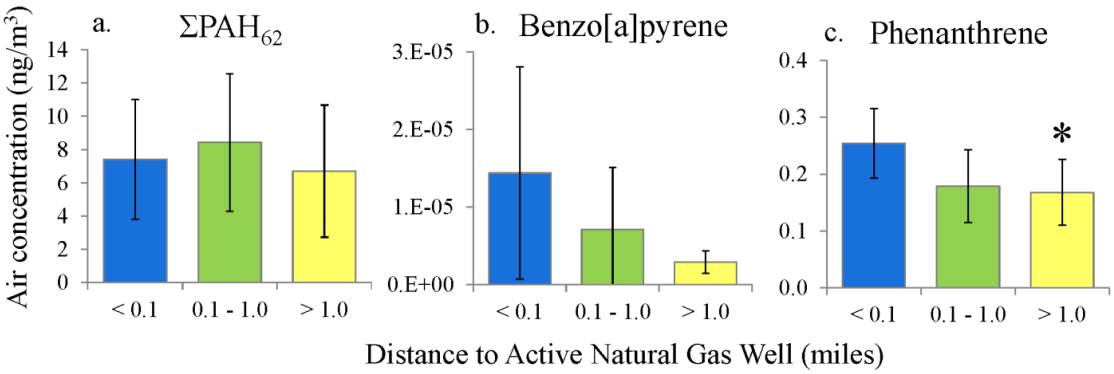

Figure 3.1. Average PAH concentrations grouped by distance to the closest active natural gas well. a. Sum of 62 PAHs, b. benzo[a]pyrene, and c. phenanthrene. The three distance groups are close (*n* = 5), middle (*n* = 12), and far (*n* = 6), defined in the text. Error bars represent one standard deviation. The asterisk indicates a significant difference between the close and far groups for phenanthrene, $p < 0.05$.

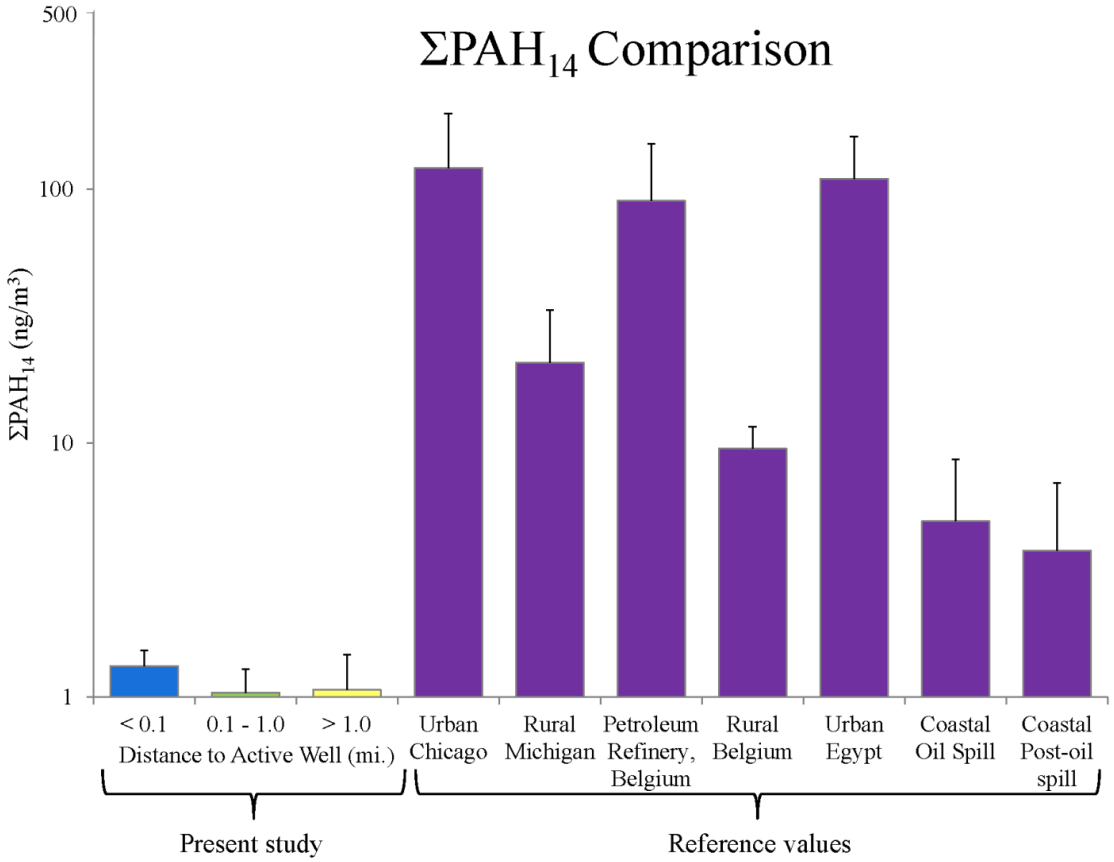

Figure 3.2. Average sum of 14 PAHs, grouped by distance to the closest active natural gas well, with comparisons to previous studies.[34, 83, 176, 177] All data represent PAHs in the vapor phase. The three distance groups in the present study are close (*n* = 5), middle (*n* = 12), and far (*n* = 6), defined in the text. To visualize all data on a log scale, a (concentration + 0.1) transformation was used on all data in this figure. Error bars represent one standard deviation.

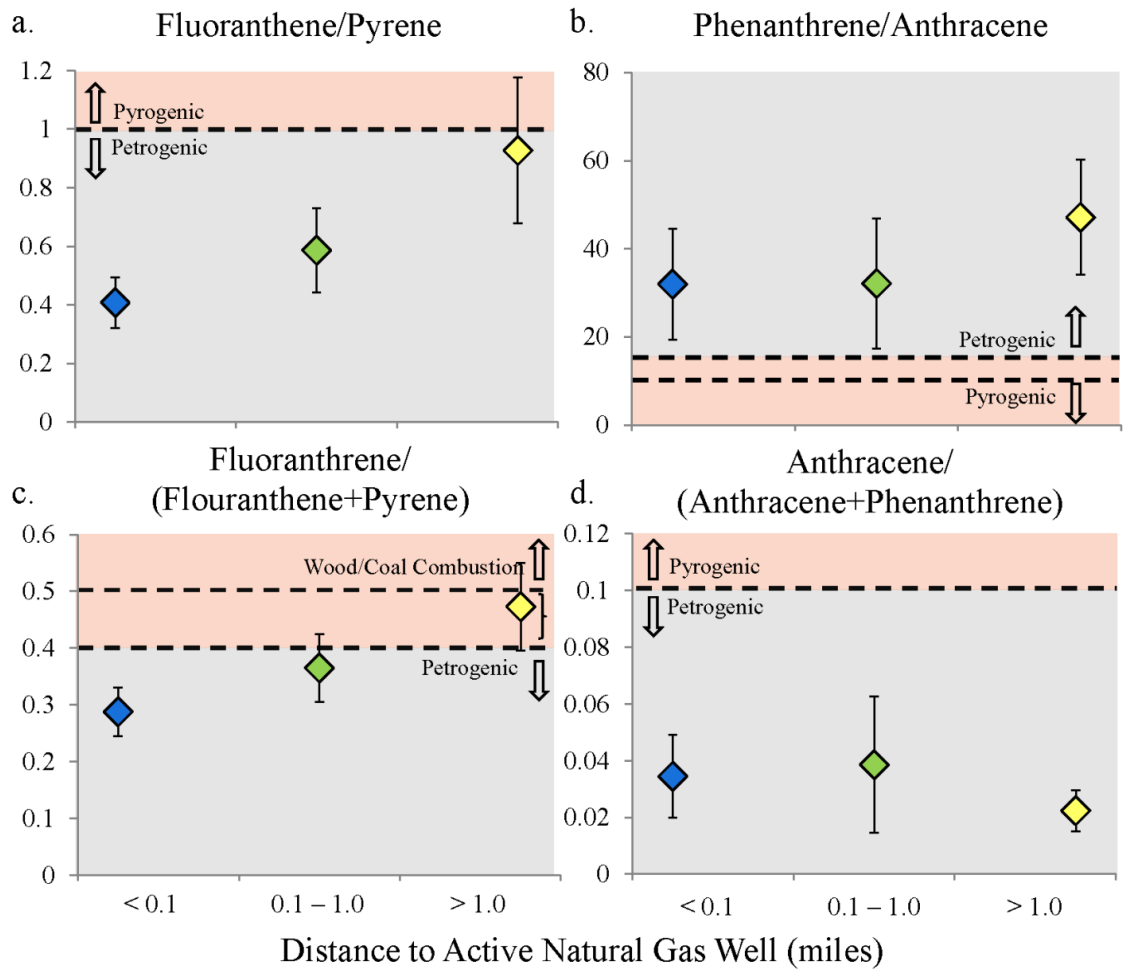

Figure 3.3. Petrogenic vs. pyrogenic sourcing ratios, grouped by distance to the closest active natural gas well. a. Fluoranthene/pyrene, b. phenanthrene/anthracene, c. fluoranthene/(fluoranthene+pyrene), and d. anthracene/(anthracene+phenanthrene) ratios. Pyrogenic and petrogenic thresholds are defined in text. The three distance groups are close (*n* = 5), middle (*n* = 12), and far (*n* = 6), defined in the text . Error bars represent one standard deviation.

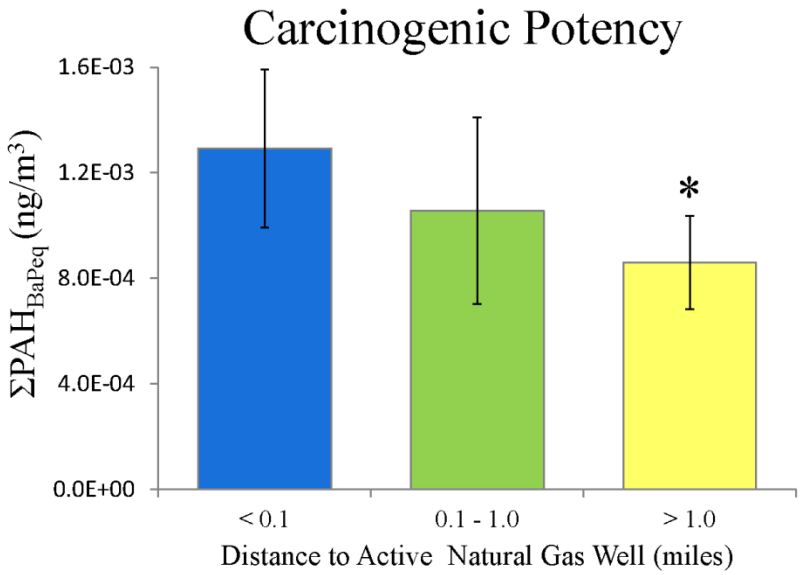

Figure 3.4. Average carcinogenic potency of measured PAHs, grouped by distance to the closest active natural gas well. The three distance groups are close (*n* = 5), middle (*n* = 12), and far (*n* = 6), defined in the text. Error bars represent one standard devaition. The asterisk indicates a significant difference between the close and far groups, $p < 0.05$.

Chapter 4: Personal Exposure to PAHs near Natural Gas Extraction

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Manuscript in preparation

Abstract

Natural gas extraction (NGE) has expanded rapidly in the United States in the last 15 years. Despite concern about environmental impacts, few studies have directly measured emissions coming from NGE. No previous study has directly measured personal exposures of people living near NGE. Recent research has suggested that NGE emits polycyclic aromatic hydrocarbons (PAHs) into air. This study used low-density polyethylene passive air samplers to measure vapor phase PAHs near active NGE wells (n=3) and sites permitted to host future wells in a rural Ohio community (n=2). At each site inner and outer rings of samplers were placed around the well pad or the proposed well pad location (n=6 samplers at each site). This study also used silicone passive wristband samplers to measure personal PAH exposures of people living near these air sampling sites (n=23). Samples were analyzed for 62 PAHs using GC-MS/MS, and isomer ratios were used to identify sources of PAH mixtures. ∑PAH levels in air were significantly higher at sites with active NGE wells than at sites without wells (Wilcoxon rank sum test, $p < 0.01$). Isomer ratios indicated that PAH mixtures at sites with active NGE wells had more petrogenic signatures, while sites without wells had more pyrogenic signatures. This is consistent with NGE well sites being more heavily affected by emissions from within the earth. ∑PAH levels were significantly higher in wristbands from participants who had NGE wells on their home properties than from participants who did not (Wilcoxon rank sum test, $p < 0.005$). Specifically, median ∑PAH was 4.5 times higher in wristbands of participants living within 0.75 km of active NGE wells than in wristbands of participants living farther than 2.0 km from any active NGE wells. There was a significant positive correlation between ∑PAH in participants' wristbands and ∑PAH in air measured at the stationary sampling site nearest to each participant's home (simple linear regression, $p < 0.01$). This work provides further evidence affirming that NGE emits PAHs into air. These findings also suggest that living on a property with an active NGE well may increase personal exposure to PAHs.

Introduction

Natural gas extraction (NGE) from shale has expanded rapidly in the United States in the last 15 years. This has been largely due to improvements to the technologies of hydraulic fracturing and horizontal drilling, which liberate previously inaccessible gas reserves from shale.[188] Due to the influence of these techniques, this activity has broadly been referred to as "fracking." Recent projections have suggested that NGE may make a 100-year supply of energy accessible at current U.S. rates.[189] Thus, NGE presents many potential advantages to the U.S. and other countries. However, NGE must be properly regulated to avoid adverse impacts on environmental and public health.[190, 191] Despite the rapid increase in NGE there has been relatively little investigation into the impacts NGE activity may have on health.[189, 192]

This lack of information is due in part to a historic lack of regulation in the U.S. For instance, the Energy Policy Act of 2005 amended portions of U.S. Safe Drinking Water Act and Clean Water Act to give gas-drilling companies more flexibility.[141] This has led to concerns about the potential for NGE to have adverse effects on environmental and human health. In the past few years this issue has begun to receive more attention from the scientific community, the public, and regulators. In response the U.S. EPA recently issued new standards increasing regulatory requirements on natural gas well sites, with the goal of reducing emissions into air.[193]

Some studies have used data from stationary monitors to estimate community-level health impacts,[148, 152, 194] while others have used results from questionnaires to approximate individual health impacts of NGE.[157, 158, 195] The majority of these studies have concluded that NGE has the potential to impact human health. Other studies have addressed that much research is still needed to assess the public health impact of NGE. Many recent studies have addressed the need for data directly measuring the environmental impact of NGE, and associated health effects.[146, 155, 196] Still other studies have measured impacts to air quality, predominantly concluding that NGE contributes contaminants to the environment at various stages of the process.[148, 149, 153, 194, 197]

Air emissions have been identified as one of the main pathways through which NGE may impact the health of nearby communities or workers. This is becoming even more relevant as the scientific community becomes increasingly aware of the impact that air pollution can have on human health.[198] Indeed, many studies have acknowledged that impact to air quality may be the most significant risk to communities living near NGE.[146-153] Shonkoff et al. concluded that NGE has the potential to pose health risks through both air and water emissions, and urged that many important data gaps remain.[147]

There is evidence that NGE emits methane,[199, 200] volatile organic compounds (VOCs),[148, 150, 201] and semi-volatile organic compounds (SVOCs).[153, 194] However, there is still limited information about concentrations of these contaminants in the environment, and whether or not those concentrations are potentially concerning to public health.[143, 194] One class of SVOCs that has been measured in the air near NGE activity is polycyclic aromatic hydrocarbons (PAHs).[153, 194] PAHs are pervasive environmental pollutants of concern, widely associated with both fossil fuel production[202] and adverse health outcomes such as increased cancer risk,[61, 134] respiratory distress,[80, 203] and developmental effects.[161, 204] Researchers, regulators, and community members would all benefit from increased understanding of the environmental movement and fate of PAHs and other contaminants that are emitted from NGE.

Low-density polyethylene (LDPE) passive samplers sequester hydrophobic compounds through passive diffusion in a time-integrated manner, and are well-suited to passively sample vapor phase PAHs from air. Since this tool's development in the 1990s, many studies have demonstrated its ability to measure PAHs.[34, 55, 162, 163] As the air quality sampling community moves toward cheaper and easier to use techniques[205], passive air sampling is emerging as an even more relevant tool. A recent study specifically demonstrated the utility of passive air samplers to measure vapor phase PAH emissions from NGE.[194]

A few studies have used stationary monitors to measure VOCs or SVOCs in the air near NGE.[148, 153, 194] However, most of these studies have had limited spatial sampling. Due to a lack of comprehensive ambient air quality monitoring in many areas heavily affected by NGE, there is a need for more data about air quality near NGE.[143] In the present study, stationary LDPE passive air samplers were used to more thoroughly measure PAH concentrations in air surrounding both active NGE well pads, and proposed sites for future NGE wells. This sampling design enabled researchers to assess environmental movement and fate of PAHs emitted from NGE wells, and to compare PAH concentrations at similar sites with and without active NGE wells.

While there is only a small amount of data regarding contaminant concentrations in the environment related to NGE, there are even less data about personal exposure to contaminants emitted from NGE. Some studies have extrapolated from data collected with stationary samplers to estimate personal exposures to NGE.[148, 153, 194] Others have used epidemiological methods to compare health records and NGE activity records.[149] Still others have predicted exposures from NGE from emissions inventories or known toxicity information of chemicals reported to be used in NGE.[145, 150] No study has directly measured the individual exposures of people living or working near NGE. There is a need for more comprehensive data assessing the relationship between personal exposure to NGE and health outcomes.[192]

It has been suggested that using personal monitors, rather than extrapolating from stationary monitors, is the best approach for assessing individuals' exposure and risk.[206] Improved understanding of the personal exposure to PAHs of people living near NGE would improve regulators' ability to mitigate the potential impact of air emissions from NGE on human health. Personal exposure data would reduce the uncertainty in the exposure assessments and projections performed by regulators and scientists. Individualized exposure data yield much more accurate risk estimates, compared to approximating exposure from questionnaires or extrapolating exposure from stationary monitoring data. Estimates from questionnaire data are fraught with potential for miscommunication between the researcher and the respondent, while estimates from

stationary monitors necessitate numerous assumptions about timing, frequency, and duration of exposure. Personal monitoring bypasses all of these uncertainties, by directly measuring the chemicals that an individual is exposed to.

Personal exposure to PAHs and other SVOCs has previously been assessed by active[15, 78, 207] and passive personal monitors.[206, 208] The passive wristband sampler (hereafter "wristband"), is a recently developed personal passive sampler that absorbs SVOCs.[33, 209, 210] The active sampling techniques are often bulky or noisy, which can negatively affect participant compliance. The wristband is lightweight and small, and does not require a motor. Thus, compared to other personal sampling technologies, such as the active sampler described above or giving blood or urine samples for use in biomonitoring, the wristband is non-invasive and easy to use. These features make it an attractive alternative to traditional personal sampling methods. This difference means the wristband may yield higher participant compliance rates than traditional methods. The wristband is well-suited to answer questions about the impact of NGE on nearby communities' exposure to PAHs.[33] The fraction of contaminants sequestered by the wristband is similar to the fraction that the participant is exposed to, making it a good surrogate for individual exposure. Thus, the wristband was used to estimate personal exposure to PAHs in this study.

This work combines passive air sampling with personal passive sampling to investigate the movement of PAHs emitted from NGE wells, and to assess the impact of those emissions on the exposures of people living or working nearby.

Materials and Methods

Sampling Site Description

This study was conducted predominantly in Carroll County, Ohio. This is a rural community that has been heavily affected by the U.S. natural gas boom. As technology has made gas in the Utica shale more accessible in the last five years, NGE in eastern Ohio has increased. In 2011, Ohio had less than 50 horizontal natural gas drilling leases.[166] As of June 2014, that number had jumped to 1386, with 421 in Carroll

County.[167] At the time of the study, this area was one of the most densely affected counties in Ohio, with more than one natural gas well pad per square mile. This county was also identified in a 2014 perspective article as an example of a county that has the highest number of active wells in its state, but which also has no routine air monitoring program.[143] Because this area was historically rural (and thus had limited pre-existing anthropogenic sources of pollution, compared to an industrial area or a city), this community presented researchers with a good opportunity to assess potential increases in air pollution related to NGE. Volunteers were identified through collaboration with a pre-existing concerned citizens group in the area.

Sampling Strategy

Stationary passive LDPE air samplers were deployed at five sites: three with active NGE wells and two with plans to build NGE wells, but with no NGE activity at the time of sampling. At each site, six passive air sampler cages were deployed. Each cage contained three PRC-infused LDPE strips. After deployment these three strips were composited as one sample for analysis. At each site cages were arranged in two nested rings of three samplers each, around either the NGE well pad or the proposed well pad site. The inner ring of samplers was deployed 55-60 m from the edge of the well pad or well pad site, and the outer ring of samplers was deployed 112-122 m from the edge of the well pad or well pad site. The six stationary air samplers deployed at each of the five sites yielded n=30 total stationary air samplers. Average temperatures during deployment were measured using Hoboware Tidbits that were placed in one of the air cages at three of the five stationary air sampling sites. Sampling was from May to June, 2014 and the average air temperature during the deployment period was 20°C.

Volunteer participants who lived or worked near these five stationary air sampling sites were identified to wear the wristband samplers. In total, 23 participants wore wristbands. Each participant wore one wristband constantly for three weeks. Each participant also filled out a questionnaire concerning lifestyle and health.

In order to ensure that the both LDPE and wristband samplers only sampled contaminants during sampler deployment, all samplers were transported between the

lab and the study area and back in airtight Teflon bags with Clip N Seal assemblies (Welch Fluorocarbon). Additionally, each sampler's bag had a detailed label. For the LDPE, these labels contained date and time of deployment and retrieval. For the wristband, these labels contained space for the participant's name, time and date the wristband was taken out of the bag to start sampling, time and date the wristband was put back in the sealed back to end the sampling period, and the participant's signature.

Citizen Scientist Training

At the time when the research team gave the participant the wristband, each participant was trained in how to properly wear the wristband, and in how to mail it back to the lab for chemical analysis. Importantly, this included education about how the technology worked, so that participants could be mindful not to accidentally bias their wristband. This included discussion of avoiding covering the wristband in lotion or any other cream (which could make it harder to measure contaminants into the wristband), and awareness that the wristband is sampling constantly while exposed to the environment. This was done to maximize the tool's ability to accurately assess only what each individual was truly exposed to. Participant training included instruction about how to return both the wristbands and the LDPE samplers. Care was taken to make sure that all participants' questions were answered.

Passive Sampler Preparation

Stationary air samplers made of LPDE and personal wristband samplers made of silicone were both deployed in this study. Details about chemicals and solvents are in Appendices 1 and 2.

Stationary LDPE Air Samplers' Preparation, Cleaning, and Extraction

Before deployment, LDPE was cleaned using hexanes as described previously.[22] Each LDPE strip was infused with performance reference compounds (PRCs) to enable calculation of *in situ* sampling rates and time-integrated air concentrations.[21] PRCs used in this study were fluorene-d10, pyrene-d10 and benzo[b]fluoranthene-d12. PRCs were

spiked into LDPE at 1-19 µg per strip. Samplers were cleaned after deployment in two isopropanol baths, stored in amber jars at -20°C, and extracted as described elsewhere.[22] Briefly, extractions were performed using two dialyses with *n*-hexane.

Personal Silicone Wristband Samplers' Preparation, Cleaning, and Extraction

Prior to deployment, passive wristband samplers (from 24hourwristbands.com) were soaked with ethyl acetate, hexane and methanol at 40°C to remove anything that might have interfered with chemical analysis, as previously described.[33] Wristbands had an average weight of 4.7 g, and were stored at -20°C after initial cleaning. After deployment, wristbands were cleaned in 18 MΩ∗cm water to remove any debris from the surface and then quickly rinsed in isopropanol as reported previously.[33] After cleaning wristbands were stored in amber jars at -20°C, and then extracted as described elsewhere.[33] Briefly, extractions were performed using two dialyses with ethyl acetate.

Chemical Analysis

Prior to extraction, both LDPE and wristbands were spiked with deuterated PAHs to act as surrogate standards, allowing for quantification of extraction efficiency. Surrogate standards are specified in **Table A1.2**. Extracts were quantitatively concentrated to 1 mL using TurboVap closed cell evaporators, transferred to amber chromatography vials, and stored at -20°C until analysis.

LDPE and wristband extracts were quantitatively analyzed for 62 PAHs using an Agilent 7890A gas chromatograph interfaced with an Agilent 7000 GC/MS-MS, as described previously.[125] Briefly, an Agilent Select PAH column was used and each PAH was calibrated with a curve of at least five points, with correlations ≥0.99. Limits of detection (LODs) range from 0.24 to 1.7 ng/mL, and limits of quantitation (LOQs) range from 1.0 to 7.1 ng/mL, with the exception of two compounds that have higher LODs and LOQs. A list of PAHs, LODs and LOQs in the instrument method is included in **Table A1.2.**

Some extracts from the deployed wristband had to be diluted prior to analysis to successfully quantify peaks. 71% of wristband extracts samples were diluted 10-fold before analysis to enable chromatographic resolution of all PAH peaks that was needed

for quantification. Thus, the LODs and LOQs for the diluted samples are 10-fold higher than for the others (**Table A3.1**).

Air Concentration Calculations for LDPE

Air concentrations (ng/m^3) of PAHs measured in LDPE were calculated from instrument concentrations (ng/mL) using PRCs. *In situ* sampling rates (R_S) were generated using calculations described by Huckins et al.[21] These calculations estimate the R_S of each PRC by incorporating deployment time, initial amount and the temperature-corrected sampler-air partition coefficient ($K_{sa(T)}$). An R_S was calculated for each PAH, using the PAH's K_{oa} and the R_S of one of the PRCs. Temperature-corrected K_{sa} values ($K_{sa(T)}$) were calculated using a modified van 't Hoff equation as described by Khairy et al.[34] Average PRC recoveries in deployed LDPE samples were 0.05, 44 and 61% for fluorene-d10, pyrene-d10 and benzo[b]fluoranthene-d12, respectively. Therefore only pyrene-d10 and benzo[b]fluoranthene-d12 were used to calculate air concentrations, as their recoveries were both between 10 and 90% for all samples. These calculations incorporate numerous compound-specific adjustments based on the physico-chemical parameters of each target compound, and based on empirical relationships between the physico-chemical parameter of a compound and its observed uptake into LDPE passive samplers. These adjustments enable accurate calculation of environmental concentrations of all target compounds, even if some compounds are at or near equilibrium. Further explanation of the air concentration calculations is in Appendix 2, and the air concentration calculations are included as **equations A2.1-A2.9.**

PAH Sourcing

Parent PAH isomer ratios were used to determine source signatures of PAH mixtures. One PAH isomer pair that is often used to diagnose whether a PAH mixture is petrogenic or pyrogenic is fluoranthene and pyrene.[85, 86, 168-172] Fluoranthene/pyrene ratios >1.0 indicate pyrogenic sources, while ratios <1.0 indicate petrogenic sources.[168, 170, 171] Ratios of one isomer to the sum of both isomers are also used in PAH sourcing. Fluoranthene/(fluoranthene+pyrene) ratios ≥0.5 indicate pyrogenic sources, and ratios

≤0.4 indicate petrogenic sources.[86, 169] Yunker et al. suggest that ratios between 0.4 and 0.5 indicate liquid fossil fuel combustion.[85]

Data Analyses

Wilcoxon rank sum tests were performed to assess statistical differences between PAH levels measured in stationary samplers at sites with and without active NGE wells, in inner and outer rings of stationary samplers at each site, and in wristbands of participants living or working at various distances from active NGE wells. Simple linear regression was used to assess whether there were correlation between PAH levels measured in participants' wristbands and distance from a wristband-wearer's home or work to the nearest active NGE well. Simple linear regression was also used to assess whether there were correlation between PAH levels measured in participants' wristbands and PAH levels measured in air nearby. Spearman's rho correlations were used to explore correlations between PAH levels in air and production of natural gas at NGE wells closest to air samplers. The statistical softwares R version 2.15.3 and JMP PRO version 12 were used to perform these statistical analyses and comparisons. For all comparisons, results were deemed significantly different when $\alpha < 0.05$.

ERSI ArcGIS version 10.2.2 was used to measure distances between stationary sampling locations and NGE wells, and distances between wristband-wearer's homes or workplaces and NGE wells, and to make site maps. The volume of natural gas produced at active NGE wells in the study area was also used in comparisons with the source signature of PAHs measured in air. These natural gas production data, reported during the quarter during which this study occurred, were obtained from the Ohio Department of Natural Resources' website in spring 2016.

Carcinogenic Potency Estimation

The carcinogenic potencies of PAH mixtures measured in air samplers were calculated by multiplying the concentration of each PAH in the mixture by the relative potency factor (RPF) it was given by the U.S. EPA in 2010.[59] RPFs that were used in these calculations are listed in **Table A1.4**. This approach normalizes the carcinogenic potency

of a PAH mixture to that of benzo[a]pyrene. This estimate is therefore referred to as the benzo[a]pyrene equivalent concentration, or BaP_{eq}.

Quality Control

During LDPE passive sampler preparation, one LDPE strip was hung in the room to account for potential contamination during PRC infusion. A trip blank of each sampler type (one LDPE and one wristband) was taken to each sampling site to account for contamination during transport. During post-deployment sampler cleaning, blank LDPE strips and blank wristbands were included in their respective post-deployment cleaning methods to act as cleaning blanks. These also doubled as blanks during sampler extractions. Perylene-d12 was spiked into all sample extracts before instrumental analysis to act as an internal standard. The analytical method was validated using its calibration, precision and accuracy, and LODs prior to use, as described previously.[125] During instrumental analysis, instrument blanks and continuing calibration verifications were run at the beginning and end of each set of samples. All laboratory and field procedures were performed according to FSES Standard Operating Procedures.

Quality Control Results

Carcinogenic PAHs were below limits of detection (BLOD) in all quality control (QC) samples. Of the non-carcinogenic PAHs, any instrument concentrations above the LODs in QC samples translated to 2.0 ng/m^3 in air and 0.95 ng/g in wristband, on average. Any measurable levels of target PAHs in QC samples were averaged and subtracted from sample concentrations. Including field and laboratory blanks >50% of analyzed samples were QC. Average recoveries of individual laboratory extraction surrogates ranged from 46 to 97% for LDPE extractions and from 50 to 87% for wristband extractions, averaging 69% for all surrogate recoveries in all extractions. All instrument concentrations were surrogate recovery-corrected. Instrument blanks were BLOD for all PAHs. Compounds were confirmed at ±20% of the true value for >80% of PAHs using verified standards before instrumental analyses of samples proceeded.

Results and Discussion

Citizen Scientist Compliance

Overall, this study had excellent compliance with citizen scientists. Citizen scientists mailed the LDPE passive air samplers and the wristbands back to the FSES lab after deployment. 97% of the LDPE samplers were received at the FSES lab in properly sealed Teflon bags. Of the 23 participants who wore wristbands, 21 mailed them back to the lab in perfect condition for analysis. One participant lost the wristband, and one mistakenly mailed the wristband outside of its Teflon bag, meaning the wristband also sampled the air during its transport via the postal service. Overall, this was over 91% compliance with the wristband. This high compliance rate suggests that the wristband was easy for participants to use. It has been observed that traditional personal sampling tools can be noisy and cumbersome.[206] The wristband is lightweight and does not require power. These features may make it easier to use and increase participant compliance.

PAH Concentrations and Comparisons

PAH concentrations in air are higher in the present study than in previous study in which PAH concentrations were measured in the same region and at similar distances from NGE, using the same sampling methods.[194] In this previous study, PAH data were grouped based on distance from the sampler to the nearest active NGE well, with the closest group being less than 0.1 mile from the nearest active well. All samples that were on sites with active NGE wells in the present study were less than 0.1 mile from the well. This makes air PAH data from active well sites in the present study comparable to air PAH data in the closest distance group in the former study. However, it should be noted that air samplers on NGE well sites in the present study were slightly closer to active NGE wells than even the closest samplers from the previous study. If the PAHs emitted from an NGE well pad quickly disperse, then these slight differences in deployment distances could be a relevant factor affecting the observed concentration differences.

In the present study, the average ∑PAH measured in air at sites with active NGE wells was 31 ng/m^3. In the previous study, average ∑PAH measured in air less than 0.1 mile from active NGE wells was 8.3 ng/m^3.[194] Another key difference between the two studies is that air samplers from the present study were deployed at 20°C colder average temperatures than in the previous study, being deployed in Ohio in February rather than May.[194] This is a 3.7-fold increase in average vapor phase ∑PAH concentrations in air, correlated with a 20°C increase in temperature. This is consistent with what has been previously proposed and observed in the literature. For instance, Huckins et al. suggested that a 2 to 4-fold increase in vapor phase PAH levels in air should be expected with each 10°C increase in air temperature.[21] Additionally, Ravindra et al., Khairy et al., and Mottelay-Massei et al. all observed higher PAH concentrations in air in warmer seasons at the majority of sampling sites in their studies.[34, 177, 211] There were some cases where the reverse was observed, with higher PAH concentrations in air with colder temperatures. In general this was explained by a corresponding increase in PAH emissions in the colder season. However, when increases in PAH concentrations were observed with warmer temperatures, this trend of about a 2-fold increase with each 10°C increase in temperature was often observed. There could also be seasonal differences in NGE activity, or in other activities in the region, that could contribute to the elevated PAH concentrations observed in spring in the present study relative to winter in the previous study.

Spatial PAH Profile in Stationary Air Samplers

Overall, PAH levels in air were higher at sites with active NGE wells than at sites without wells (**Figure 4.1**). Specifically, median ∑PAH concentrations were significantly higher in air at sites with active NGE wells than at sites without them (Wilcoxon rank sum test, $p = 0.0058$). Median ∑PAH levels were 43% higher in air measured by stationary samplers at sites with wells than at sites without. This trend was even more pronounced when comparing only PAH levels measured in air by samplers in the inner rings at sites with and without active NGE wells. Median ∑PAH levels measured by the inner rings of LDPE samplers in air were even more significantly higher at sites with wells

than at sites without wells (Wilcoxon rank sum test, p = 0.00080). Median ∑PAH levels in air measured by inner rings of stationary samples were 50% higher at sites with wells than at sites without. In contrast, ∑PAH levels were not significantly different in air measured by the outer rings of LDPE samplers at sites with and without NGE wells (Wilcoxon rank sum test, p = 0.46). This may suggest that vapor phase PAHs in air diffused quickly enough after leaving the NGE well that they significantly impacted the inner ring of samplers, but not the outer ring of samplers.

There appeared to be spatial trends in PAH levels in air at 2 of the 3 active NGE well sites. In general, these sites appeared to have higher ∑PAH levels in samplers that were closer to the NGE well pad (**Figure 4.1 a,c**). At the third active NGE site, PAH levels were highest in air samplers closest to a service road leading to the NGE well pad (**Figure 4.1b**). At sites without NGE wells, spatial trends among the levels of ∑PAH in samplers were less straight forward (**Figure 4.1 d,e**). At site d, ∑PAH levels were relatively consistent in air measured by all six samplers. However, the two highest PAH levels at site d were measured near a service road on the site. At site e, there was no clear spatial trend with PAH levels measured in the six air samplers. Median ∑PAH measured in the inner ring and outer rings of samplers were not significantly different at any of the five sites, with or without NGE wells (Wilcoxon rank sum tests, p > 0.05).

Spatial Profile in Wristbands Related to NGE

Simple linear regression estimated that ∑PAH in a participant's wristband decreases significantly as the distance from that participant's home or workplace to the nearest active NGE well increases (simple linear regression, p = 0.00015, R-squared = 0.58) (**Figure 4.2**). Specifically, the slope of this regression line suggests that there was an average of 500 ± 100 ng/g decrease in ∑PAH in a participant's wristband with each 1.0 km increase in distance from the participant's home or work to the nearest active NGE well (**Figure 4.2**). Additionally, the three highest ∑PAH levels were measured in wristbands that were worn by the only three people who reported having active NGE wells on their home properties. The median ∑PAH concentration was 4.1-fold higher in wristbands of participants who reported having active NGE wells on their home

properties than in wristbands of participants who did not. ∑PAH concentrations in wristbands of participants who reported having active NGE wells on their home properties were significantly higher than in wristbands of participants who did not (Wilcoxon rank sum test, $p = 0.0044$). This suggests that personal exposure to PAHs in this study was affected by the proximity of participants' homes and workplaces to active NGE wells.

Interestingly, median ∑PAH in wristbands of participants who reported having active NGE wells on their neighbors' property was closer to median ∑PAH in wristbands of participants who did not report having an active NGE well near their home or work, than to ∑PAH in wristband of participants who had active wells on their property. Median ∑PAH in wristbands of participants whose neighbors had active NGE wells was 3.0-fold smaller than of participants with active NGE wells on their own property, and was only 1.7-fold larger than ∑PAH in wristbands of participants reporting no well nearby. The difference between ∑PAH levels in wristbands of participants with wells on their home property and in wristbands of participants with wells on their neighbors' property was just above the significance level (Wilcoxon rank sum test, $p = 0.057$). Additionally, ∑PAH levels in wristbands of participants who had active wells on neighbors' property were only slightly significantly different than in wristband of participants who did not report having any wells nearby (Wilcoxon rank sum test, $p = 0.013$). In contrast, ∑PAH in wristband of participants who had active wells on their home property were strongly significantly different than in wristband of participants who did not report having any wells nearby (Wilcoxon rank sum test, $p = 0.0044$).

This suggests that PAH exposures for people living or working as neighbors of active NGE wells may be more similar to those of people living far from NGE activity than to those of people living with active NGE wells on their home properties. In this group of study participants, these results suggest that PAHs emitted from NGE wells may be diluted quickly after leaving an NGE well, and that they minimally affect the PAH exposure of a neighbor living more than about 0.75 km from the well. This is further evidence to suggest that PAHs emitted from NGE may dissipate quickly after leaving the

well pad, making them most available for exposures in a small spatial range. It is important to consider, however, that this study was conducted in a rural area where individual landowners' properties sizes are variable and often quite large. This "neighbor" comparison may look different in a more densely populated suburban or urban region.

PAH Sourcing in Air

PAH isomer ratio sourcing suggested that PAH mixtures in air have more petrogenic or mixed signatures at sites with NGE wells, and more pyrogenic or mixed signatures at sites without NGE wells (**Figure 4.3**). PAH mixtures measured in air at sites with active NGE wells were more petrogenic (**Figure 4.3 a-c)** than at sites without active NGE wells (**Figure 4.3 d,e**). Additionally, at the two sites that appeared to have the strongest spatial association between ∑PAH and NGE well activity (**Figure 4.1 a,c**), there was also the strongest petrogenic signature in air samples (**Figure 4.3 a, c**). In addition to showing overall more petrogenic signatures, both of these sites appeared to have spatial trends, where samplers closer to the active NGE well pad generally had stronger petrogenic signatures than samplers farther away. However, the sourcing ratio told a different story at the active NGE well site that had a weaker spatial relationship between proximity to the active NGE well pad and PAH levels (**Figure 4.1b**). At this site, where highest PAH levels were measured near the service road, PAH signatures were more mixed or slightly pyrogenic (**Figure 4.3b**). Additionally, sites without NGE wells had predominantly pyrogenic signatures (**Figure 4.3 d,e**). This suggests that PAH emissions at sites a and c were heavily affected by direct emissions from the earth, which could have been fugitive emissions from NGE wells. In contrast, air samples from site b were affected by both direct emissions from the earth and also emissions from combustion, perhaps in part from traffic on the service road. Air samples from the sites without active NGE wells, d and e, were more heavily affected by the myriad combustion sources that produce stronger pyrogenic signatures. Additionally, the prevalence of naphthalene (~70%) in the air in the present study is consistent with previous work that

has measured naphthalene as the most abundant PAH in the vapor phase near NGE wells.[153, 194]

The relationship between daily natural gas production at the NGE well nearest to each passive air sampler, and source signature of the PAH mixture measured in that passive air sampler, was also used to assess the impact of NGE activity on source of PAH mixtures. Natural gas production data were obtained from the Ohio Department of Natural Resources' database. Average daily production rates were calculated from amounts of natural gas produced in the quarter in which this sampling campaign occurred. For this comparison, the source signature was again defined by the fluoranthene/pyrene isomer ratio. There was a significant negative correlation between daily natural gas production at the NGE well nearest to the sampler, and the fluoranthene/pyrene ratio of the PAH mixture in that sampler (Spearman rho correlation = 0.58, p = 0.0028). Given that smaller values of this ratio suggest more petrogenic signatures of PAH mixtures, this suggests that there is a positive correlation between the amount of natural gas produced by an NGE well, and stronger petrogenic signatures of PAH mixtures measured nearby.

The predominant petrogenic signature measured in air near NGE activity is different from the majority of what is reported in the literature for PAH signatures in air. Khairy et al., for instance, used LDPE passive air samplers to measure PAH levels in urban Egypt, and reported pyrogenic fossil fuel combustion source signatures consistently at all sites.[34] The authors used these sourcing ratios as evidence to suggest that PAHs in air came predominantly from the vehicle emissions and other combustion activities in the urban areas where the study was conducted.[34]

These findings suggest that PAH mixtures in air at sites with active NGE wells are affected by direct releases from the earth. This is consistent with these PAH mixtures being affected by fugitive emissions during natural gas extraction. This is consistent with what was seen in a previous study assessing the impact of NGE on PAH levels in air, where signatures of PAH mixtures in air were observed to be petrogenic at all sites within 0.1 mi of active NGE wells.[194]

PAH Sourcing in Wristbands

PAH sourcing suggests that PAH mixtures in wristbands worn by participants who reported having active NGE wells on their property had more petrogenic or mixed signatures, while neighbors of NGE activity had pyrogenic signatures (**Figure A3.1**). The majority (86%) of people who did not report having NGE on their home or work property had pyrogenic signatures in their wristbands. Of the two participants without NGE wells nearby who did not have pyrogenic signatures, one had a mixed signature and one had a petrogenic signature. These two participants also lived 0.4 km from a traditional natural gas well. This may partly explain the more petrogenic signatures in their wristbands, although this well was not producing during the sampling period (**Figure A3.1**).

The more convoluted petrogenic to pyrogenic gradient of signatures in the wristband than in the stationary air samples, and the more predominantly pyrogenic signatures observed in the wristband than in the stationary air samplers, can likely be explained by the mobility of the wristband-wearing participants (**Figure A3.1**). It is important to consider that PAHs are pervasive, and thus people are constantly exposed to PAH mixtures.[212] In the modern age, the majority of these PAH exposures are likely to a mixture of PAHs with a predominantly pyrogenic source. There are many combustion processes that people encounter daily, and that would likely produce pyrogenic PAH signatures. Thus, even if there is a strongly petrogenic source of PAHs at an individual's home, it is reasonable to think that this individual's overall PAH exposure may be more mixed or even pyrogenic, given that many of the other inputs into this exposure are likely pyrogenic. It is therefore interesting that any of the participants' wristbands had predominantly petrogenic signatures. This reinforces the observation that participants' PAH exposures were affected by NGE emissions in this study.

Data from participants' responses to lifestyle questionnaires suggested that the number of times they were exposed to cigarette smoke during the study was not correlated with ∑PAH in their wristbands (simple linear regression, R-squared = 0.0066, p = 0.74) (**Figure A3.2**). This cigarette smoke exposure metric included both number of

cigarettes smoked, and number of reported exposures to secondhand cigarette smoke. This lack of correlation indicates that exposure to cigarette smoke was not a driver of ∑PAH levels in the wristband.

Comparison between PAH levels in wristband and in nearby air

There was a significant positive correlation between ∑PAH in wristbands and in the air measured closest to the wristband-wearer's home or work (**Figure 4.4**). Specifically, simple linear regression estimated that an increase of 1.0 ng/m^3 in ∑PAH in air was associated with an increase of 50 ± 16 ng/g in ∑PAH in wristbands of participants living or working nearby (simple linear regression, R-squared = 0.33, p = 0.0062). This suggests that personal exposure to PAHs in this study was affected by PAH levels in air near participants' homes or workplaces.

The significant correlation between PAH levels in wristbands and in nearby air is compelling. Wristband-wearing participants are mobile, and PAHs are pervasive pollutants that can come from myriad sources. There is also the potential for multiple exposure pathways to contribute PAHs to the wristband. It should therefore not be taken for granted that PAH levels in wristbands would be correlated with PAH levels in the air nearby. In addition to further elucidating the potential impact of NGE on PAH exposures of people living nearby, the significant positive correlation observed in **Figure 4.4** is another piece of evidence in support of the previously observed utility of the wristband for assessing personal exposure to environmental contaminants.[33, 209]

Carcinogenic Potency of PAH Mixtures

The highest average BaP_{eq} measured in air at any of the sites was 0.0085 ng/m^3 and was measured in the inner ring of samplers at one of the sites with an NGE well. On average, BaP_{eq} levels were about 2-fold higher in air at sites with NGE wells than at sites without. Additionally, average BaP_{eq} measured in air at sites with active NGE wells was about 3-fold higher than was reported within a tenth of a mile of active NGE wells in a previous study conducted in the same county.[194]

Even the highest BaP_{eq} level measured in the air in the present study (0.0085 ng/m^3) is more than 100 times smaller than the 1.0 ng/m^3 regulatory level that was

suggested by the World Health Organization as a level of concern for carcinogenic PAHs in ambient air.[182] This suggests that measured PAH levels would not be concerning with regard to carcinogenic risk associated with chronic inhalation exposure. These PAH levels would therefore not be expected to increase inhalation-based cancer risk of participants living or working near NGE in this community.

Additional Considerations

This work employed volunteer citizen scientist participants who each lived near one of the five stationary air sampling stations. Thus, the sampling design does not necessarily represent a random sample of the population, and findings may not be directly applicable to other regions affected by NGE. Additionally, none of the participants were employees of the NGE companies. Employees of these companies could potentially have higher exposures. The exposures of employees working directly on NGE well pads would be a useful future line of investigation.

The PAH sourcing analysis presented in this study relies only on ratios between one pair of isomers, fluoranthene and pyrene. Ideally, more than one ratio would have been used. However, other PAHs that are commonly used in sourcing ratios were not consistently detected in this study and thus other ratios were not used.

One challenge of assessing emissions from NGE is the uncertainty of exactly where the point source is on any given well pad. Well pads are roughly between 1 and 2 hectares (3-5 acres),[213] and there are multiple locations on each pad that could produce emissions. Some evidence suggests that PAHs and other SVOCs are present in the subsurface along with the lighter molecular weight hydrocarbons that make up the majority of natural gas, and that these may therefore be emitted from the wellhead as fugitive emissions.[178, 194] Other evidence suggests that flaring of excess gas may be a major source of VOCs and SVOCs, including PAHs, to air.[197] Furthermore, there may be PAH emissions from vehicle traffic traveling to and from the well pad to set up the well pad and then to remove product and waste from the site.

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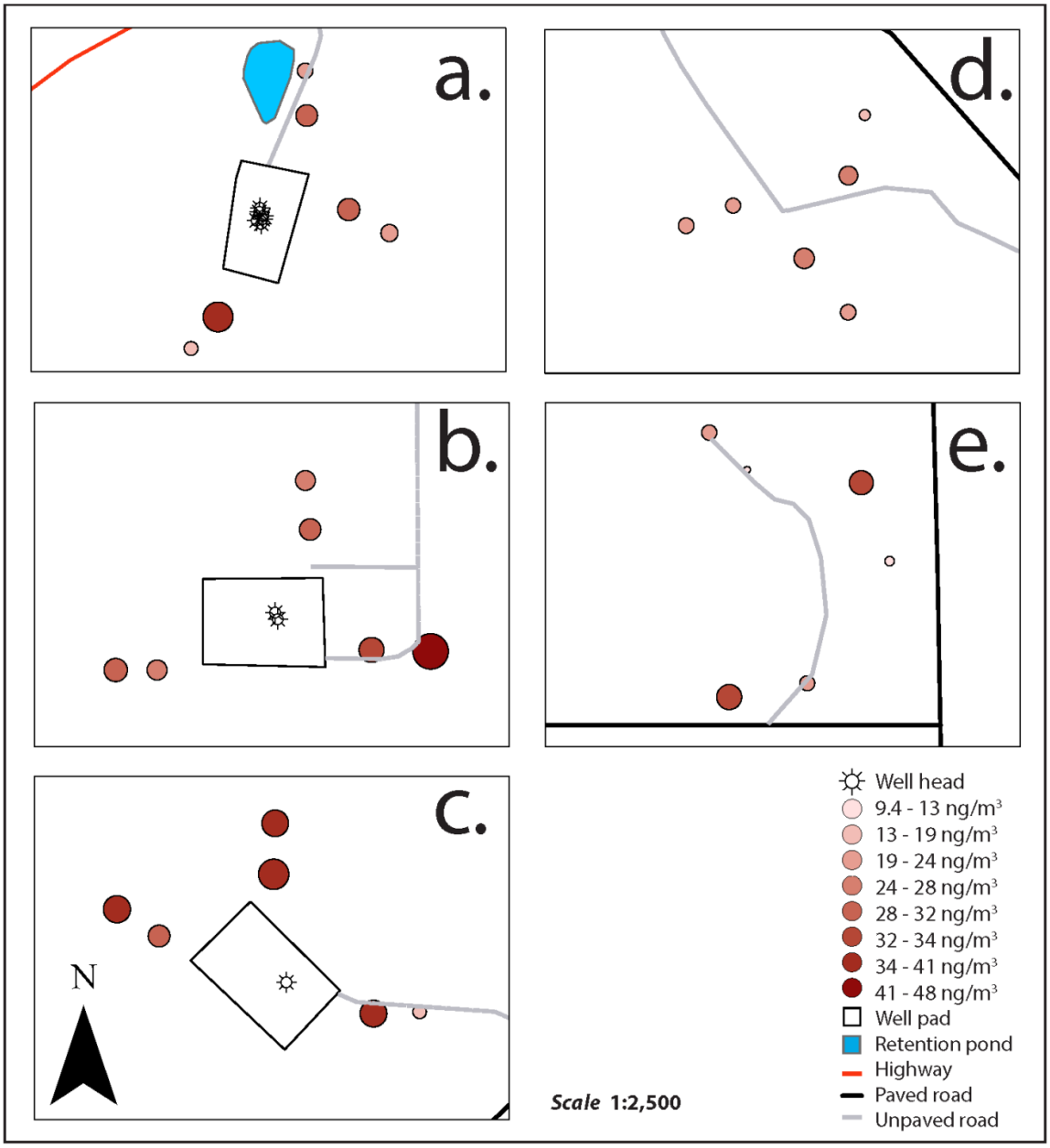

Figure 4.1. Maps of ∑PAH concentrations (ng/m^3) measured in air at the five stationary sampling sites. Each filled circle represents one stationary passive air sampler. Each circle is color-coded and sized based on the ∑PAH concentration (ng/m^3) measured by the air sampler at that location. Sites a-c had active natural gas well pads, represented by black squares.

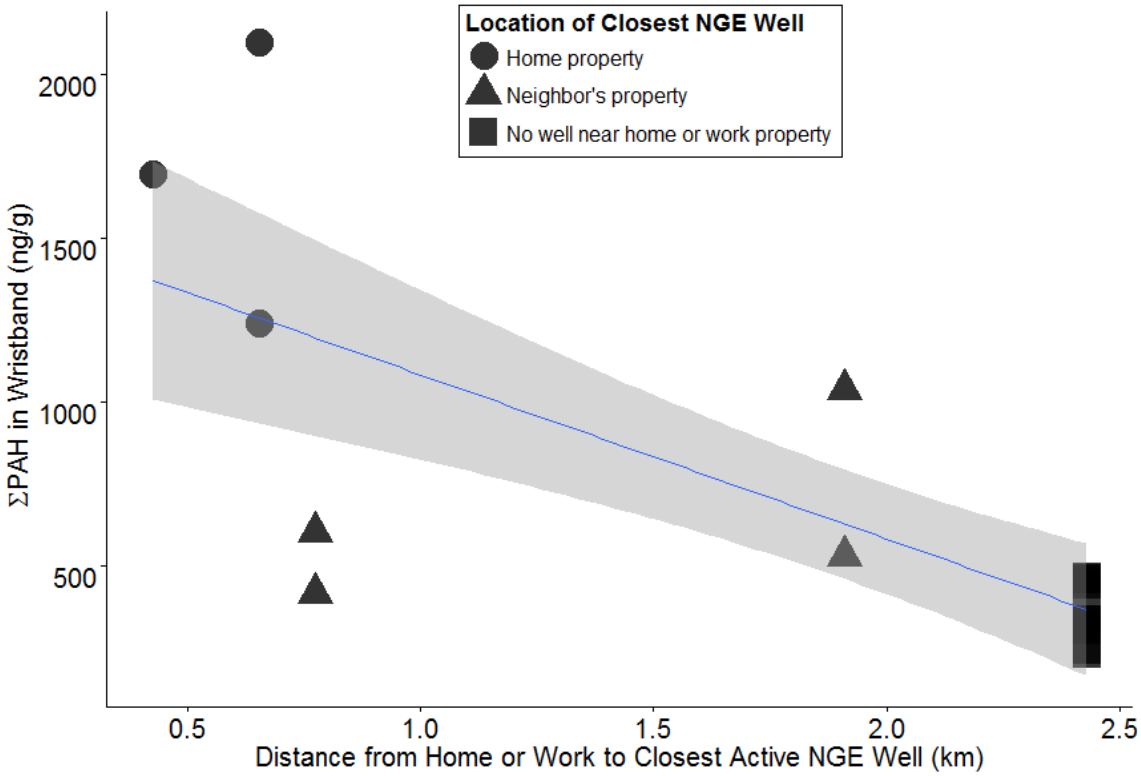

Figure 4.2. ∑PAH in each participant's wristband (ng/g) vs. distance from each participant's home or workplace to the nearest active NGE well (km). There is a significant negative correlation between ∑PAH in a participant's wristband and distance from that participant's home or work to the nearest active NGE well (simple linear regression, p = 0.00015). R-squared = 0.58, correlation coefficient = - 0.76. The shape of the symbol represents whether participants had active NGE wells on their home properties, their neighbor's properties, or neither.

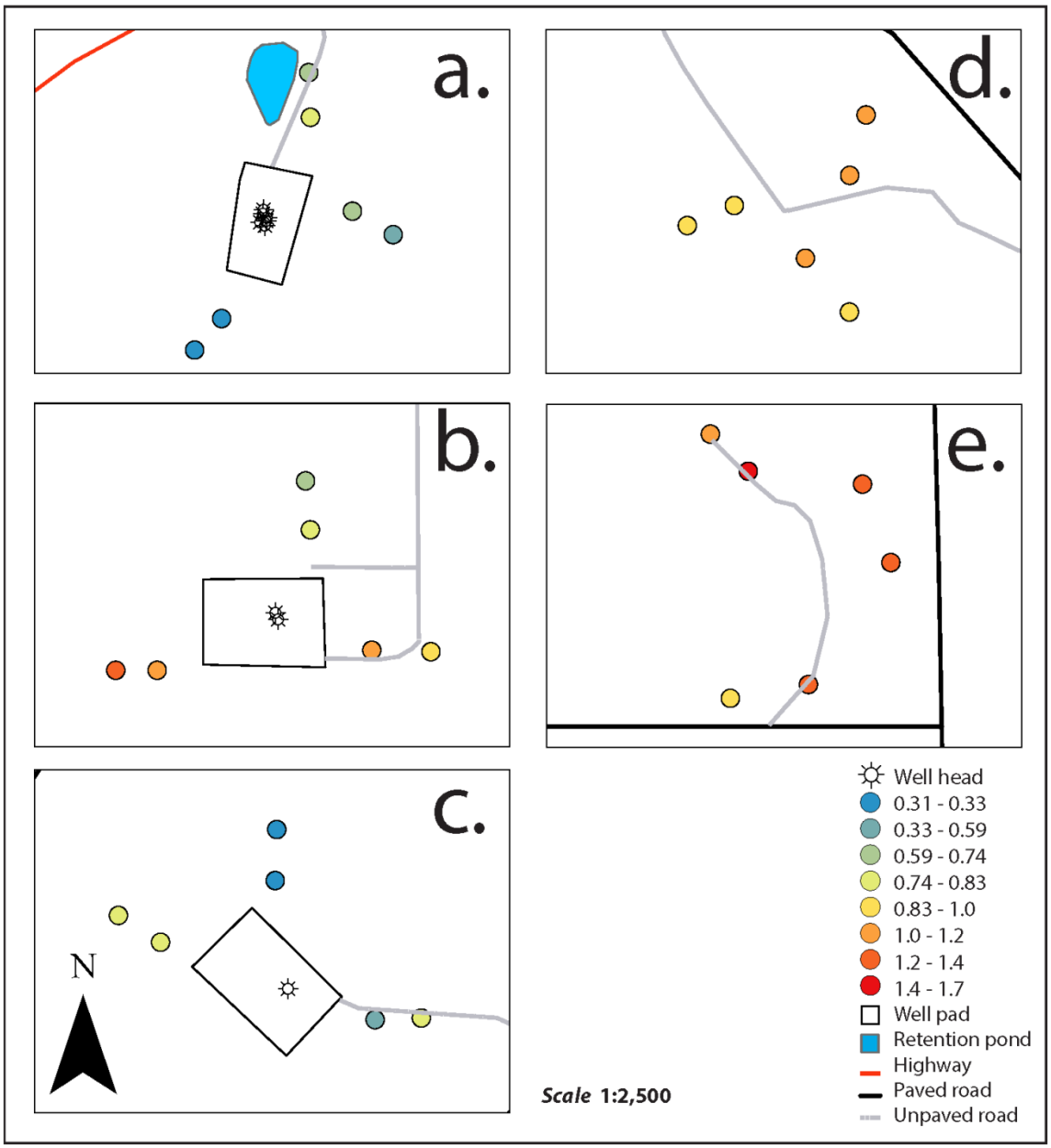

Figure 4.3. Maps of the petrogenic or pyrogenic source signatures of PAH mixtures measured at the five stationary air sampling sites. Each filled circle represents one stationary passive air sampler. Each circle is color-coded based on the source signature of the PAH mixture measured in the air sampler at that location. Source signatures of PAH mixtures are based on the fluoranthene/pyrene ratio. For this ratio, values <1.0 are petrogenic and values >1.0 are pyrogenic. Sites a-c had active natural gas well pads, represented by black squares.

Figure 4.4. ∑PAH in each participant's wristband (ng/g wristband) vs. ∑PAH measured at the nearest stationary air sampling site (ng/m^3), with source signature measured in air. There is a significant positive correlation between ∑PAH in a participant's wristband and ∑PAH in the inner ring of air samplers at the nearest stationary air sampling site (simple linear regression, p = 0.0062). R-squared = 0.33, correlation coefficient = 0.57. Symbols are color-coded based on the source signatures of PAH mixtures in air at the associated stationary air sampling site. Source signatures are based on the fluoranthene/pyrene ratio. For this ratio, values <1.0 are petrogenic and values >1.0 are pyrogenic. Here, values of this ratio of 1.0 ± 0.1 are interpreted as a "mixed" signature and shown in yellow. The shape of the symbol represents whether participants had active NGE wells on their home properties, their neighbor's properties, or neither.

Chapter 5: Conclusions and Future Directions

This dissertation presents considerations for assessing human exposure to mixtures of environmental chemicals, and illustrates these considerations through three studies measuring PAHs in air, water, and crayfish. These studies assess human health risks associated with exposure to PAH mixtures while eating crayfish in the Portland Harbor Superfund Megasite (PHSM) and breathing air near natural gas extraction (NGE) wells. The work presented here advances the fields of exposure science and environmental toxicology by presenting new observations and insights about environmental PAH mixtures and human exposures.

Chapter 2 affirms the ability of passive water samplers to accurately predict PAH concentrations in resident crayfish. This study predicted concentrations of 34 PAHs in crayfish tissues within an average factor of 2.4 of concentrations measured in crayfish tissues, using only PAH concentrations measured by passive water samplers and a simple linear regression model. It is noteworthy that this model did not require any other inputs, as many traditional methods also require inputs estimating other parameters of the system. These additional inputs are often estimates, and thus can increase uncertainty in predicted values. The combination of simplicity and accuracy of the present model further confirms that passive samplers are useful tools to assess PAH contamination in shellfish. Given the simplicity of the model, it would be interesting to explore whether it could be used to predict PAH contamination in other shellfish. It would also be interesting to explore whether this, or a similar model, could predict concentrations of other SVOCs, such as PCBs, in crayfish and in other shellfish. Because PCBs are a major driver of remedial action in the PHSM and in many Superfund sites, this could lead to predictive tools that would be extremely useful to U.S. risk assessors. It would also be interesting to investigate similar predictive approaches for emerging contaminant classes, such as oxygenated PAHs (OPAHs). Additionally, multiple studies have suggested that passive sediment samplers accurately predict SVOC contamination of benthic organisms, such as clams. This would also be a useful relationship to explore in future work.

Chapters 3 and 4 suggest that PAHs are emitted from NGE. This was shown by the increased PAH concentrations measured in air closer to active NGE wells in Chapter 3, and by the increased PAH concentrations in air at sites with active NGE wells in Chapter 4. This was also shown by the predominant petrogenic signature of PAH mixtures measured closer to active NGE wells in Chapters 3 and 4. This signature suggested that PAH mixtures closer to active NGE wells were affected by direct emissions from the earth, rather than exclusively by the myriad combustion sources that would produce pyrogenic signatures. In Chapter 4, the positive correlation between PAH concentrations in participants' personal environments and in air measured near their home or workplaces suggested that living or working closer to an active NGE well increases personal PAH exposure. Overall average ∑PAH concentrations were 3.7-fold higher in air in Chapter 4 than in Chapter 3. The study presented in Chapter 3 was conducted in winter, with an average air temperature of -1°C, while the study presented in Chapter 4 was conducted in spring, with an average air temperature of 20°C. Thus, the elevated PAH concentrations measured in air in Chapter 4 could partly be due to the higher ambient air temperature during the sampling period. The low-density polyethylene passive air samplers used in these studies sequester only PAHs in the vapor phase, and many studies have suggested that vapor phase PAH concentrations decrease with decreasing air temperature. However, it is also important to note that the inner rings of samplers in Chapter 4 were slightly closer to active NGE wells than even the closest samplers from Chapter 3. If the PAHs emitted from an NGE well pad quickly disperse, then these differences in deployment distances could also have affected the observed concentration differences. Thus, further research is needed to continue elucidating the relationship between NGE and PAH emissions. One relevant future direction of NGE-related exposure science research would be to assess the PAH exposures of individuals working directly on NGE well pads. These individuals likely have different exposures than people living or working nearby.

All of the carcinogenic risk assessments presented in this dissertation used the U.S. EPA's 2010 Relative Potency Factors to estimate the carcinogenic potency of

measured PAH mixtures. This approach normalizes the carcinogenic potency of each PAH in a mixture relative to that of benzo[a]pyrene, and then sums these values to yield a benzo[a]pyrene equivalent carcinogenic potency value. While this approach is the state of the science, it is also well-known that the toxicity of a chemical mixture is seldom simply equal to the summed toxicities of its constituents. Thus, future research directly measuring the toxicities of PAH mixtures, and reporting any greater or less than additive toxic responses, would help researchers and regulators more accurately estimate the toxicities of PAH mixtures found in the environment. This type of mixtures research would also be informative for other chemical classes.

This dissertation adds information to discussions surrounding the most effective tools for assessing shellfish contamination, and regulatory and research needs related to the environmental health impacts of NGE. Much work is still needed to give scientists, members of the public, and policymakers enough information to fully minimize risks associated with exposure to environmental pollutants.

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APPENDICES

Appendix 1: Supporting Information to Chapter 2 – Passive Samplers Accurately Predict PAH Levels in Resident Crayfish

Crayfish Morphology

Crayfish collected in 2013 had average carapace lengths of 3.5 ± 0.57 cm, average body lengths of 10 ± 1.4 cm, and average body weights of 31 ± 13 g. Half of the crayfish were female and half were male. On average, viscera contributed 13 ± 4% to the total wet weight of each crayfish, while tails contributed 11± 2%.

Crayfish Tissue Extraction

All tissues were extracted with a 2:2:1 solution of ethyl acetate, acetone and iso-octane, and dried using QuEChERS AOAC salts. Viscera samples were cleaned using flow-through solid-phase extraction (SPE) cartridges containing primary-secondary amines, as described in Forsberg 2014 [25], while tail samples were cleaned with AOAC Fatty Samples dispersive SPE tubes, as described in Forsberg 2011 [122].

Passive Water Sampler Preparation

LDPE strips were cut from pre-sized polyethylene tubing that was approximately 2.7 cm wide. Each polyethylene strip was approximately 100 cm long and had a volume of 5.1 cm^3. LDPE was dried under filtered vacuum in stainless steel kegs, from AEB Kegs in Delebio, Italy. TurboVap® evaporators were from Biotage, in Charlotte, NC.

2013 Passive Water Sampler Deployment Dates

Samplers were deployed at river miles 18.5, 12E, 3.5W and 1NW from September 30th to October 17th, 2013, and at RM 11E from October 17th to November 7th, 2013.

2012 Passive Water Sampler Deployment Information

In 2012, three sampler cages with 5 strips of LDPE each were individually deployed at RM 7E (McCormick & Baxter). They were in the water from Nov 30, 2012 to

Jan 17, 2013. In this sampling campaign, p,p'-DDE-d4 was used as a PRC instead of pyrene-d10.

Chemical Information

Single PAH standards were purchased from Sigma Aldrich, in St. Louis, MO, Chiron, in Trondheim, Norway, or Fluka (part of Sigma-Aldrich). PAH mixes were purchased from Accustandard, in New Haven, CT. Labeled compounds used as performance reference compounds (PRCs), laboratory surrogates, or instrument internal standards were obtained from either CDN Isotopes, in Pointe-Claire, Quebec, Canada, Cambridge Isotope Laboratories, in Tewksbury, MA, or Fisher Scientific in Pittsburgh, PA. All solvents were Optima-grade (from Fisher Scientific, Pittsburgh, PA) or equivalent, and all laboratory glassware and other tools were baked at 450°C for 12 hours and/or solvent-rinsed before use. Water used to clean LDPE was filtered through a D7389 purifier purchased from Barnstead International, in Dubuque, IA.

Chemical Analysis

An Agilent Select PAH column was used to chromatographically separate PAHs. Each PAH was calibrated with a curve of at least five points, with correlations ≥0.99. The temperature profile in the GC-MS/MS analytical method was as follows: 60°C for 1 minute, increasing 40°C per minute to reach 180°C, then increasing 3°C per minute to reach 230°C, then increasing 1.5°C per minute to reach 235°C, then increasing 15°C per minute to reach 280°C, staying at 280°C for 10 minutes, then increasing 6°C per minute to reach 298°C, and finally ramping up 16°C per minute to reach 350°C with a hold time of 4 minutes. The dimensions of the Agilent Select PAH column were: 30 m, 0.25 mm, 0.15 µm. Continuing calibration standards were run nominally every 10 samples, and/or at the end of the sample set. If a closing standard did not meet the criteria, samples were re-run after the standard was verified.

Differences in Processing of Crayfish Collected in 2003

In the 2003 data set, the entire mass of each crayfish viscera was homogenized, extracted and analyzed. Additionally, the viscera tissue from each organism was homogenized, extracted, and analyzed separately. No compositing was done, and no tail tissues were retained for analysis. 2003 crayfish viscera were reanalyzed for 62 PAHs using an Agilent 6890N gas chromatograph coupled with an Agilent 5975B mass spectrometer.

An important site during the 2003 sampling campaign was RM 7E. This is the site of the former McCormick & Baxter creosote company. This site has been under investigation by the Oregon Department of Environmental Quality since 1990, and was added to the U.S. EPA's National Priority List in 1994, before the rest of the Portland Harbor Superfund [128].

2012 PAH Concentrations in Water

Average ∑PAH measured in water (C_{free} calculated from water-deployed LDPE) at RM 7E (McCormick & Baxter) in 2012 was 56 ± 55 ng/L. These data were used in the model to predict PAH levels in crayfish viscera at this site in 2012.

Water Concentration Calculations

Freely dissolved water concentrations (C_{free}) were determined through an empirical uptake model, as described below. Sampling rates were derived by measuring loss of performance reference compounds (PRCs) during deployment. PRCs allow for accurate assessment of *in situ* uptake rates for a wide range of analytes in variable environmental conditions [126, 127, 214]. The uptake calculations do not make any assumptions about the analyte being at equilibrium, so this model was used for water concentration calculations for all PAHs. PRCs shared similar physical and chemical properties with the target PAHs in this study and spanned a range of log K_{ow} values from 4.18 to 5.78 [127]. Water concentrations (C_w) of PAHs were determined using equations S1-S6, all presented in Huckins *et al* [21]:

Sampler-water partitioning coefficients (K_{sw}) were calculated for both PRCs and target PAHs using this quadratic equation:

$$\boldsymbol{Eq\ A1.1.}\ logK_{SW} = a_0 + (2.321 * logK_{OW}) - \left(0.1618 * logK_{OW}{}^2\right)$$

The a_0 term was determined by Huckins *et al* to be equal to -2.61 for PAHs and other similarly nonpolar compounds. To determine PRC sampling rates, a depuration rate (k_e) was needed. The following equation was used to calculate k_e, assuming first-order kinetics:

$$\boldsymbol{Eq\ A1.2.}\ k_e = \frac{-ln\left(\frac{PRC_t}{PRC_i}\right)}{t}$$

PRC_t is the amount of PRC remaining after a deployment period (t), and PRC_i is the initial amount spiked into the LDPE. Each PRC's sampling rate (R_{sPRC}) was calculated using:

$$\boldsymbol{Eq\ A1.3.}\ R_{sPRC} = k_e * K_{sw} * V_s$$

V_s is the volume of the LDPE sampler. Sampling rates for target analytes (R_s) were determined using:

$$\boldsymbol{Eq\ SA1.4.}\ R_s = R_{sPRC} * \frac{\alpha_{analyte}}{\alpha_{PRC}}$$

The α terms are compound-specific adjustments made to account for differing chemistry between the PRC and the target analyte. This model is a best-fit polynomial, which gives α values for target analytes and PRCs, based on $logK_{ow}$. These α terms were calculated using:

$$\boldsymbol{Eq\ A1.5.}\ log\ \alpha = \left(0.013 * logK_{ow}{}^3\right) - \left(0.3173 * logK_{ow}{}^2\right) + (2.244 * logK_{ow})$$

Compound uptake during deployment was not assumed to be in any particular phase (kinetic, linear, or equilibrium), and no assumptions are necessary. C_w for target analytes were calculated using:

$$\boldsymbol{Eq\ A1.6.}\ C_w = \frac{N_{analyte}}{V_s K_{sw}\left(1 - \exp\left(-\frac{R_s t}{V_s K_{sw}}\right)\right)}$$

$N_{analyte}$ is the concentration of analyte measured in LDPE, and t is the length of the deployment in days.

Quantitative Risk Assessment Calculations

Risk assessment was performed using equations S7-S9. The benzo[a]pyrene equivalent concentration (BaP_{eq}) was used in these calculations. This was determined using:

$$\boldsymbol{Eq\ A1.7.}\ \sum BaP_{eq} = \sum(C_i * RPF_i)$$

C_i is the concentration of a given PAH in a crayfish sample, and RPF_i is the EPA's Relative Potency Factor of that PAH [59]. Average daily dose (ADD) was calculated using:

$$\boldsymbol{Eq\ A1.8.}\ ADD = \frac{\sum BaP_{eq} * CF * IR * EF * ED}{BW * AT}$$

ADD is the average daily dose, in mg/kg-day, C is the concentration in crayfish in ng/g, CF is a conversion factor, EF is the exposure frequency in days/year, ED is the exposure duration in years, BW is body weight in kg, and AT is the averaging time. The AT includes the lifetime in years multiplied by 365 days/year. In this work, $\sum BaP_{eq}$ for a given sample was used as the concentration in crayfish tissue. The IR was set at both 3.3 and 18 g/day, which are the average and 95th percentiles for crayfish consumption that were used in ATSDR's Portland Harbor Public Health Assessment to evaluate risks to local crayfish consumers [117]. To mimic what was done in the ATSDR Public Health Assessment for Portland Harbor, average adult body weight was set at 70 kg, average lifetime (used in the AT) was set at 70 years, EF was set at 365 days/year and ED was set at 30 years [117].

$$\boldsymbol{Eq\ A1.9.}\ ELCR = ADD * SF$$

ELCR is an estimate of excess lifetime cancer risk and SF is an oral slope factor. In this study, a SF of 7.3 mg/kg-d was used, based on the EPA's 2010 guidance [59].

There were 11 PAHs that were above the detection limits and had nonzero relative potency factors (RPFs) in crayfish (see **Table A1.4** for RPFs). Thus, these were the 11 PAHs used in the carcinogenic risk assessment. These PAHs were benzo[c]fluorene, benzo[b]fluoranthene, fluoranthene, benzo[a]pyrene, benzo[a]anthracene, chrysene, benzo[j]fluoranthene, indeno[1,2,3-c,d]pyrene, benzo[k]fluoranthene, anthanthrene, and benzo[g,h,i]perylene.

Assumptions in this risk assessment that may bias risk estimates high include assuming 1) exposure occurs every day for 30 years 2) 100% of crayfish eaten are from the stretch of river discussed in this study, 3) ingestion rates are accurate, and do not change over time, 4) PAHs are 100% bioavailable via ingestion, 5) and the cancer slope factor from high-dose animal data are predictive of low-dose effects in the general population However, these parameters were chosen to mimic assessment done in ATSDR's 2006 PHA [23]. Due to the dearth of data regarding the toxicity of the majority of PAHs, especially of alkylated PAHs, there is inherent uncertainty in PAH risk assessment. This risk assessment was conducted for adults, with no adjustments being made for the different exposures of children.

Heterogeneity in Crayfish PAH Levels: 2003 vs. 2013

The 2003 RM averages span two orders of magnitude, while the 2013 RM averages only differ by a factor of four (**Figure A1.6**). The comparatively small variability in PAH levels in crayfish seen in 2013 is partly explained by 2003 crayfish data representing averages of contaminants measured in individual organisms (n=3 at all sites except RM 7E, where n=7), while data for 2013 crayfish represent averages of 3 composites of tissue from 4 crayfish.

Heterogeneity is to be expected among PAH levels in crayfish. Indeed, reduced variability is one of the main selling points for the use of PSDs to estimate organismal concentrations in lieu of collecting and analyzing the organisms themselves [23, 25]. While their home range is relatively small, crayfish are still mobile organisms. Additionally, many of the known point sources in the Superfund sites in this study include sediment

contamination, which is notoriously heterogeneous. If crayfish are feeding on detritus near contaminated sediment, they are likely exposed to a wide range of contamination levels [121]. Crayfish are opportunistic omnivores, so their range of diets could further exaggerate the variation in their internal contaminant levels. Forsberg *et al* discussed this phenomenon, reasoning that the substantial variation measured in crayfish collected near the McCormick & Baxter site was likely related to the crayfish being exposed to heterogeneous sediment contamination resulting from the creosoting operations that took place on the nearby shore [25]. This is consistent with the data in the present study, in which crayfish collected at McCormick and Baxter in 2003 have by far the greatest variability, with one crayfish having ∑PAH levels 2 orders of magnitude greater than the rest, making the average for this site three times larger than the median (**Figure A1.6**). Fernandez and Gschwend made a similar point, suggesting that the atypically heterogeneous PAH levels in clams collected at one site were due to coal tar contamination in the sediment [26]. Levengood *et al* observed large variability in PAH levels measured in crayfish at a site with elevated sediment contamination, and suggested that this was partly due to the spatial heterogeneity of sediment contamination [121].

PAH Profiling: 2,6-dimethylnaphthalene Discussion

Specifically, 2,6-DMN contributed 49 to 85% to ∑PAH in crayfish viscera and 55-95% to ∑PAH in crayfish tails, while it only contributed 1.9 to 5.7% to ∑PAH in water. In a previous study comparing co-deployed SPMDs and Asiatic clams, *Corbicula fluminea*, 2,6-DMN was one of only three PAHs measured in the clams, while 24 PAHs were measured in SPMDs [215]. Thus, it is possible that shellfish preferentially accumulate 2,6-DMN relative to other PAHs. Additionally, Eisler reported that the BCF for dimethylnaphthalenes in crustaceans was two orders of magnitude higher than the BCF for naphthalene in clams, and one order of magnitude higher than the BCF for naphthalene in crustaceans [216]. This could begin to explain why 2,6-DMN was an order of magnitude higher in crayfish tissues than the rest of the PAHs in the present work, but more investigation is needed. For instance, Eisler's BCF does not explain why this

heightened bioconcentration would occur for 2,6-DMN, and not with any of the six other dimethylnaphthalenes present in the analytical method.

The dominance of 2,6-DMN in 2013 crayfish tissue is at odds with the positive correlation between BAF and $\log K_{ow}$ that has been observed in mussels [23]. This disparity could be due in part to differential PAH uptake and/or metabolism in mussels and crayfish. This idea is supported by the significant negative correlation between BAF and $\log K_{ow}$ that has been observed in crayfish [121]. PAH accumulation differ between bivalves and crustaceans, predominantly due to crustaceans' more mobile lifestyles and increased capacity for PAH metabolism [217]. Studies have suggested that crayfish metabolize xenobiotics through oxidative metabolism with P450s [120, 132, 218]. Perhaps the increased oxidative metabolism of xenobiotics in crayfish relative to bivalves reduces crayfish' load of higher molecular weight PAHs, but enriches crayfish tissues in 2,6-DMN. Additionally, a negative correlation has been observed between PAH uptake rate (from water into *P. leniusculus*) and $\log K_{ow}$. This begins to explain the heightened uptake of PAHs with lower $\log K_{ow}$s in crayfish, in both viscera and tail tissue [133], However, it is still unclear why this effect was so dramatic with 2,6-DMN specifically,

It is worth noting that this phenomenon was much less dramatic in 2003 crayfish tissue, in which 2,6-DMN was not measured in all samples, and its contribution to ∑PAH ranged from 0 to 30%.

Linear Range of the Predictive Model

The predictive model was built using individual PAH concentrations from the 2013 sampling campaign. Measured individual PAH concentrations used to build the model ranged from 0.01 to 5.7 ng/L in water and from 0.11 to 18 ng/g in crayfish viscera.

Validation of Predictive Model with Training Set and Test Set

A training set (80% of the paired PAH data measured in crayfish viscera and in water in the 2013 sampling campaign) was used to build a model to validate the main

predictive model. The equation of the line of best fit in the model built by this training set was: $[PAH]_{crayfish} = 0.89 \times [PAH]_{water} + 0.57$. This model is very similar to the main model, which was built using all of the data, and which is presented as Equation 1 in the main text. Additionally, the test set (the remaining 20% of the data) was used to compare PAH concentrations predicted by the training model, to what was actually measured in crayfish tissues (**Figure A1.5**). Root mean squared errors (RMSE) were computed to assess the models, by taking the square root of the average of the squared residuals. RMSE values were computed to compare how well the full model and the training model predicted concentrations for the test set, relative to what was measured in crayfish. These root mean squared errors were very similar for the full model and the training model (0.222 and 0.226, respectively).

Risk Assessment: EPA 2010 vs. EPA 16 Priority Pollutants

There are 23 PAHs with EPA 2010 RPFs included in the present analytical method, while only 11 of the EPA Priority Pollutants have RPFs. When all 23 of these PAHs are included, average $\sum BaP_{eq}$ doubles for crayfish collected at RM 7E and in the PHSM in 2003. For 2013 crayfish collected within the PHSM, average $\sum BaP_{eq}$ increases by 45% when the additional PAHs are included. This trend did not hold true for crayfish viscera collected upriver of the Superfunds in both 2003 and 2013, or for crayfish tails. $\sum BaP_{eq}$ was not affected by the additional PAHs in crayfish viscera collected upstream of the Superfunds (no change in 2003; 3.7% increase in 2013). Additionally, including the longer PAH list did not change $\sum BaP_{eq}$ for crayfish tails. This is explained by the fact that the only 2 PAHs contributing to $\sum BaP_{eq}$ (fluoranthene and benzo[ghi]perylene) in tails are both on the 16 PP list.

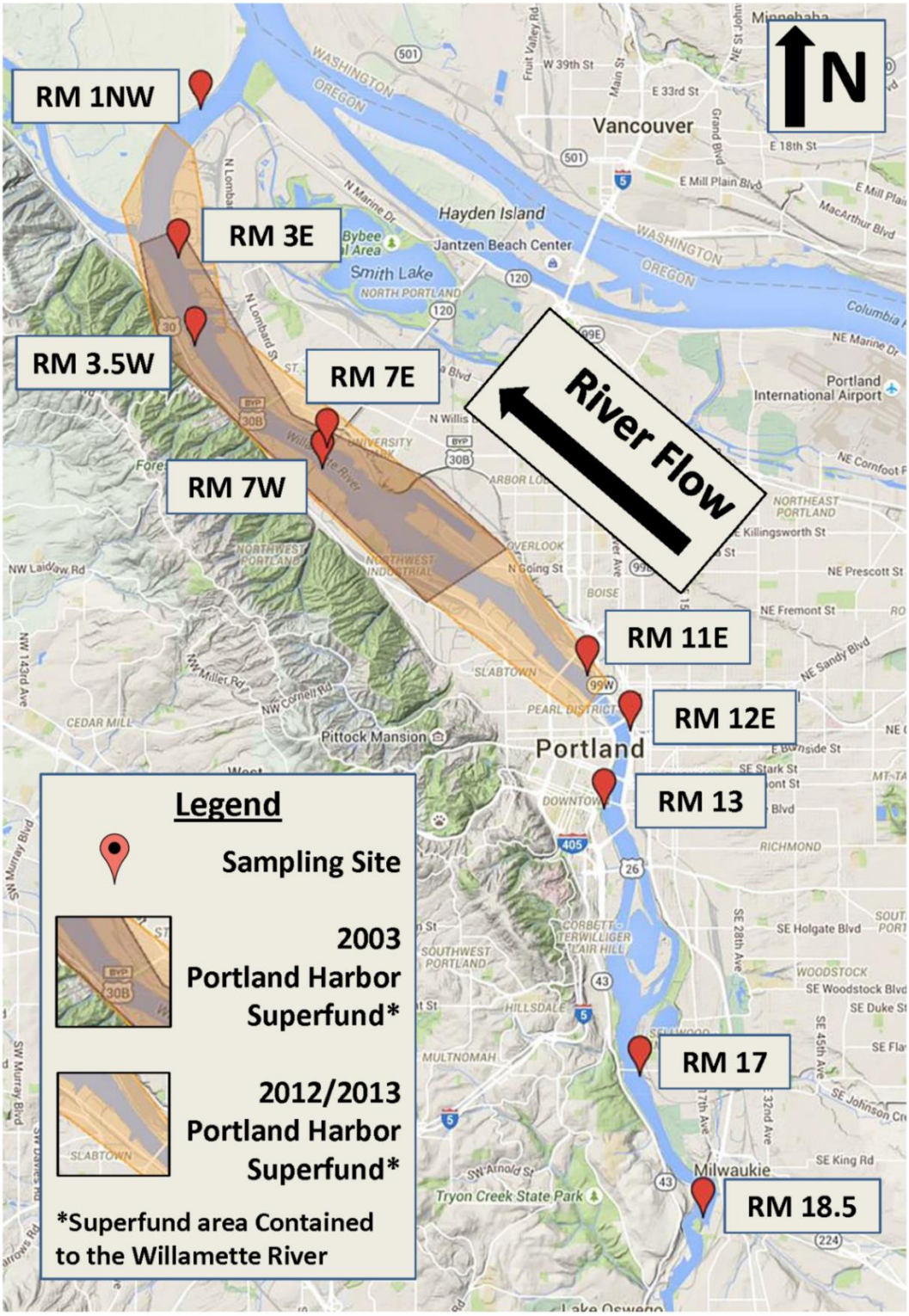

Figure A1.1 Map of crayfish and water sampling sites. This map depicts the Portland Harbor study area in 2003, 2012, and 2013, and all sampling sites referenced in the present work.

Table A1.1. GPS coordinates for Portland Harbor sampling sites. Coordinates are given for locations of crayfish collection and LDPE deployment presented in this study from a. 2013 and b. 2003. Deployment of LDPE at RM 7E in 2012 was at approximately the same location as crayfish collection at that site in 2003.

a. 2013

Approximate River Mile (used as sampling site identifier)	Latitude	Longitude
RM 18.5	45° 26' 14.88"N	122° 38' 49.05"W
RM 12E	45° 31' 34.87"N	122° 39' 57.88"W
RM 11E	45° 32' 11.50"N	122° 40' 37.74"W
RM 3.5W	45° 35' 51.59"N	122° 46' 51.70"W
RM 1NW	45° 38' 30.24"N	122° 46' 46.35"W

b. 2003

Approximate River Mile (used as sampling site identifier)	Latitude	Longitude
RM 17	45° 27' 47.67"N	122° 39' 49.72"W
RM 13	45° 30' 43.21"N	122° 40' 21.70"W
RM 7W	45° 34' 28.96"N	122° 44' 48.94"W
RM 7E	45° 34' 43.05"N	122° 44' 45.03"W
RM 3E	45° 36' 50.38"N	122° 47' 7.55"W

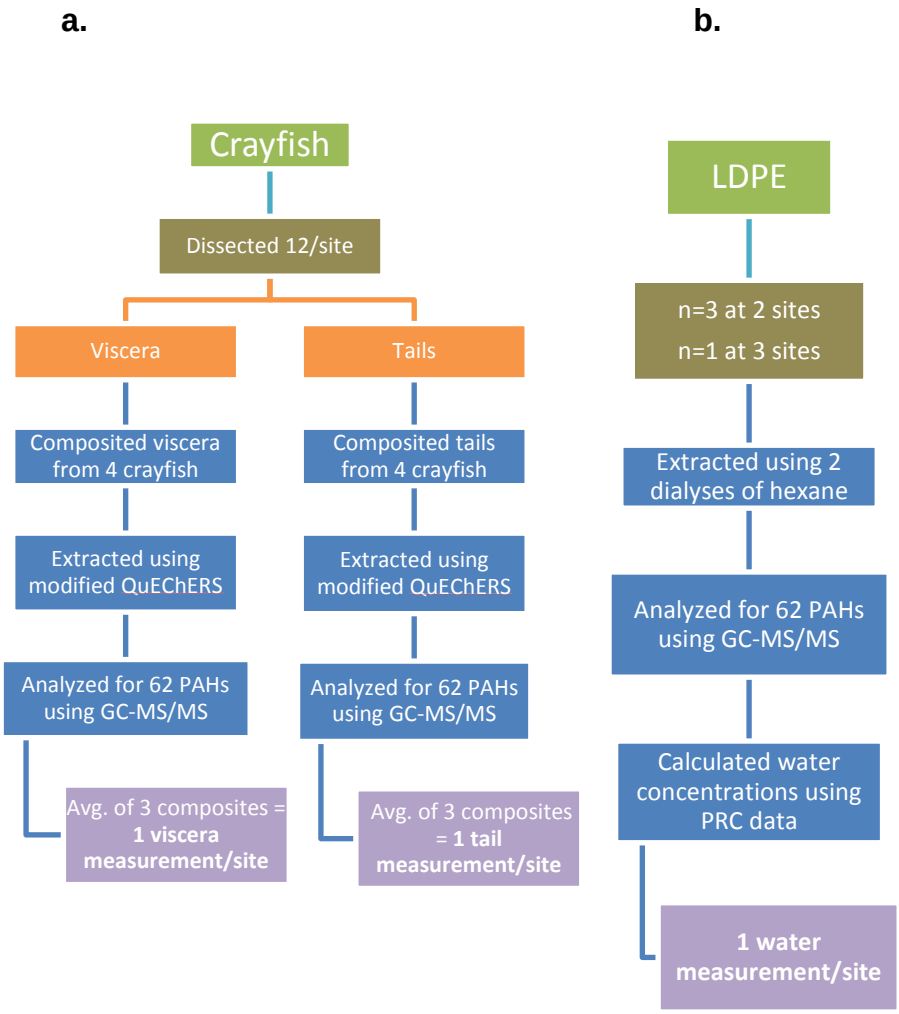

Figure A1.2. Conceptual diagram of in-lab sample processing of crayfish and passive water samplers. Diagram shows processing steps for **a.** crayfish and **b.** LDPE passive water samplers.

Table A1.2. List of QC and target PAHs in GC-MS/MS method. Performance reference compounds (PRCs), internal standard (IS), surrogates, and target polycyclic aromatic hydrocarbons (PAH) are given for the GC-MS Triple Quad method used for PAH analysis in this study, with instrument limits of detection (LOD) and limits of quantitation (LOQ).

PAH	Category	CAS #	LOD (ng/mL)	LOQ (ng/mL)
Fluorene-d10	PRC	81103-79-9	0.33	1.0
Pyrene-d10	PRC	1718-52-1	0.42	2.1
Benzo[b]fluoranthene-d12	PRC	205-99-2	1.7	5.0
Perylene-d12	IS	1520-96-3	1.7	N/A
Naphthalene-d8	Surrogate	1146-65-2	0.33	1.0
Acenaphthylene-d8	Surrogate	93951-97-4	0.33	1.0
Phenanthrene-d10	Surrogate	1517-22-2	1.7	5.0
Fluoranthene-d10	Surrogate	93951-69-0	1.7	5.0
Chrysene-d12	Surrogate	1719-03-5	1.7	5.0
Benzo[a]pyrene-d12	Surrogate	63466-71-7	1.7	5.0
Benzo[ghi]perylene-d12	Surrogate	93951-66-7	1.7	5.0
Naphthalene	Target	91-20-3	1.0	5.2
2-Methylnaphthalene	Target	91-57-6	0.70	3.5
1-Methylnaphthalene	Target	90-12-0	0.28	1.4
2-Ethylnaphthalene	Target	939-27-5	0.97	4.8
2,6-Dimethylnaphthalene	Target	581-42-0	0.89	4.4
1,6-Dimethylnaphthalene	Target	575-43-9	0.81	4.1
1,4-Dimethylnaphthalene	Target	571-58-4	1.2	6.2
1,5-Dimethylnaphthalene	Target	571-61-9	1.2	5.9
1,2-Dimethylnaphthalene	Target	573-98-8	0.94	4.7
1,8-Dimethylnaphthalene	Target	569-41-5	0.83	4.2
2,6-Diethylnaphthalene	Target	59919-41-4	0.81	4.1
Acenaphthylene	Target	208-96-8	2.3	12
Acenaphthene	Target	83-32-9	1.1	5.4
Fluorene	Target	86-73-7	0.79	4.0
Dibenzothiophene	Target	132-65-0	0.24	1.2
Phenanthrene	Target	85-01-8	0.46	2.3
Anthracene	Target	120-12-7	1.1	5.2
2-Methylphenanthrene	Target	2531-84-2	0.39	1.9
2-Methylanthracene	Target	613-12-7	0.47	2.4
1-Methylphenanthrene	Target	832-69-9	1.1	5.3
9-Methylanthracene	Target	779-02-2	0.87	4.4
3,6-Dimethylphenanthrene	Target	1576-67-6	0.42	2.1
2,3-Dimethylanthracene	Target	613-06-9	0.34	1.7
Fluoranthene	Target	206-44-0	0.54	2.7

Table A1.2., continued.

9,10-Dimethylanthracene	Target	781-43-1	0.85	4.2
Pyrene	Target	129-00-0	0.42	2.1
Retene	Target	483-65-8	0.84	4.2
Benzo[a]fluorene	Target	238-84-6	1.7	5.0
Benzo[b]fluorene	Target	243-17-4	1.7	5.0
Benzo[c]fluorene	Target	205-12-9	0.30	1.5
1-Methylpyrene	Target	2381-21-7	0.38	1.9
Benz[a]anthracene	Target	56-55-3	0.75	3.8
Cyclopenta[c,d]pyrene	Target	27208-37-3	0.53	2.7
Triphenylene	Target	217-59-4	0.41	2.0
Chrysene	Target	218-01-9	0.50	2.5
6-Methylchrysene	Target	1705-85-7	0.89	4.4
5-Methylchrysene	Target	3697-24-3	1.7	5.0
Benzo[b]fluoranthene	Target	205-99-2	0.37	1.9
7,12-Dimethylbenz[a]anthracene	Target	57-97-6	0.94	4.7
Benzo[k]fluoranthene	Target	207-08-9	0.53	2.6
Benzo[j]fluoranthene	Target	205-82-3	0.56	2.8
Benz[j]&[e]aceanthrylene	Target	202-33-5 and 199-54-2	1.7	5.0
Benzo[e]pyrene	Target	192-97-2	0.71	3.5
Benzo[a]pyrene	Target	50-32-8	1.2	5.9
Indeno[1,2,3-c,d]pyrene	Target	193-39-5	0.26	1.3
Dibenzo[a,h]pyrene	Target	53-70-3	1.0	5.1
Picene	Target	213-46-7	0.74	3.7
Benzo[ghi]perylene	Target	191-24-2	0.34	1.7
Anthanthrene	Target	191-26-4	0.33	1.7
Naphtho[1,2-b]fluoranthene	Target	5385-22-8	1.7	5.0
Naphtho[2,3-j]fluoranthene	Target	205-83-4	1.7	5.0
Dibenzo[a,e]fluoranthene	Target	5385-75-1	0.47	2.4
Dibenzo[a,l]pyrene	Target	191-30-0	0.48	2.4
Naphtho[2,3-k]fluoranthene	Target	207-18-1	1.7	5.0
Naphtho[2,3-e]pyrene	Target	193-09-9	1.7	5.0
Dibenzo[a,e]pyrene	Target	192-65-4	6.4	32
Coronene	Target	191-07-1	0.7	3.5
Dibenzo[e,l]pyrene	Target	192-51-8	1.7	5.0
Naphtho[2,3-a]pyrene	Target	196-42-9	1.7	5.0
Benzo[b]perylene	Target	197-70-6	1.7	5.0
Dibenzo[a,i]pyrene	Target	189-55-9	1.4	7.1
Dibenz[a,h]anthracene	Target	189-64-0	0.52	2.6

Table A1.3. List of PAH detection limits in samples. LODs for PAHs measured in water, crayfish tails, and crayfish viscera collected in 2003 and 2013. All tissue concentrations are in ng/g wet weight.

PAH	Water (ng/L)	Crayfish Viscera 2013 (ng/g)	Crayfish Tails 2013 (ng/g)	Crayfish Viscera 2003 (ng/g)
Naphthalene	0.03	0.12	1.0	0.15
2-Methylnaphthalene	0.01	0.08	0.7	0.15
1-Methylnaphthalene	0.002	0.03	0.28	0.15
2-Ethylnaphthalene	0.002	0.11	0.97	0.15
2,6-Dimethylnaphthalene	0.002	0.10	0.89	0.15
1,6-Dimethylnaphthalene	0.002	0.09	0.81	0.15
1,4-Dimethylnaphthalene	0.003	0.14	1.2	0.15
1,5 Dimethylnaphthalene	0.003	0.13	1.2	0.15
1,2-Dimethylnaphthalene	0.003	0.10	0.94	0.15
1,8-Dimethylnaphthalene	0.003	0.09	0.83	0.15
2,6-Diethylnaphthalene	0.002	0.09	0.81	0.15
Acenaphthylene	0.01	0.26	2.3	0.15
Acenaphthene	0.007	0.12	1.1	0.15
Fluorene	0.003	0.09	0.79	0.15
Dibenzothiophene	0.001	0.03	0.24	0.15
Phenanthrene	0.001	0.05	0.46	0.78
Anthracene	0.002	0.12	1.1	0.78
2-Methylphenanthrene	0.001	0.04	0.39	0.78
2-Methylanthracene	0.001	0.05	0.47	0.78
1-Methylphenanthrene	0.001	0.12	1.1	0.78
9-Methylanthracene	0.001	0.10	0.87	0.78
3,6-Dimethylphenanthrene	0.0004	0.05	0.42	0.78
Fluoranthene	0.001	0.06	0.54	0.78
2,3-Dimethylanthracene	0.0003	0.04	0.34	0.78
9,10-Dimethylanthracene	0.001	0.09	0.85	0.78
Pyrene	0.001	0.05	0.42	0.78
Retene	0.001	0.09	0.84	0.78
Benzo[a]fluorene	0.002	0.19	1.7	0.78
Benzo[b]flourene	0.002	0.19	1.7	0.78
Benzo[c]fluorene	0.0004	0.03	0.30	0.78

Table A1.3., continued.

1-Methylpyrene	0.0004	0.04	0.38	0.78
Benz[a]anthracene	0.001	0.08	0.75	0.78
Cyclopenta[c,d]pyrene	0.001	0.06	0.53	0.78
Triphenylene	0.0004	0.05	0.41	0.78
Chrysene	0.0005	0.06	0.50	0.78
6-Methylchrysene	0.001	0.10	0.89	0.78
5-Methylchrysene	0.002	0.19	1.7	0.78
Benzo[b]fluoranthene	0.0004	0.04	0.37	0.78
7,12-Dimethylbenz[a]anthracene	0.001	0.10	0.94	0.78
Benzo[k]fluoranthene	0.001	0.06	0.53	0.78
Benzo[j]fluoranthene	0.001	0.06	0.56	0.78
Benz[j]+[e]aceanthrylene	NA	0.19	1.7	0.78
Benzo[e]pyrene	0.001	0.08	0.71	0.78
Benzo[a]pyrene	0.001	0.13	1.2	0.78
Indeno[1,2,3-c,d] pyrene	0.0003	0.03	0.26	0.78
Dibenz[a,h]anthracene	0.001	0.11	1.0	0.78
Picene	0.001	0.08	0.74	0.78
Benzo[ghi]perylene	0.0004	0.04	0.34	0.78
Anthanthrene	0.001	0.04	0.33	0.78
Naptho[1,2-b]fluoranthene	0.003	0.19	1.7	0.78
Naptho[2,3-j]fluoranthene	0.003	0.19	1.7	0.78
Dibenzo[a,e]fluoroanthene	0.001	0.05	0.47	0.78
Dibenzo[a,l]pyrene	0.001	0.05	0.48	0.78
Naptho[2,3-k]fluoranthene	0.003	0.19	1.7	0.78
Naptho[2,3-e]pyrene	0.003	0.19	1.7	0.78
Dibenzo[a,e]pyrene	0.01	0.72	6.4	0.78
Coronene	0.002	0.08	0.7	0.78
Dibenzo[e,l]pyrene	0.002	0.19	1.7	0.78
Naptho[2,3-a]pyrene	0.003	0.19	1.7	0.78
Benzo[b]perylene	0.003	0.19	1.7	0.78
Dibenzo[a,i]pyrene	0.003	0.16	1.4	0.78
Dibenzo[a,h]pyrene	0.001	0.06	0.52	0.78

Table A1.4. List of EPA Relative Potency Factors (RPFs), or "PAHs with final RPFs based on tumor bioassay data," from the U.S. EPA's 2010 Development of a Relative Potency Factor (RPF) Approach for Polycyclic Aromatic Hydrocarbon (PAH) Mixtures.[59]

PAH	Relative Potency Factor	Relative Confidence Level
Anthanthrene	0.4	Medium
Anthracene	0	Medium
Benz[a]anthracene	0.2	Medium
Benz[b,c]aceanthrylene	0.05	Low
Benzo[b]fluoranthene	0.8	High
Benzo[c]fluorene	20	Medium
Benz[e]aceanthrylene	0.8	Low
Benzo[g,h,i]perylene	0.009	Low
Benz[j]aceanthrylene	60	Low
Benzo[j]fluoranthene	0.3	High
Benzo[k]fluoranthene	0.03	Medium
Benz[l]aceanthrylene	5	Low
Chrysene	0.1	High
Cyclopenta[c,d]pyrene	0.4	Medium
Cyclopenta[d,e,f]chrysene	0.3	Low
Dibenzo[a,e]fluoranthene	0.9	Low
Dibenzo[a,e]pyrene	0.4	Low
Dibenz[a,h]anthracene	10	High
Dibenzo[a,h]pyrene	0.9	Low
Dibenzo[a,i]pyrene	0.6	Low
Dibenzo[a,l]pyrene	30	Medium
Fluoranthene	0.08	Low
Indeno[1,2,3-c,d]pyrene	0.07	Low
Naphtho[2,3-e]pyrene	0.3	Low
Phenanthrene	0	High
Pyrene	0	Medium
Benzo[a]pyrene	1	N/A

Table A1.5. Average ∑PAH measured in crayfish viscera, tails, and water (using water-deployed LDPE passive samplers), in the Willamette River in Portland, Oregon in 2013 **(a.)** and 2003 **(b.)**. All tissue concentrations are in ng/g wet weight.

a.

2013					
Sample Type	**Average ∑PAH ± Standard Deviation (Sample Size)**				
	RM 18.5	**RM 12E**	**RM 11E**	**RM 3.5W**	**RM 1NW**
Crayfish Viscera (ng/g)	250 ± 51 (n=3)	290 ± 44 (n=3)	110 ± 38 (n=3)	210 ± 100 (n=3)	390 ± 90 (n=3)
Crayfish Tails (ng/g)	37 ± 3.0 (n=3)	38 ± 14 (n=3)	20 ± 7.3 (n=3)	26 ± 3.6 (n=3)	18 ± 2.0 (n=3)
Water (ng/L)	8.4 ± 1.3 (n=3)	4.2 ± 0.76* (n=1)	3.8 ± 0.68* (n=1)	20 ± 4.0 (n=3)	15 ± 2.7* (n=1)

b.

2003					
Sample Type	**Average ∑PAH ± Standard Deviation (Sample Size)**				
	RM 17	**RM 13**	**RM 7W**	**RM 7E**	**RM 3E**
Crayfish Viscera (ng/g)	31 ± 14 (n=3)	80 ± 35 (n=3)	270 ± 306 (n=3)	2700 ± 5000 (n=7)	360 ± 350 (n=3)

* Where n=1, standard deviations were calculated using the average relative standard deviation calculated at sites where sampling was replicated (n=3) in that sampling campaign.

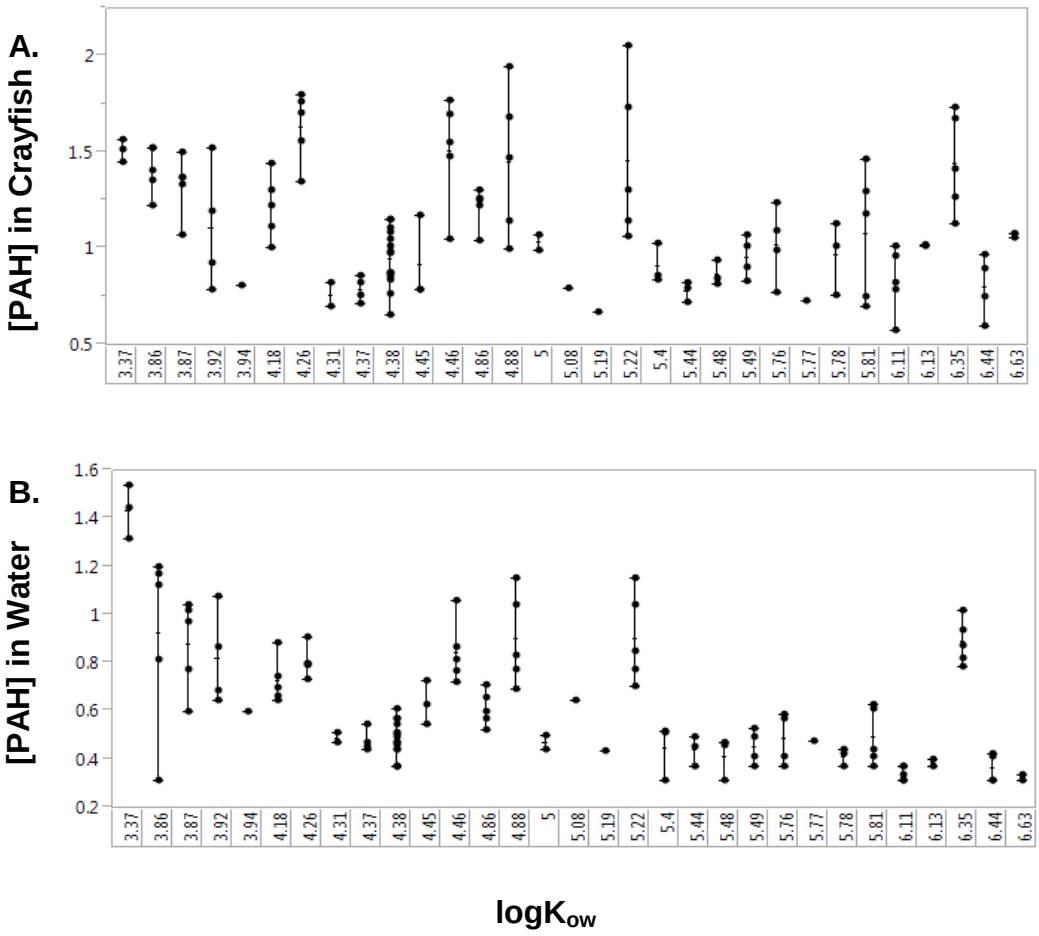

Figure A1.3. Profiles of PAHs measured in A. crayfish viscera and B. water, organized by logK$_{ow}$, from the 2013 sampling campaign.

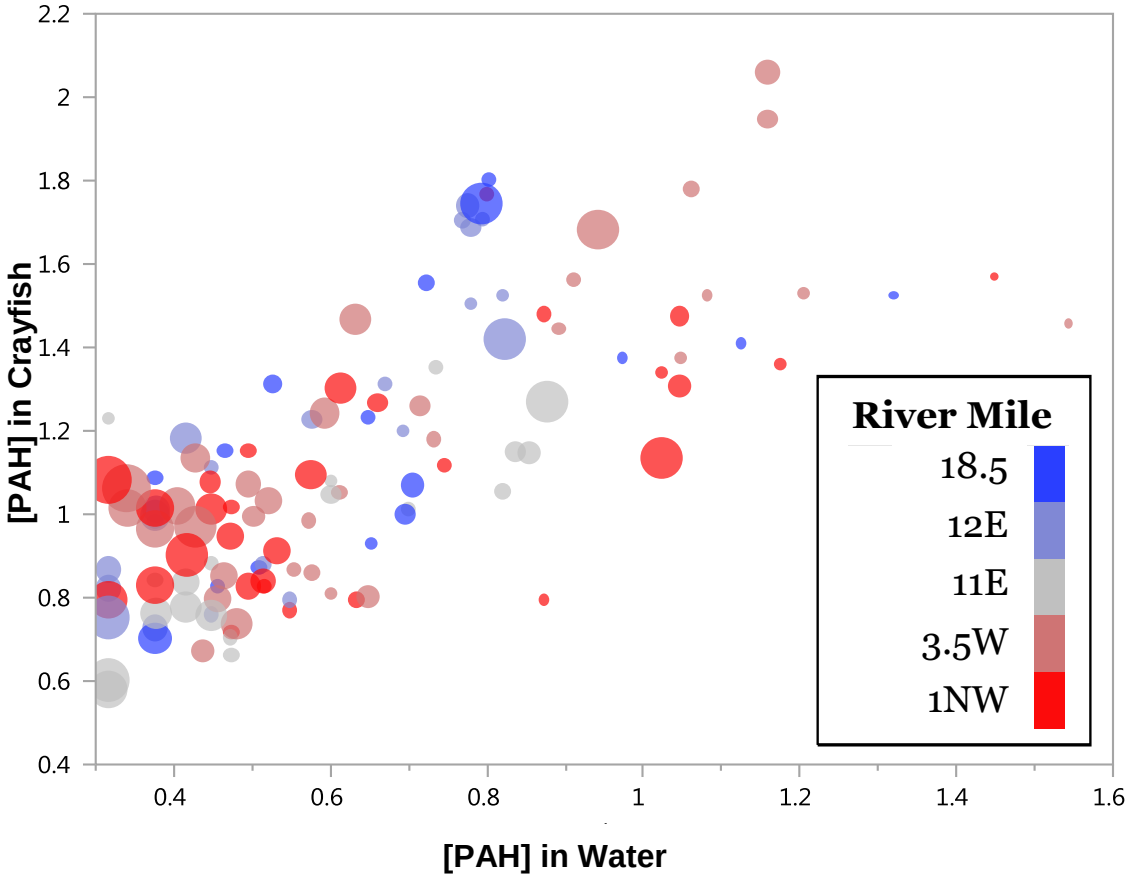

Figure A1.4. PAHs measured in 2013 crayfish viscera vs. water. Bubble size indicates $\log K_{ow}$ (larger bubbles indicate larger $\log K_{ow}$), while the bubble color indicates the sampling site associated with that data point.

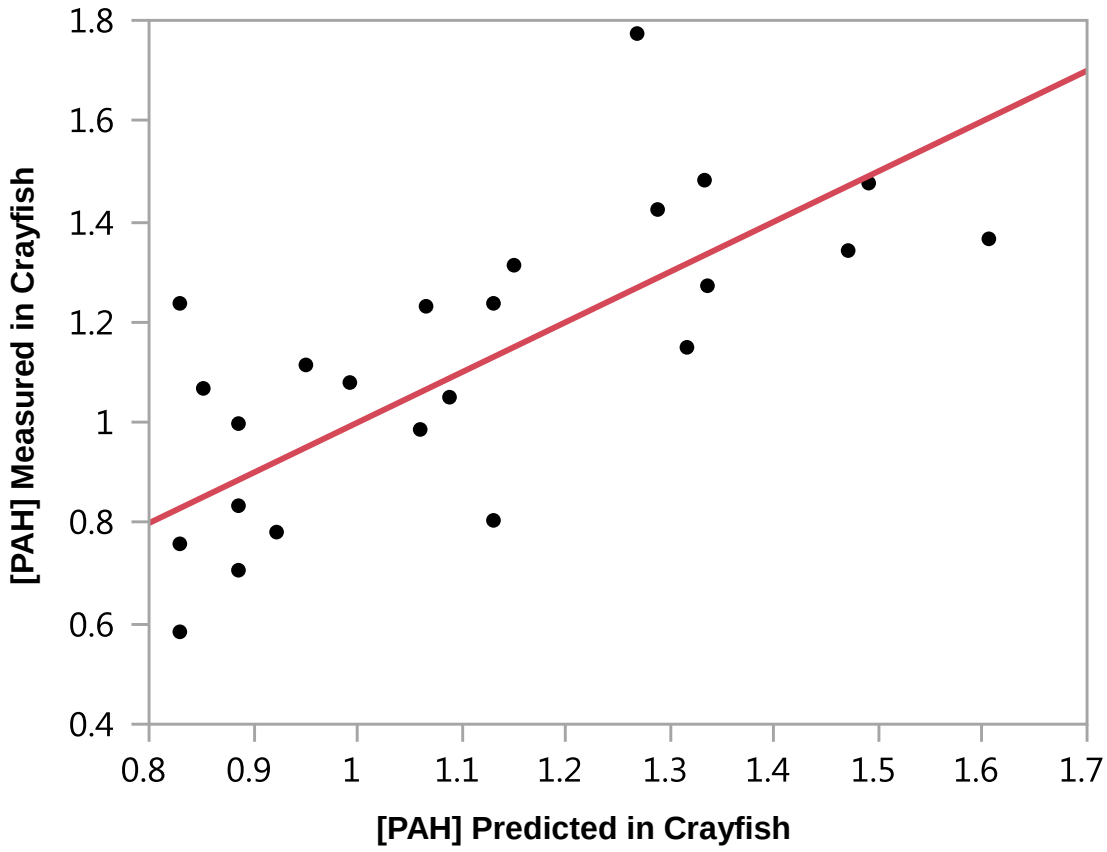

Figure A1.5. Predictions generated using test set (20% of the data). A model built only with a training set (80% of the paired 2013 crayfish and water data), was used to predict these PAH levels in crayfish for a test set (the other 20% of the data). These predicted values are compared to PAH levels measured in the crayfish. The diagonal reference line represents a predicted:measured ratio of 1:1.

Table A1.6. Measured and predicted PAH concentrations (ng/g) in 2013 crayfish viscera, within and outside of the Portland Harbor Superfund Megasite. Predicted:measured factor differences shaded light green are less than a factor of 1.5, ratios shaded yellow are between a factor of 1.5 and a factor of 4, and ratios shaded light red are more than a factor of 4. BLOD = below limit of detection.

	Inside Superfund			Outside Superfund		
	Measured (ng/g) ± (Standard Deviation)	Predicted (ng/g)	Factor difference between predicted & measured	Measured (ng/g) ± (Standard Deviation)	Predicted (ng/g)	Factor difference between predicted & measured
Naphthalene	3.3 (1.7)	14	4.2	5.5 (0.49)	10	1.9
2-Methylnaphthalene	3.9 (2.2)	3.7	1.0	4.3 (1.0)	5.1	1.2
1-Methylnaphthalene	2.5 (1.6)	3.2	1.3	4.0 (1.0)	3.7	1.1
2-Ethylnaphthalene	0.37 (0.25)	1.1	3.0	0.51 (0.14)	1.1	2.2
2,6-Dimethylnaphthalene	95 (10)	NA	NA	250 (73)	NA	NA
1,6-Dimethylnaphthalene	4.7 (1.9)	2.8	1.6	9.6 (1.0)	2.6	3.7
1,4-Dimethylnaphthalene	0.28 (0.40)	1.2	4.3	0.35 (0.10)	0.88	2.5
1,5-Dimethylnaphthalene	0.78 (0.24)	1.1	1.4	1.7 (0.13)	0.91	1.9
1,2-Dimethylnaphthalene	0.12 (0.17)	0.92	7.5	0.16 (0.27)	1.1	6.9
1,8-Dimethylnaphthalene	BLOD	NA	NA	BLOD	NA	NA
2,6-Diethylnaphthalene	BLOD	NA	NA	BLOD	NA	NA
Acenaphthylene	0.22 (0.31)	1.4	6.6	BLOD	NA	NA
Acenaphthene	2.7 (3.8)	5.3	2.0	1.1 (0.88)	2.2	NA
Fluorene	2.7 (2.3)	2.6	1.0	2.3 (0.70)	1.9	1.2

Table A1.6., continued.

Dibenzothiophene	0.87 (0.51)	1.1	1.2	1.1 (0.24)	0.73	1.5
Phenanthrene	5.6 (6.2)	3.9	1.4	6.4 (1.9)	2.5	2.5
Anthracene	1.0 (1.4)	2.1	2.2	0.27 (0.23)	1.4	5.2
2-Methylphenanthrene	1.9 (0.93)	1.7	1.1	2.6 (0.35)	1.4	1.9
2-Methylanthracene	0.62 (0.52)	1.0	1.6	1.3 (0.25)	0.8	1.5
1-Methylphenanthrene	0.21 (0.29)	1.7	8.0	BLOD	NA	NA
9-Methylanthracene	BLOD	NA	NA	BLOD	NA	NA
3,6-Dimethylphenanthrene	0.20 (0.29)	0.87	4.3	0.25 (0.24)	0.8	3.3
2,3-Dimethylanthracene	BLOD	NA	NA	BLOD	NA	NA
Fluoranthene	9.9 (12)	4.7	2.1	4.5 (4.1)	3.1	1.4
9,10-Dimethylanthracene	BLOD	NA	NA	BLOD	NA	NA
Pyrene	8.1 (9.0)	4.6	1.8	4.6 (3.6)	3.1	1.5
Retene	5.3 (3.8)	3.5	1.5	5.0 (3.9)	3.3	1.5
Benzo[a]fluorene	0.57 (0.80)	1.1	1.9	0.36 (0.31)	0.79	2.2
Benzo[b]fluorene	0.15 (0.21)	0.95	6.5	BLOD	NA	NA
Benzo[c]fluorene	0.10 (0.15)	0.81	7.8	BLOD	NA	NA
1-Methylpyrene	0.26 (0.37)	0.90	3.4	0.42 (0.40)	0.71	1.7
Benzo[a]anthracene	1.4 (1.4)	1.1	1.3	0.81 (0.74)	1.0	1.2
Cyclopenta[cd]pyrene	BLOD	NA	NA	BLOD	NA	NA

Table A1.6., continued.

Triphenylene	0.91 (0.59)	0.87	1.0	0.58 (0.54)	0.89	1.5
Chrysene	2.5 (3.1)	1.2	2.1	1.7 (1.3)	1.0	1.8
6-Methyl chrysene	BLOD	NA	NA	0.08 (0.14)	NA	NA
5-Methylchrysene	BLOD	NA	NA	BLOD	NA	NA
Benzo[b]fluoranthene	1.0 (0.94)	0.71	1.4	0.69 (0.46)	0.8	1.2
7,12-Dimethylbenz[a]anthracene	BLOD	NA	NA	0.06 (0.11)	NA	NA
Benzo[k]fluoranthene	0.61 (0.65)	0.55	1.1	0.22 (0.20)	0.50	2.3
Benzo[j]fluoranthene	0.49 (0.53)	0.57	1.2	0.26 (0.24)	0.64	2.5
Benz[j]and[e]aceanthrylene	BLOD	NA	NA	BLOD	NA	NA
Benzo[e]pyrene	0.51 (0.53)	0.64	1.3	0.33 (0.33)	0.62	1.9
Benzo[a]pyrene	0.54 (0.77)	0.71	1.3	0.45 (0.55)	0.64	1.4
Indeno[1,2,3-c,d]pyrene	0.23 (0.33)	NA	NA	BLOD	NA	NA
Dibenzo[a,h]anthracene	BLOD	NA	NA	BLOD	NA	NA
Benzo[a]chrysene	BLOD	NA	NA	BLOD	NA	NA
Benzo[g,h,i]perylene	0.75 (0.74)	0.55	1.4	0.65 (0.69)	0.50	1.3
Anthanthrene	BLOD	NA	NA	BLOD	NA	NA
Naphtho[1,2-b]fluoranthene	BLOD	NA	NA	BLOD	NA	NA
Naphtho[2,3-j]fluoranthene	BLOD	NA	NA	BLOD	NA	NA

Table A1.6., continued.

Dibenzo [a,e] fluoranthene	BLOD	NA	NA	BLOD	NA	NA
Dibenzo[a,l]pyrene	BLOD	NA	NA	BLOD	NA	NA
Naphtho[2,3-k]fluoranthene	BLOD	NA	NA	BLOD	NA	NA
Naphtho[2,3-e]pyrene	BLOD	NA	NA	BLOD	NA	NA
Dibenzo[a,e]pyrene	BLOD	NA	NA	BLOD	NA	NA
Coronene	0.10 (0.14)	NA	NA	0.09 (0.15)	NA	NA
Dibenzo[e,l]pyrene	BLOD	NA	NA	BLOD	NA	NA
Naphtho[2,3-a]pyrene	BLOD	NA	NA	BLOD	NA	NA
Benzo[b]perylene	BLOD	NA	NA	BLOD	NA	NA
Dibenzo[a,i]pyrene	BLOD	NA	NA	BLOD	NA	NA
Dibenzo[a,h]pyrene	BLOD	NA	NA	BLOD	NA	NA

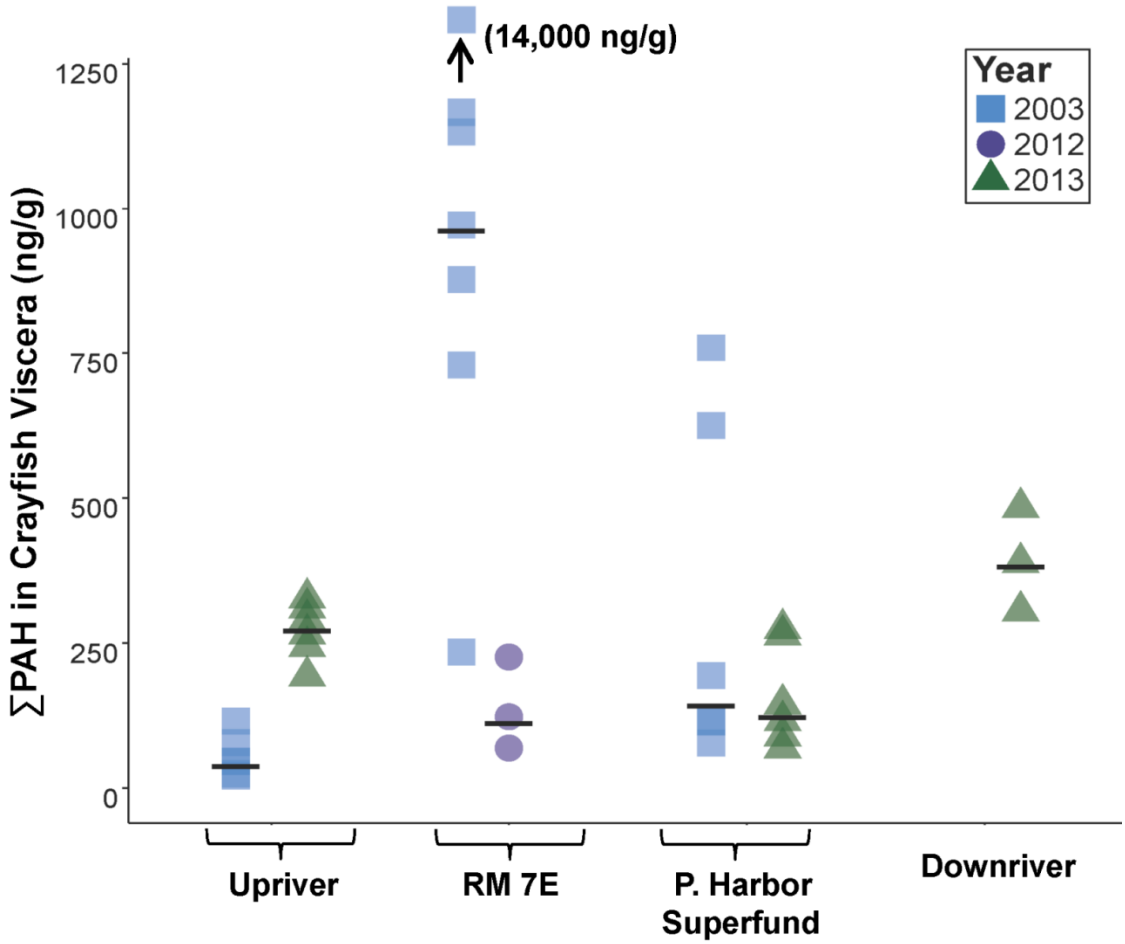

Figure A1.6. Spatial and temporal comparison of ∑PAH in crayfish viscera. ∑PAH are presented in crayfish viscera (ng/g) that were collected upriver of Superfund sites, at RM 7E (the McCormick and Baxter Superfund site), in the greater Portland Harbor Superfund Megasite (PHSM), and downriver of the Superfund sites, in the Willamette River in Portland, Oregon. ∑PAH in crayfish were measured in crayfish collected in 2003 (blue squares), and in 2013 (green triangles). For 2012, ∑PAH in crayfish were predicted from water data, using the linear regression described in this study (purple circles). Horizontal black lines represent the median of ∑PAH in each spatiotemporal group of samples.

Appendix 2: Supporting Information to Chapter 3 – Emissions of Polycyclic Aromatic Hydrocarbons from Natural Gas Extraction into Air

Chemical Information

Single PAH standards were purchased from Sigma Aldrich, in St. Louis, MO, Chiron, in Trondheim, Norway, or Fluka (part of Sigma-Aldrich). PAH mixes were purchased from Accustandard, in New Haven, CT. Labeled compounds used as performance reference compounds (PRCs), laboratory surrogates, or instrument internal standards were obtained from either CDN Isotopes, in Pointe-Claire, Quebec, Canada, Cambridge Isotope Laboratories, in Tewksbury, MA, or Fisher Scientific in Pittsburgh, PA. All solvents were Optima-grade (from Fisher Scientific, Pittsburgh, PA) or equivalent, and all laboratory glassware and other tools were baked at 450°C for 12 hours and/or solvent-rinsed before use. Water used to clean LDPE was filtered through a D7389 purifier purchased from Barnstead International, in Dubuque, IA.

Passive Sampler Handling

LDPE strips were cut from pre-sized polyethylene tubing that was approximately 2.7 cm wide. Each polyethylene strip was approximately 100 cm long and had a volume of 5.1 cm^3. LDPE was dried under filtered vacuum in stainless steel kegs, from AEB Kegs in Delebio, Italy. TurboVap® evaporators were from Biotage, in Charlotte, NC.

Site Description and Sampling Design

A significant fraction of the Carroll County's residents earn their livings through farming. Carroll County also sits on both the Marcellus and Utica Shales. It has therefore been part of the natural gas boom occurring in the United States in recent years. The exact number of days that samplers were deployed ranged from 18 to 28. Welch Fluorocarbon, Inc. is in Dover, NH.

Volunteer Training

Volunteer landowners were trained in passive sampler handling, retrieval, and documentation by Oregon State University and University of Cincinnati community

outreach specialists. Training included demonstration of retrieving samplers, practicing the technique, and time for discussion of questions and concerns. Volunteers were given handouts with step-by-step instructions of the sampler retrieval process. Handouts included a website with access to training videos, as well as contact information for OSU and UC trainers who would be available to answer questions.

Chemical Analysis

Agilent is located in Alpharetta, GA. The temperature profile in the GC/MS-MS analytical method was as follows: 60°C for 1 minute, increasing 40°C per minute to reach 180°C, then increasing 3°C per minute to reach 230°C, then increasing 1.5°C per minute to reach 235°C, then increasing 15°C per minute to reach 280°C, staying at 280°C for 10 minutes, then increasing 6°C per minute to reach 298°C, and finally ramping up 16°C per minute to reach 350°C and stay there for 4 minutes. The dimensions of the Agilent Select PAH column were: 30 m, 0.25 mm, 0.15 µm. Continuing calibration standards were run nominally every 10 samples, and/or at the end of the sample set. If a closing standard did not meet the criteria, samples were re-run after the standard was verified.

Air Concentration Calculations

Vapor phase air concentrations were determined using an empirical uptake model. Sampling rates were derived by measuring PRC loss, as described in Huckins *et al*.[21] PRCs allow for an accurate assessment of *in situ* uptake rates for a wide range of analytes in variable environmental conditions.[126, 127, 214] The uptake calculation does not make any assumptions about the analyte being at equilibrium, so this model was used for air concentration calculations for all PAHs. PRCs had logK_{oa} values ranging from 6.59 to 10.35, and had similar physical and chemical properties as the target PAHs.[127] Air concentrations (C_a) of PAHs were determined using equation A2.1:

Eq. A2.1
$$C_a = \frac{N_{analyte}}{V_S \times K_{sa(T)} \times \left(1 - \mathrm{e}^{\left(-\frac{R_{S,analyte} \times t}{V_S \times K_{sa(T)}}\right)}\right)}$$

In equation A2.1, C_a is the air concentration, $N_{analyte}$ is the mass of the target analyte in the sampler, V_s is the sampler volume, $K_{sa(T)}$ is the temperature-adjusted sampler-air partition coefficient, R_s is the compound-specific sampling rate, and t is the duration of sampling, in days. Sampling rates (R_s) of the PRCs were determined using equation A2.2:

Eq. A2.2 $$R_{S,PRC} = -\frac{\ln(\frac{N}{N_0})}{t} \times K_{sa(T)} \times V_s$$

In equation A2.2, N_0 ad N are the mass of PRC present at the beginning and end of the sampling period, respectively. The sampling rate (R_s) for each analyte was calculated based on the R_s of the PRC with the most similar K_{oa}, using Equation A2.3:

Eq. A2.3 $$R_{s,analyte} = R_{s,PRC} \times \frac{\alpha_{analyte}}{\alpha_{PRC}}$$

In equation A2.3, α is a compound class specific modifier. Equation A2.4 was used to calculate α for both target analytes and PRCs:

Eq. A2.4 $$\log\alpha = 0.0130(\log K_{oa})^3 - 0.3173(\log K_{oa})^2 + 2.244(\log K_{oa})$$

In Huckins et al., Equation A2.4 uses K_{ow}.[21] K_{oa} was used here to estimate air concentrations instead of water concentrations. An analyte-specific sampler-air partition coefficient (K_{sa}) at the reference temperature (298 K) was calculated for each target PAH and PRC, using equation A2.5:

Eq. A2.5 $$K_{sa(298)} = \frac{K_{sw} \times R \times 298}{K_{aw}}$$

In equation A2.5, K_{sw} is the sampler-water partition coefficient, K_{aw} is the Henry's law constant in atm*m^3*mol^{-1}, R is the ideal gas constant in m^3*atm*K^{-1}*mol^{-1}, and 298 K is the reference temperature used in this study. $K_{sw}$was calculated using equation A2.6, from Huckins et al.[21]:

Eq. A2.6 $$\log K_{sw} = a_0 + 2.321(\log K_{ow}) - 0.1618(\log K_{ow})^2$$

In equation A2.6, a_0 = – 2.61 for PAHs.[21] Temperature-corrected K_{sa} values ($K_{sa(T)}$) were calculated using an approach described in Khairy et al.[34] This approach uses a modified van 't Hoff equation to adjust each compound's K_{sa} for the temperature

during the sampling period. Equation A1.7 is the modified van 't Hoff equation used in this study:

Eq. A2.7 $$K_{sa(T)} = K_{sa(298)} \times e^{\frac{\Delta H_{vap}}{R} \times (\frac{1}{T} - \frac{1}{298})}$$

In equation A1.7, ΔH_{vap} is the enthalpy of vaporization, *T* is the average temperature during deployment in K, 298 is the reference temperature in K, and *R* is the ideal gas constant in kJ*K^{-1}*mol^{-1}. ΔH_{vap}values for the target PAHs were calculated using Equation S8:

Eq. A2.8 $$\Delta H_{vap} = 69.354 - 9.3891 \times \log P_L$$

In equation S8 P_L is the subcooled liquid vapor pressure. This equation is a relationship between $logP_L$ and ΔH_{vap} that was reported by Khairy et al.[34] It uses empirically-derived ΔH_{vap} from Roux et al.,[219] and $\log P_L$ that were estimated using Equation S9:

Eq. A2.9 $$\log P_L = 8.52 - 0.054 \times MW$$

In equation S9 *MW* is the molecular weight. This equation is a relationship between $\log P_L$ and MW that was empirically derived by Ma et al.[220]

Sourcing Ratios

Petrogenic PAH sources are typically enriched in the more thermodynamically stable isomer[85, 168]. In the two isomer pairs of PAHs used for PAH sourcing, phenanthrene and pyrene are the more thermodynamically stable isomers. Thus, a higher phenanthrene/anthracene, and a lower fluoranthene/pyrene ratio each indicate that the sample is predominantly petrogenic. A few samples had slightly pyrogenic signatures according to one ratio. However, more than one ratio should be used to confirm PAH source, as interpretation of values near the boundaries between sources can be less certain.[168]

PAHs Used in Comparison

The 14 PAHs used in the comparison in Figure 2 were were acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene,

benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[g,h,i]perylene, and indeno[1,2,3-cd]pyrene. PAHs measured during the same season as the present study were used in this comparison where possible.

Quantitative Risk Assessment Calculations

There were 12 PAHs that were above the detection limits and had nonzero RPFs (see Table A2.2). Thus, these were the 12 PAHs that were used in the carcinogenic risk assessment. These 12 PAHs were benzo[c]fluorene, fluoranthene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[a]anthracene, chrysene, benzo[j]fluoranthene, cyclopenta[c,d]pyrene, indeno[1,2,3-c,d]pyrene, anthanthrene, benzo[k]fluoranthene, and benzo[g,h,i]perylene.

Risk assessment was performed using equations from the EPA's 2009 Risk Assessment Guidance for Superfund[173], using equations A2.10 and A2.11:

Eq. A2.10
$$\mathrm{EC} = \frac{(\mathrm{CA\ x\ ET\ x\ EF\ x\ ED})}{\mathrm{AT}}$$

In equation A2.10, EC is the exposure concentration in ng/m^3, CA is the contaminant concentration in air in ng/m^3, ET is the exposure time in hours/day, EF is the exposure frequency in days/year, ED is the exposure duration in years, and AT is the averaging time. The AT includes the lifetime in years multiplied by 365 days/year and 24 hours/day.

Eq. A2.11
$$\mathrm{ELCR} = \mathrm{IUR\ x\ EC}$$

In equation A2.11, ELCR is excess lifetime cancer risk, IUR is inhalation unit risk, and EC is the exposure concentration from equation A2.10. In this study, an IUR of 8.7×10^{-5} ng/m^3 was used. This is an IUR that was estimated for benzo[a]pyrene by the World Health Organization.[182] This was used because the U.S. EPA has not established an IUR for PAHs.

Overview and Discussion of Exploratory Principle Components Analysis (PCA)

Exploratory data analysis was performed using a Principle Component Analysis (PCA) biplot showing scores and loadings plots together. All PAH variables that were above limits of detection were used, in addition to the distance to closest well variable. Only sites classified as "close" or "far" were included. The data were mean centered and scaled. PC1 and PC2 were chosen as graph axes because the resulting PCA graph displayed good delineation between close and far sites. PCA was performed using Primer-E version 6.1.13. A list of labels used in the PCA plot and the PAHs they correspond to can be found in **Table A2.1**.

Figure A2.1 shows that the majority of the detected PAHs have negative correlations with distance to the nearest active NGE well. This reinforces the results of Figures 2.1b, 1c, 2 and 4 in the main text, suggesting that distance to the nearest NGE well is negatively correlated with PAH levels in air.

Figure A2.1 also shows samples in the close and far groups clustering separately. Looking closer at the clustering in the plot is also interesting. For instance, there is one sample from the close group that is clumping with the samples from the far group ("Close 5"). Interestingly, this sample was farther from an active NGE well than any others in the close group (located 0.09 miles from the nearest NGE well). This may partially explain why this sample's profile is more similar to samples in the far group than to the other samples in the close group.

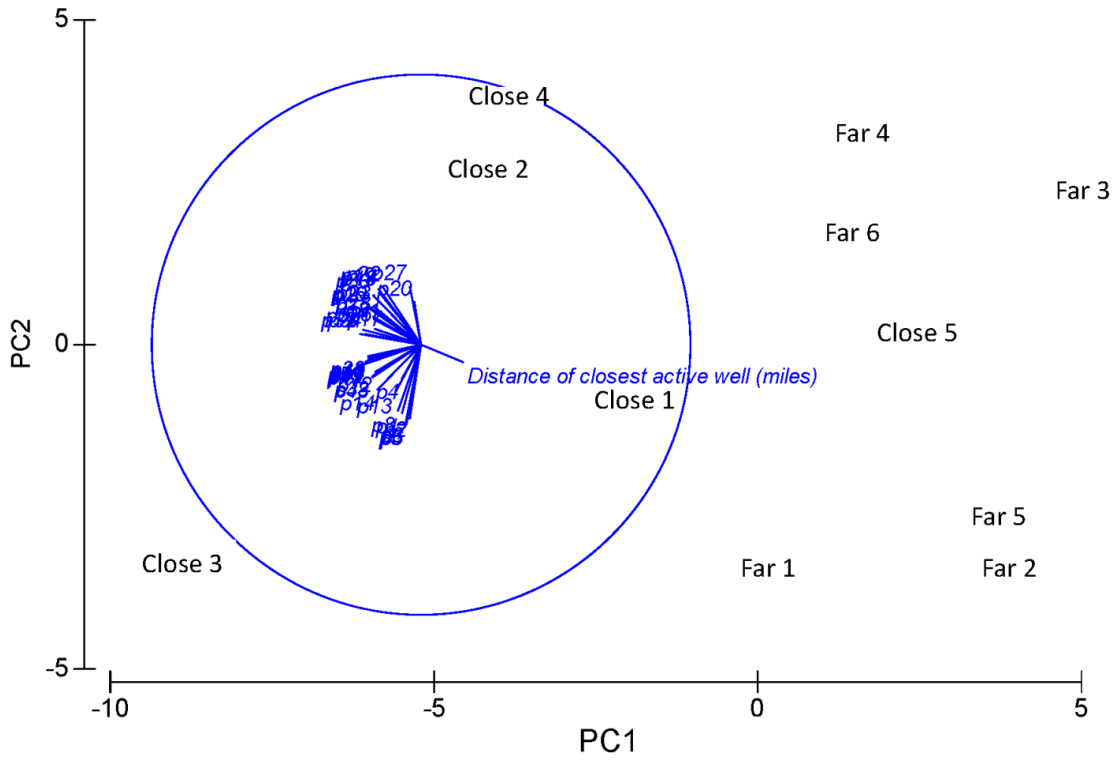

Figure A2.1. Exploratory principle components analysis (PCA) using PAHs and distance to closest active NGE well treated as covariates. The graph only includes data for samples in the close (*n* = 5) and far (*n* = 6) distance groups.

Table A2.1: Labels used in the PCA plot (Figure A2.1) and their corresponding PAH names.

PCA Label	PAH
p1	Naphthalene
p2	2-Methylnaphthalene
p3	1-Methylnaphthalene
p4	2-Ethylnaphthalene
p5	2,6-Dimethylnaphthalene
p6	1,6-dimethylNaphthalene
p7	1,4-dimethylnaphthalene
p8	1,5-dimethylnaphthalene
p9	1,2-dimethylnaphthalene
p10	1,8-Dimethylnaphthalene
p11	2,6-Diethylnaphthalene
p12	Acenaphthylene
p13	Acenaphthene
p14	Fluorene
p15	Dibenzothiophene
p16	Phenanthrene
p17	Anthracene
p18	2-Methylphenanthrene
p19	2-Methylanthracene
p20	1-Methylphenanthrene
p21	9-Methylanthracene
p22	3,6-Dimethylphenanthrene
p23	Fluoranthene
p24	2,3-Dimethylanthracene
p25	9,10-Dimethylanthracene
p26	Pyrene
p27	Retene
p28	Benzo[a]fluorene
p29	Benzo[b]fluorene
p30	Benzo[c]fluorene
p31	1-Methylpyrene
p32	Benzo[a]anthracene
p33	Cyclopenta[cd]pyrene
p34	Triphenylene
p35	Chrysene
p36	6-Methyl chrysene
p37	5-Methylchrysene
p38	Benzo [b] fluoranthene
p39	7,12-Dimethylbenz[a]anthracene

Table A2.1., continued.

PCA Label	PAH
p40	Benzo [k] fluoranthene
p41	Benzo [j] fluoranthene
p42	Benzo [e] pyrene
p43	Benzo [a] pyrene
p44	Indeno [1,2,3-c,d] pyrene
p45	Dibenzo [a,h] anthracene
p46	Benzo [a] chrysene
p47	Benzo [g,h,i] perylene
p48	Anthanthrene
p49	Naphtho[1,2-b]fluoranthene
p50	Naphtho[2,3-j]fluoranthene
p51	Dibenzo [a,e] flouranthene
p52	Dibenzo [a,l] pyrene
p53	Naphtho[2,3-k]fluoranthrene
p54	Naphtho[2,3-e]pyrene
p55	Dibenzo [a,e] pyrene
p56	Coronene
p57	Dibenzo[e,l]pyrene
p58	Naphtho[2,3-a]pyrene
p59	Benzo [b] perylene
p60	Dibenzo [a,i] pyrene
p61	Dibenzo [a,h] pyrene

Appendix 3: Supporting Information to Chapter 4 – Personal Exposure to PAHs near Natural Gas Extraction

Table A3.1. List of LODs and LOQs of target PAHs in GC-MS/MS method, for 10x diluted sample extracts. Limits of detection (LODs) and limits of quantitation (LOQs) are given for the GC-MS Triple Quad instrument method used for PAH analysis in this study. These LODs and LOQs apply in cases when sample extracts have been diluted 10-fold prior to analysis.

Target PAH	CAS #	LOD (ng/mL)	LOQ (ng/mL)
Naphthalene	91-20-3	10	52
2-Methylnaphthalene	91-57-6	7	35
1-Methylnaphthalene	90-12-0	2.8	14
2-Ethylnaphthalene	939-27-5	9.7	48
2,6-Dimethylnaphthalene	581-42-0	8.9	44
1,6-Dimethylnaphthalene	575-43-9	8.1	41
1,4-Dimethylnaphthalene	571-58-4	12	62
1,5-Dimethylnaphthalene	571-61-9	12	59
1,2-Dimethylnaphthalene	573-98-8	9.4	47
1,8-Dimethylnaphthalene	569-41-5	8.3	42
2,6-Diethylnaphthalene	59919-41-4	8.1	41
Acenaphthylene	208-96-8	23	120
Acenaphthene	83-32-9	11	54
Fluorene	86-73-7	7.9	40
Dibenzothiophene	132-65-0	2.4	12
Phenanthrene	85-01-8	4.6	23
Anthracene	120-12-7	11	52
2-Methylphenanthrene	2531-84-2	3.9	19
2-Methylanthracene	613-12-7	4.7	24
1-Methylphenanthrene	832-69-9	11	53
9-Methylanthracene	779-02-2	8.7	44
3,6-Dimethylphenanthrene	1576-67-6	4.2	21
2,3-Dimethylanthracene	613-06-9	3.4	17
Fluoranthene	206-44-0	5.4	27
9,10-Dimethylanthracene	781-43-1	8.5	42
Pyrene	129-00-0	4.2	21
Retene	483-65-8	8.4	42
Benzo[a]fluorene	238-84-6	17	50
Benzo[b]fluorene	243-17-4	17	50
Benzo[c]fluorene	205-12-9	3	15
1-Methylpyrene	2381-21-7	3.8	19
Benz[a]anthracene	56-55-3	7.5	38
Cyclopenta[c,d]pyrene	27208-37-3	5.3	27
Triphenylene	217-59-4	4.1	20
Chrysene	218-01-9	5	25
6-Methylchrysene	1705-85-7	8.9	44
5-Methylchrysene	3697-24-3	17	50

Table A3.1., continued.

Target PAH	CAS #	LOD (ng/mL)	LOQ (ng/mL)
Benzo[b]fluoranthene	205-99-2	3.7	19
7,12-Dimethylbenz[a]anthracene	57-97-6	9.4	47
Benzo[k]fluoranthene	207-08-9	5.3	26
Benzo[j]fluoranthene	205-82-3	5.6	28
Benz[j]&[e]aceanthrylene	202-33-5 and 199-54-2	17	50
Benzo[e]pyrene	192-97-2	7.1	35
Benzo[a]pyrene	50-32-8	12	59
Indeno[1,2,3-c,d]pyrene	193-39-5	2.6	13
Dibenzo[a,h]pyrene	53-70-3	10	51
Picene	213-46-7	7.4	37
Benzo[ghi]perylene	191-24-2	3.4	17
Anthanthrene	191-26-4	3.3	17
Naphtho[1,2-b]fluoranthene	5385-22-8	17	50
Naphtho[2,3-j]fluoranthene	205-83-4	17	50
Dibenzo[a,e]fluoranthene	5385-75-1	4.7	24
Dibenzo[a,l]pyrene	191-30-0	4.8	24
Naphtho[2,3-k]fluoranthene	207-18-1	17	50
Naphtho[2,3-e]pyrene	193-09-9	17	50
Dibenzo[a,e]pyrene	192-65-4	64	320
Coronene	191-07-1	7	35
Dibenzo[e,l]pyrene	192-51-8	17	50
Naphtho[2,3-a]pyrene	196-42-9	17	50
Benzo[b]perylene	197-70-6	17	50
Dibenzo[a,i]pyrene	189-55-9	14	71
Dibenz[a,h]anthracene	189-64-0	5.2	26

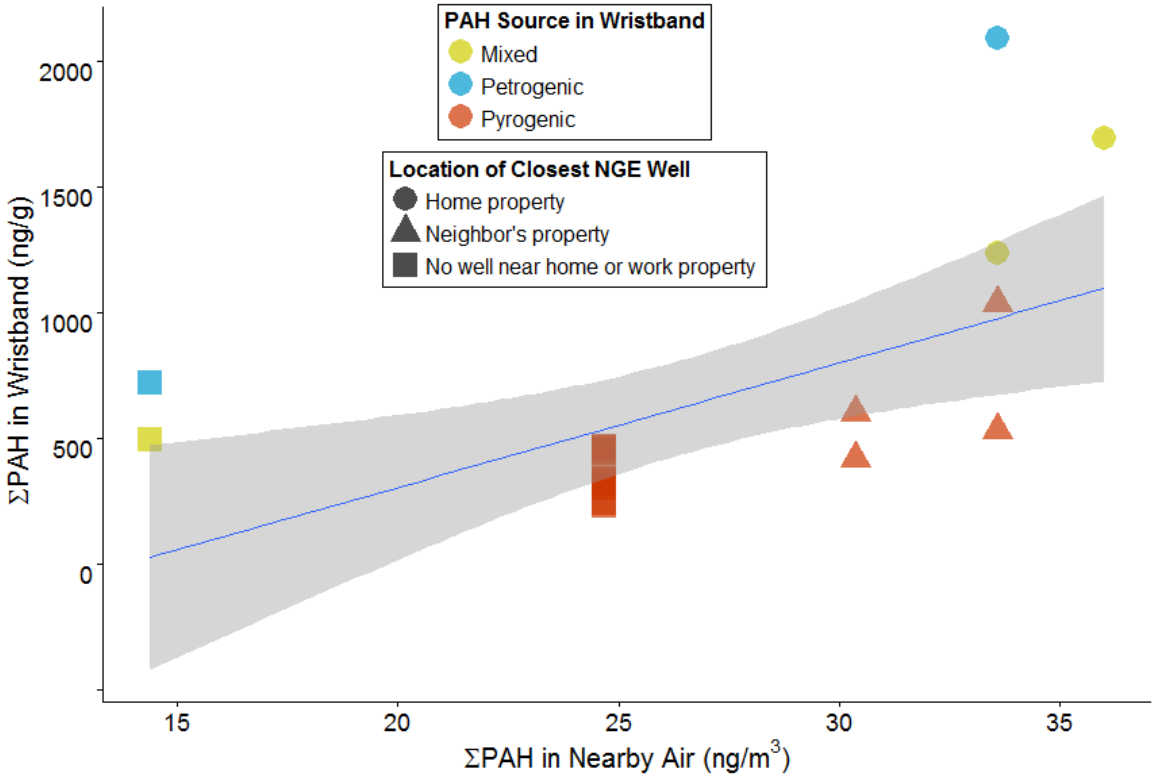

Figure A3.1. ∑PAH in each participant's wristband (ng/g wristband) vs. ∑PAH measured at the nearest stationary air sampling site (ng/m^3), with source signature measured in wristbands. There is a significant positive correlation between ∑PAH in a participant's wristband and ∑PAH in the inner ring of air samplers at the nearest stationary air sampling site (simple linear regression, p = 0.0062). R-squared = 0.33, correlation coefficient = 0.57. Symbols are color-coded based on the source signatures of PAH mixtures measured in participants' wristbands. Source signatures are based on the fluoranthene/pyrene ratio. For this ratio, values <1.0 are petrogenic and values >1.0 are pyrogenic. Here, values of this ratio of 1.0 ± 0.1 are interpreted as a "mixed" signature and shown in yellow. The shape of the symbol represents whether participants had active NGE wells on their home properties, their neighbor's properties, or neither.

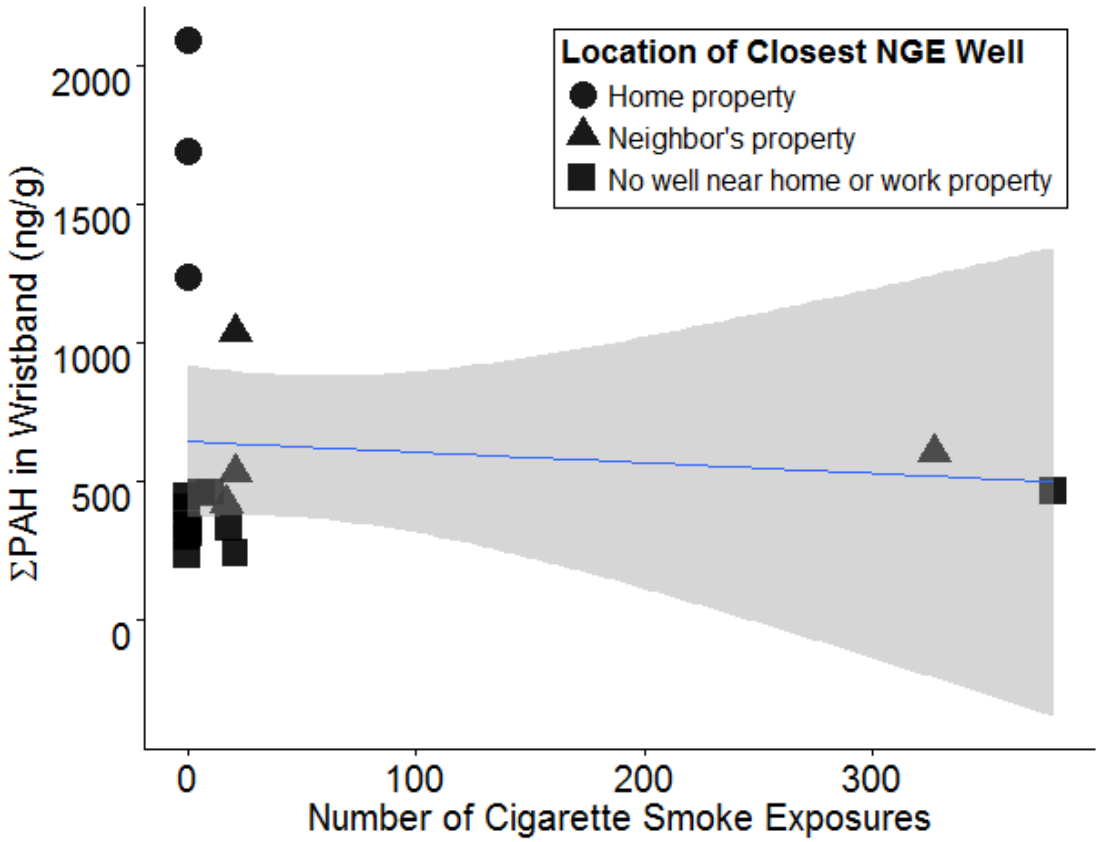

Figure A3.2. ∑PAH in each participant's wristband (ng/g wristband) vs. number of cigarette smoke exposures each participant reported during the study. There was no correlation between number of cigarette smoke exposures reported by participants and ∑PAH measured in their wristbands (simple linear regression, p = 0.74). R-squared = 0.0066.