Published in final edited form as:

Environ Toxicol Chem. 2019 April ; 38(4): 748–759. doi:10.1002/etc.4361.

Aryl hydrocarbon receptor-mediated activity of gas-phase ambient air derived from passive sampling and an *in vitro* bioassay

Carrie A. McDonough^{a,*}, Diana G. Franks^b, Mark E. Hahn^b, and Rainer Lohmann^a

^aUniversity of Rhode Island Graduate School of Oceanography, Narragansett, RI, USA

^bBiology Department, Woods Hole Oceanographic Institution, Woods Hole, MA, USA

Abstract

The gaseous fraction of hydrophobic organic contaminants (HOCs) in ambient air appears to be responsible for a significant portion of aryl hydrocarbon receptor (AhR)-mediated activity, but the majority of compounds contributing to this activity remain unidentified. This study investigated the use of polyethylene passive samplers (PEs) to isolate gaseous HOCs from ambient air for use in *in vitro* bioassays and to improve our understanding of the toxicological relevance of the gaseous fraction of ambient air in urban and residential environments. Concentrations of polycyclic aromatic hydrocarbons (PAHs) and organic flame retardants (OFRs) were measured in PE extracts. Extracts were also analyzed using an *in vitro* bioassay to measure AhR-mediated activity. Bioassay-derived benzo[a]pyrene (BaP) equivalents (BaP-Eq_{bio}), a measure of potency of HOC mixtures, were greatest in the downtown Cleveland area and lowest at rural/residential sites further from the city center. BaP-Eq_{bio} was weakly correlated with concentrations of 2-ring alkyl/substituted PAHs and one organophosphate flame retardant, ethylhexyl diphenyl phosphate (EHDPP). Potency predicted based on literature-derived induction equivalency factors (IEFs) explained only 2–23% of the AhR-mediated potency observed in bioassay experiments. This study suggests that health risks of gaseous ambient air pollution predicted using data from targeted chemical analysis may underestimate risks of exposure, most likely due to augmentation of potency by unmonitored chemicals in the mixture, and the lack of relevant IEFs for many targeted analytes.

Keywords

aryl hydrocarbon receptor; passive sampler; mixture toxicology; polycyclic aromatic hydrocarbons (PAHs); organophosphate esters; flame retardants

* Address correspondence to carrie.a.mcd@gmail.com.

Data Accessibility: Data and calculation tools can be made available upon request by contacting the corresponding author (carrie.a.mcd@gmail.com).

Supplemental Data: The Supplemental Data, including a map of study locations, list of all target analytes, summary of concentrations in dosing solutions for all analytes, positive control dose-response curve, and correlation analyses between compounds, are available on the Wiley Online Library at DOI: 10.1002/etc.xxxx.

INTRODUCTION

Hydrophobic organic contaminants (HOCs) sorbed to particulate matter in ambient air pose a health risk to humans via several pathways, and activation of the aryl hydrocarbon receptor (AhR) by polycyclic aromatic hydrocarbons (PAHs) is strongly associated with the carcinogenicity of ambient atmospheric particulate matter (Matsumoto et al. 2007; Andrysík et al. 2011). However, health risks associated with HOCs in the gaseous phase remain poorly understood. Humans are exposed to gaseous air pollution directly via respiration and dermal uptake (Weschler and Nazaroff 2012). This is especially concerning in urban areas with heavier vehicular traffic and greater population density, as well as in indoor environments. Furthermore, gaseous HOCs are freely available to partition into other media, including plants (Kobayashi et al. 2007), and dietary uptake from crops has been identified as a route of human exposure (Kobayashi et al. 2008).

The gaseous fraction of ambient air has a distinct composition compared to the particle-bound fraction (Boström et al. 2002). The summed mass of PAHs in the gaseous phase is typically greater than in the particulate phase. However, gaseous PAHs are generally dominated by lower molecular weight 2–3-ring PAHs, while the particulate-bound fraction is dominated by more hydrophobic 4–5-ring PAHs (Boström et al. 2002; Klein et al. 2005; Ramírez et al. 2011; Barrado et al. 2013; Gungormus et al. 2014).

In addition to PAHs, recent studies have demonstrated that many organic flame retardant compounds (OFRs) are also ubiquitous in ambient urban air, and that one particular class, the organophosphate esters (OPEs), are present at unexpectedly high levels in urban ambient air (Salamova et al. 2014; Shoeib et al. 2014). Furthermore, some currently-used chlorinated OPEs are expected to be present predominantly in the gaseous phase (Brommer et al. 2014; Salamova et al. 2014; Peverly et al. 2015). O'Connell et al. used silicone wristbands as personal monitoring devices for exposure to gas-phase HOCs and frequently detected OPEs, along with several 2–3-ring PAHs (O'Connell et al. 2014).

Chronic exposure to gas-phase OPEs and other OFRs in ambient air is of concern because several studies have provided evidence that many OPEs, including tris(1,3-dichloro-2-propyl) phosphate (TDCIPP), tris(1-chloro-2-propyl) phosphate (TCIPP), triphenyl phosphate (TPHP), and tris(2-ethylhexyl) phosphate (TEHP), can disrupt normal development, metabolism, immune response, and hormone function (Farhat et al. 2013; Liu et al. 2013; Farhat et al. 2014; Porter et al. 2014). Studies have also indicated that TDCIPP is carcinogenic and/or mutagenic (Gold et al. 1978; Farhat et al. 2014), and, along with tris(2-chloroethyl) phosphate (TCEP), it has been designated a carcinogen under California Proposition 65 (California OEHHA, 2017). Some OFRs, including tris(methylphenyl) phosphate (meta; TmMPP) and TDCIPP, have also been associated with changes in expression of genes regulated by AhR in a few past studies, though evidence of this is sparse (Liu et al. 2013; Porter et al. 2014). Previous studies indicate that some polybrominated diphenyl ether (PBDE) congeners are also weak or moderate AhR agonists, and that binding affinity appears to depend on the degree and position of bromination (Chen and Bunce 2003; Gu et al. 2012). Recent work has also indicated that concentrations of PBDEs may be

positively correlated with dioxin-like activity in dust samples, possibly due to the cooccurrence of polybrominated dioxins/furans (PBDD/Fs) (Wong et al. 2016).

Activation of AhR is linked to induction and repression of a large number of genes, including modulation of cell growth and proliferation, tumor promotion, immunological effects, cardiotoxicity, and endocrine disruption, with the severity and type of response dependent upon the specific ligand and its binding affinity (Denison et al. 2011). Previous studies on health risks of ambient air pollution have used induction equivalency factors (IEFs) to represent the AhR-mediated potency of PAHs relative to benzo[a]pyrene (BaP) (Kennedy et al. 2010; Ramírez et al. 2011). This IEF-based approach assumes an additive, rather than synergistic or antagonistic, relationship between multiple ligands. AhR is activated by binding with variable affinity to several PAHs, with 4–5-ring PAHs generally more potent than the 2–3-ring PAHs that dominate gas-phase air pollution (Boström et al. 2002). Highly potent PAHs such as (BaP) are typically present only at very low concentrations in the gas phase due to low volatility. The lower molecular weight PAHs, especially phenanthrene, fluoranthene, and the methylated phenanthrenes/anthracenes, may contribute more significantly to the potency of the gaseous fraction due to their high gas-phase concentrations (Boström et al. 2002).

Despite low concentrations of potent high molecular weight PAHs in the gaseous fraction of ambient air pollution, previous studies have shown that this fraction appears to be responsible for a significant portion of the AhR-mediated activity associated with ambient air. In studies of gas-phase air pollution, Ramirez et al. found that, while concentrations of PAHs known to be most potent with respect to cytochrome P450 1A1 (CYP1A1) induction were low in the gaseous fraction, this fraction was estimated to contribute 34–86% of total carcinogenicity associated with 16 PAHs based on potency relative to BaP (Ramírez et al. 2011). Previous studies by Klein et al. and Novak et al. also observed significant AhR activation from the gaseous, as well as particulate, fraction of ambient air pollutants (Klein et al. 2005; Novák et al. 2009). Kennedy et al. found a statistically significant relationship between PAH concentrations and AhR activity in samples of gaseous and fine particulate contaminants, but determined that the specific PAHs targeted in the study accounted for less than 3% of the observed AhR activity (Kennedy et al. 2010). Similarly, Érsekóvá et al. found that quantified PAHs accounted for only 3–33% of measured AhR activity from ambient air samples (Érsekóvá et al. 2014). While some of these studies considered contributions of compound groups besides PAHs, including polychlorinated biphenyls (PCBs) and organochlorine pesticides, none have investigated whether OFRs may explain some fraction of AhR activity.

Previous studies have noted that gaseous HOCs should not be ignored in risk assessments, but all of this work was carried out using high-volume air samplers or passive polyurethane foam (PUF) samplers, which are less selective for gaseous HOCs than polyethylene passive samplers (PEs) (Melymuk et al. 2011). Studies using less selective sampling strategies could not fully rule out that some fraction of particulate-bound HOCs may have contributed to the measured AhR activity. PEs accumulate only gas-phase HOCs and have an affinity for HOCs that is similar to that of fatty tissue, so they have been used in many studies predicting the extent to which HOCs will bioaccumulate (Joyce et al. 2016). The present study is the

first to our knowledge to investigate AhR activation caused by the freely gaseous fraction of HOCs taken up by a single-phase sampler (pre-cleaned polyethylene), and will help contribute to our understanding of the biological relevance of the truly gaseous fraction of ambient air in urban and residential environments.

PEs were deployed throughout the Cleveland (OH) area on the southern shore of Lake Erie from June to September of 2013. Extracts from PEs were analyzed by gas chromatography coupled with mass spectrometry (GC/MS) for a suite of PAHs and OFRs and were also analyzed via an *in vitro* bioassay to measure AhR activation. The objectives of this study were to (i) investigate the use of PEs as a viable vehicle for isolating gaseous HOCs for use in *in vitro* bioassays, (ii) explore whether AhR-mediated activity of PE extracts correlated significantly with any PAHs or OFRs measured in the extracts, and (iii) determine what portion of AhR-mediated activity measured via *in vitro* bioassays could be predicted based on targeted chemical analysis of commonly monitored PAHs.

We expected that AhR-mediated potency and gaseous concentrations of OFRs and PAHs in PE extracts would be greatest at densely populated urban sites located near the city center and that some correlation would be seen between gaseous PAH concentrations and potency. However, based on previous studies, we expected that BaP-equivalents calculated from targeted PAH chemical analysis (BaP-Eq_{chem}) would likely underestimate the potency observed in bioassay experiments. We also expected that, unlike in particulate air samples, AhR-mediated potency of PE extracts would not correlate significantly with BaP concentrations, as BaP was not expected to be present at significant levels in the gaseous phase. Furthermore, we hypothesized that gas-phase OFRs may account for some fraction of AhR activity unexplained by commonly monitored PAHs, and that this would be indicated by significant correlation between OFR concentrations and AhR activity.

MATERIALS AND METHODS

Passive air sampler deployment

800- μ m-thick low-density polyethylene sheeting (United Plastics, Inc.) was cut into approximately 7.5 cm x 13 cm pieces and cleaned in solvent (DCM and hexane) to remove background contamination. At each of nine sampling sites throughout the Cleveland area, four PEs were fastened inside an inverted stainless steel bowl using zip-ties and the bowl was suspended so that the PEs were hanging at approximately 2 m height.

In order to calculate ambient air concentrations from concentrations measured in deployed PEs, performance reference compounds (PRCs) are often added to the PE for *in situ* calibration of sampling rates. However, PRCs could not be added to the PEs intended for bioassays because these compounds would interfere with bioassay response. Therefore, 50- μ m-thick PEs, preloaded with PRCs by incubation in an 80:20 methanol:water solution, were co-deployed at each site, and sampling rates determined for these 50- μ m PEs were used to interpret results from 800- μ m PEs.

A map of the study region is shown in the Supplementary Information (SI Figure S1) and characteristics of the deployment sites are summarized in Table 1. Deployments took place

from June to September of 2013, with each set of PEs deployed for about 60 days. After deployment, PEs were removed from the protective bowl, wrapped in precombusted aluminum foil, and shipped on ice overnight to the University of Rhode Island Graduate School of Oceanography, where they were kept frozen until extraction.

Sample preparation

Each 800- μ m PE was extracted twice in pentane, each time for 18–24 hours. 50- μ m PEs were extracted once for 18–24 hours in pentane. Every batch of PEs was extracted along with a laboratory blank, which was a PE that had been cleaned alongside the field samples and then stored frozen in precombusted aluminum foil. All four 800- μ m PEs deployed simultaneously at the same site were composited into one extract and concentrated to 1 mL in a warm water bath under a gentle stream of nitrogen. Extracts from 800- μ m PEs appeared to contain a white precipitate, possibly from co-extracted polyethylene material. To remove the particulate, extracts were serially frozen, causing the precipitate to solidify at the bottom of the vial, and the overlying liquid was removed via Pasteur pipet and reconstituted to 1 mL with pentane. Two aliquots were removed from the 1 mL solution: one for chemical analysis and the other for biological analysis. A schematic summarizing sample preparation is shown in the Supplementary Material (Figure S2).

Chemical analysis by GC/MS

The fraction of PE extract intended for chemical analysis was spiked with internal standards acenaphthene- d_{10} , phenanthrene- d_{10} , chrysene- d_{12} , and perylene- d_{12} and analyzed on an Agilent 6890 GC coupled to an Agilent 5973 MSD in electron impact (EI, 70 eV) mode for 22 PAHs, 18 alkylated PAHs, and (in a separate GC/MS run) 12 organophosphate esters (OPEs) using an Agilent J&W DB-5 fused capillary column (30 m x 0.25 mm I.D.). PAHs were quantified using an 8-point calibration curve with linearity $r^2 > 0.990$ for all compounds. OPEs were quantified using a 10-point calibration curve with linearity $r^2 > 0.997$ for all compounds except TDBPP, which was not detected in samples and is omitted from discussion.

Extracts were also spiked with non-native polybrominated diphenyl ethers (BDEs 35, 77, 128, and 183) and analyzed on an Agilent 7890 GC coupled to an Agilent 5977 MSD in negative chemical ionization (NCI) mode with methane reagent gas for 12 polybrominated diphenyl ethers (BDEs) and 8 novel halogenated flame retardants (NHFRs), as well as 3 polybrominated biphenyls (PBBs), which were used as PRCs in sampling rate determination for co-deployed thin PEs. A complete list of target compounds and abbreviations is available in the Supplementary Material (Table S1). BDEs and NHFRs were quantified using an 8-point calibration curve with linearity $r^2 > 0.995$.

To avoid interference with biological assays, samples were not spiked with internal standard prior to extraction and so were not corrected for internal standard recoveries. Concentrations presented for PE extracts were not blank-subtracted before use in data interpretation. This was considered appropriate as our primary interest was in determining the actual concentration present in the bioassay exposure solution.

Calculation of ambient air concentrations

The composition of HOCs accumulated in polyethylene differs from the ambient composition of gas-phase HOCs in air because the concentration in polyethylene is dependent not only on gas-phase concentrations, but also on the affinity of each compound for the PE matrix and the rate at which the compound is absorbed into the PE. To compare the composition of solutions used in bioassay experiments to the actual composition of gaseous HOCs expected in ambient air, gaseous HOC concentrations were calculated based on the results of chemical analysis of PE extracts and PE sampling rates determined from co-deployed PRC-loaded PEs. Concentrations were blank-subtracted using the co-extracted laboratory PE Blank. After blank subtraction, concentrations below 25% of the PE Blank were considered <DL, and all <DL values were replaced with 0.

To translate concentrations within the PE to concentrations in ambient air, the volume of air sampled by each PE during deployment was estimated using data on the percent loss of labeled PRCs from co-deployed 50- μ m thick PEs. From the PRC loss data, the best-fit value for the thickness of the diffusive boundary layer (DBL) at the air-PE interface was determined. Because all PEs were deployed under the same conditions and the thickness of the PE sheet does not affect air-side resistance, the DBL thickness determined for thin sheets was then used in a two-film model describing PE-side and air-side mass transfer rates to calculate the percent equilibration reached by each target compound in the 800 μ m-thick PEs. This approach for estimation of percent equilibration from PRC loss data has been described in detail in previous work (McDonough et al. 2016).

Biological analysis by reporter cell bioassay

Aliquots for biological analysis were mixed with 200 μ L of DMSO and blown down under a gentle stream of nitrogen to constant volume. This stock solution was then used to create a 10-point dilution curve (0.01 g PE/mL – 120 g PE/mL) for each sample, including the PE blank (Figure S1).

The AhR reporter cell line used was H1G1.1c3, a murine hepatoma cell line consisting of Hepa-1c1c7 cells stably transfected with AhR-responsive green fluorescent protein (GFP) reporter gene (Nagy et al. 2002). Cells were plated in 96-well plate (3×10^5 cells per well; Costar 96-well black plate with a clear bottom) and allowed to attach overnight at 37°C in selective medium (Nagy et al. 2002). The medium was then changed to non-selective medium and the cells in 100 μ L of medium were treated with 1 μ L of each sample dilution for a final vehicle concentration of 1% DMSO. All wells were prepared in triplicate and incubated at 33°C. For each test extract, the cells in three wells were treated with 1 μ L of DMSO as a negative control, and the cells in another set of three wells were left untreated to control for any natural cell fluorescence. On each plate, three wells were treated with BaP at a final well concentration of 120 nM dissolved in DMSO as a positive control. On one plate, a 10-point dilution curve was also run for BaP (1.2×10^{-5} – 12000 nM), and results were normalized to the positive control 120 nM BaP (Figure S3).

AhR-mediated activity was measured by reading the GFP fluorescence emitted by the cells at 515 nm using a Spectra Max M3 plate reader at 24 and 48 hours post dosing (hpd). The

mean fluorescence value of the DMSO-treated negative control triplicate wells was subtracted from each sample's fluorescence reading, and the response was expressed as a ratio over the mean fluorescence value for the triplicate 120 nM BaP positive controls run on the same plate to control for plate-to-plate differences in cell response.

Calculation of extract potency

Data from 48-hpd readings were fitted to a four-parameter log-logistic concentration-response model with the lower bound set to 0 using R package *drc* (Ritz et al. 2015). The response f occurring as a result of concentration x is modeled as in Equation 1, where c is the lower bound value (set to 0), d is the upper bound value, b determines slope steepness, and e is the concentration achieving 50% of maximum efficacy (EC_{50}). The upper bound was set to the maximum observed response in cases where response reached a plateau or decreased at highest dosages, but was not defined for the extract from site Cleveland Lakefront 1 because response continued increasing up to the maximum extract concentration.

$$f(x) = \frac{d - c}{1 + \exp(b(\log(e) - \log(x)))} \quad \text{Eq 1}$$

In addition to the EC_{50} , the EC_{BaP50} was calculated as an alternative measure of potency. The EC_{BaP50} is the concentration resulting in 50% of the effect observed for the plate-specific positive control (120 nM BaP). The EC_{BaP50} was identified as a more useful metric than EC_{50} because the extracts' concentration-response curves were not parallel and maximum efficacy varied among curves.

Dosing solutions were prepared so that each sample was representative of the same amount of extracted PE to facilitate comparison with the PE blank and control for any interference caused by background contamination in the PE matrix. However, due to site-to-site variability in sampling rates, the volume of air represented by each sample differed among sites (Table 1). For this reason, after determination of EC_{BaP50} from the concentration-response curve fit, EC_{BaP50} values were normalized based on the volume of air sampled at each site. Aliquots of PE extracts used in dosing solutions were representative of 1900–3100 m³ of air, and were all normalized to 2000 m³.

To compare predicted AhR-mediated potency based on chemical composition to observed potency based on bioassay experiments, BaP equivalents were calculated for both sets of data. For concentrations measured via chemical analysis, BaP equivalents in each mixture ($BaP-Eq_{chem}$) were determined as in Equation 2 by multiplying the concentration of each compound in the PE extract (C_n) by the compound's potency relative to BaP (expressed as induction equivalency factor, IEF_n) using values from Machala et al. (2001) and summing results for all compounds. Benzo[b]fluoranthene, benzo[k]fluoranthene, and benzo[j]fluoranthene could not be quantitated separately with confidence via the chromatographic method used, so the IEFs for these three compounds were averaged as an

estimated of the IEF for benzo[b,j,k]fluoranthene. Concentrations in the PE extract were normalized based on volume of air sampled before BaP-Eq_{chem} calculations were done.

$$BaP - Eq_{chem} = \Sigma(IEF_n \cdot C_n)(ng/\mu L) \quad \text{Eq 2}$$

For the bioassay results, the BaP equivalent of each sample extract was expressed as the amount of BaP needed to achieve the same response as the extract. The bioassay-derived toxic equivalency (BaP-Eq_{bio}) was calculated as in Equation 3 as the ratio of the amount of BaP needed to achieve a response of 50% the maximum efficacy (EC₅₀ for the BaP curve) over the volume of PE extract added to the well to achieve that same effect (the EC_{BaP50} of the extract).

$$BaP - Eq_{bio} = \frac{EC_{BaP50}[BaP](ng/well)}{EC_{BaP50}[Extract](\mu L/well)} \quad \text{Eq 3}$$

The degree to which chemical analysis explained observed potency (%_{chem}) was then expressed as in Equation 4.

$$\%_{chem} = \frac{BaP - Eq_{chem}}{BaP - Eq_{bio}} \cdot 100 \quad \text{Eq 4}$$

RESULTS AND DISCUSSION

Chemical composition of passive sampler extracts

Concentrations of all compounds in PE extracts are presented in the Supplementary Material for PAHs (Table S2), OPEs (Table S3), and halogenated flame retardants (HFRs; Table S4). Concentrations of PAHs and OPEs in the PE extracts are displayed in Figure 1 (left side) along with estimated ambient air concentrations (right side). All concentrations for field samples were normalized to an air volume of 2000 m³ to facilitate comparison between sites.

Total alkyl and parent PAHs (Σ_{40} PAH) in PE extracts ranged from 3.6 ng/ μ L for the extract from Cuyahoga National Park to 34 ng/ μ L for a residential suburban area in University Heights. Concentrations of PAHs were dominated by phenanthrene (0.6–16.3 ng/ μ L; 10–57%), fluoranthene (0.1–6 ng/ μ L; 1–18%), 2-methylphenanthrene (0.1–1 ng/ μ L; 1–6%), and fluorene (0.3–1 ng/ μ L; 3–9%).

Concentrations of OPEs were much greater than those of halogenated organic flame retardants (HFRs). Total OPEs (Σ_{12} OPE) ranged from 0.4 ng/ μ L for the extract from Cuyahoga National Park to 2.0 ng/ μ L for a residential area in Kent. Σ_{12} OPE was dominated by TPHP at all downtown Cleveland sites (0.09–0.78 ng/ μ L; 28–69%), while Cuyahoga National Park and Fairport Harbor were dominated by TEHP (0.30 – 0.57 ng/ μ L; 68%), and

University Heights and Kent were dominated by tri-n-butyl phosphate (TNBP; 0.50 ng/ μ L; 59%) and TCIPP (1.60 ng/ μ L; 78%), respectively. Concentrations of total BDEs (Σ_{12} BDE) ranged from 10 pg/ μ L in Cuyahoga National Park to 46 pg/ μ L at Downtown Cleveland Site 2, and were dominated by BDE 47 and 154. Concentrations of total NHFRs (Σ_{18} NHFRs) were greatest in the PE blank due to the presence of 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) and Dechlorane Plus, which were not found in any of the field sample extracts.

2-ring, 3-ring, and 4-ring PAHs, as well as their alkylated and substituted counterparts, were generally correlated in the different extracts ($0.3 < r^2 < 0.9$), while 5–6-ring PAHs did not exhibit significant correlation among themselves or with any other group of PAHs (Table S5). Correlation among individual PAHs was expected, as they are typically emitted from the same sources. Correlation among PAHs was further confirmed by principal component analysis (PCA), which showed that 76% of variation in samples was explained by two principal components, the first with loadings primarily from 3–4-ring PAHs, and the second with loadings primarily from 2-ring and 4–5-ring PAHs (Figure S4). In contrast, individual OPEs were generally not significantly correlated, though some degree of correlation ($r^2 < 0.3$) was observed between TDCIPP and TNBP (Table S6). Additionally, TNBP, TDCIPP, and ethylhexyl diphenyl phosphate (EHDPP) exhibited some correlation with PAHs (Table S7).

Ambient air concentrations

Ambient gaseous concentrations of Σ_{40} PAH ranged from 7.1 ng/ m^3 in Cuyahoga National Park to 36.2 ng/ m^3 at urban site Cleveland Downtown 1 and were dominated by the methylnaphthalenes (1.7–8.8 ng/ m^3 ; 18–33%), phenanthrene (0.3–9.8 ng/ m^3 ; 2–33%), and fluorene (0.5–2.6 ng/ m^3 ; 5–14%). Concentrations were similar in range to those measured by Peverly et al. in Chicago using polyurethane foam passive samplers (PUFs) in 2012 – 2014 (Σ_{16} PAH = 9 – 52 ng/ m^3), and by Melymuk et al. in Toronto in 2007 – 2008 (Σ_{27} PAH = 0.3 – 51 ng/ m^3), also using PUFs (Melymuk et al. 2012; Peverly et al. 2015).

Concentrations in this study were similar but lower than previous measurements of total gaseous PAHs using PEs in the downtown Cleveland area by McDonough et al. in 2012 (Σ_{15} PAH = 23–80 ng/ m^3 ; McDonough et al. 2014). In larger-scale regional studies, atmospheric concentrations of PAHs have often been found to correlate with population density (Hafner et al. 2005; McDonough et al. 2014), but here no significant ($p < 0.05$) correlation between gaseous PAH concentrations and population density within 5–30 km was observed.

Gaseous concentrations of Σ_{12} OPE ranged from 0.01 ng/ m^3 in Cuyahoga National Park to 1.1 ng/ m^3 in Kent. This was similar in range to measurements by Peverly et al. in Chicago using PUFs in 2012–2014 (Σ_{13} OPE = 0.5 – 1.5 ng/ m^3), and slightly lower than measurements of particulate Σ_{12} OPE in the Cleveland area by Salamova et al. in 2012 (mean Σ_{12} OPE = 2.1 ± 0.4 ng/ m^3 ; Salamova et al. 2014; Peverly et al. 2015). TCIPP was the most abundant OPE at all sites (0.01–1.0 ng/ m^3 ; 9–98%) except University Heights, where TNBP dominated (0.6 ng/ m^3 ; 87%). TCIPP was also found to be most abundant in Cleveland particulate Σ_{12} OPE in a previous study (0.85 ± 0.3 ng/ m^3 ; Salamova et al. 2014).

Figure 1 compares the chemical composition of the PE extracts used in bioassay experiments and of ambient gaseous PAHs and OPEs. Extracts used in bioassays were enriched in moderately hydrophobic compounds, such as fluoranthene and TDCIPP, which make up a lower percentage of total HOCs in the gaseous fraction of ambient air but have a greater affinity for the PE matrix. The different HOC composition in the gas-phase and in the PE extract illustrates that it is not possible to estimate the total AhR-mediated potency of the mixture that is present in gas-phase air. However, AhR-mediated potency results based on the HOC mixture found in PE extracts is still an important step in understanding the biological relevancy of gas-phase compounds. Furthermore, the composition in PE extracts is expected to be more similar to the composition of HOCs diffusing into plant material or skin from air, or accumulating in the body via other mechanisms.

Concentration-response curves

Extracts from all PEs, including the PE Blank, induced concentration-dependent activation of AhR-dependent GFP. All concentration-response data are displayed along with curve fits and 95% confidence intervals in Figure 2, with response represented as a ratio compared to response elicited by the plate-specific positive control. For all extracts, an initial increase in GFP induction was seen with increasing concentration. However, there was a precipitous decline in the fluorescence for all extracts (except Cleveland Lakefront 1) at the greatest concentrations, possibly due to cytotoxicity or inhibition of fluorescence response at high concentrations of PE extract. These points were omitted during concentration-response curve fitting, as we were interested in determining only the induction potencies of the extracts. Most extracts did not exhibit a clear plateau in response, making determination of maximum efficacy, as well as EC_{50} , somewhat uncertain. Furthermore, maximum efficacy of the samples varied from 94%–230% of positive control response (Table 2). For this reason, EC_{BaP50} , measured relative to the plate-specific positive control, was used to compare the potencies of the samples.

The EC_{BaP50} of each extract, normalized based on the volume of air sampled at each site, is displayed in Table 2 along with each extract's maximum observed efficacy. Values of EC_{BaP50} ranged from 0.5 ± 0.1 g PE/mL at Downtown Cleveland 1 to 6.6 ± 1.2 g PE/mL at Cuyahoga National Park.

The three rural/residential sites had the lowest potency (greatest EC_{BaP50} values), ranging from 2.6 – 6.6 g PE/mL, followed by the two Cleveland Lakefront sites. The most potent extracts were from the three Cleveland Downtown sites and one semi-urban residential site (University Heights, a densely populated suburb). This contrasts with work by Klein et al., where no change in potency of gaseous extracts was observed between urban and rural samples with distinct chemical compositions, but is consistent with work by Ersekova et al, where extracts from impacted sites were found to be more potent in AhR bioassays than extracts from rural sites (Klein et al. 2005; Erseková et al. 2014). The potency of the PE Blank ($EC_{BaP50} = 23 \pm 5$ g PE/mL) was significantly lower than all field samples. Blank comparisons were done before normalizing for the volume of air sampled so that each sample would be representative of the same mass of extracted polyethylene.

The potency and maximum efficacy of the extracts did not appear to be correlated. This is most likely due to a complex interplay between the unique composition of ligands in each sample, their affinity for the AhR, the resulting ligand-receptor complex's ability to bind other necessary transcription factors, and cytotoxicity of specific components. Response could also be affected by ligands interacting with other pathways that could amplify or dampen AhR response. Klein et al. also observed a lack of correlation between potency of extracts and maximum efficacy with respect to AhR binding of gas-phase extracts from active air sampling (Klein et al. 2005).

Initial bioassay experiments demonstrated that the treated cells' fluorescence responses increased over time from 16 to 48 hpd, so all responses reported here were measured at 48 hpd. This is in contrast to other studies of AhR activation for environmental samples, most of which have used a luciferase reporter rather than the GFP reporter used here. For example, Machala et al. measured greatest potency at 6 hpd, most likely due to PAH metabolism (Machala et al. 2001) and Kennedy et al. observed steadily decreasing potency in extracts from 24 to 72 hpd (Kennedy et al. 2010). This discrepancy is most likely due to differences in induction kinetics and increased stability of the GFP reporter compared to the luciferase reporter (Han et al. 2004). It is also possible that some of the response observed in this study was due to compounds that were less readily metabolized than PAHs and OPEs.

Bioassay-derived BaP equivalents for PE extracts

A map of results for BaP-Eq_{bio} is displayed alongside maps of total concentrations of PAHs and OPEs in the PE extracts (Σ_{40} PAH and Σ_{12} OPE) in Figure 3. BaP-Eq_{bio} values ranged from 21–283 ng/ μ L BaP equivalents and were generally greatest in the downtown Cleveland area and lowest at the rural/residential sites further from the city center.

BaP-Eq_{bio} values were compared to concentrations of PAHs and organic flame retardants (OPEs, PBDEs, and NHFRs) in the PE extracts to determine whether there was any significant correlation between potency and chemical composition. Though some correlations were found, few were likely to be driving potency. No correlations with PBDE and NHFR concentrations were observed. BaP-Eq_{bio} weakly correlated only with 2-ring alkyl/substituted PAHs ($r^2 = 0.42$; $p < 0.1$; SE = 64; N = 9) and also displayed some correlation with EHDPP ($r^2 = 0.66$; $p < 0.01$; SE = 49; N = 9). Maximum efficacy of PE extracts showed some correlation with concentrations of 3-ring ($r^2 = 0.61$; $p < 0.05$; SE = 31; N = 9) and 4-ring ($r^2 = 0.48$; $p < 0.05$; SE = 36; N = 9) parent PAHs. Correlations between BaP-Eq_{bio} and alkyl/substituted PAHs were only investigated by grouping compounds (2-ring alkyl/substituted PAHs; 3–4-ring alkyl/substituted PAHs) because quantitative standards were not available for all alkylated PAHs. However, it is important to note that AhR-mediated potency differs greatly between PAH isomers. Because there is a high degree of correlation observed between different low molecular weight PAHs at different locations in this study (Table S5), it was expected that the composition of alkyl/substituted PAHs is most likely similar between sites, so correlations with BaP-Eq_{bio} are likely driven by the same compounds at all sites.

There is little information available regarding the biological effects of alkylated PAHs. Recent studies using a yeast reporter assay system and a H4IIE-*luc* reporter-gene assay

suggest that methyl- and dimethyl-substituted phenanthrenes are in some cases more potent with respect to AhR activation than their unsubstituted counterparts (Sun et al. 2014; Lam et al. 2018). The statistically significant correlation between BaP-Eq_{bio} and EHDPP suggests that this compound, or unmonitored compounds with which it covaries spatially, could be contributing to AhR activity. As no compelling evidence is available for EHDPP as an AhR activator, the presence of other AhR activators that covary with EHDPP is somewhat more likely. Previous studies have shown that levels of OPEs and other OFRs can correlate in air due to their historical use in the same formulations (Salamova et al. 2014). Additionally, some OPEs that were not targeted in this study, including mono-substituted isopropyl triaryl phosphate (mITP), have been shown to have relatively strong AhR activity (Gerlach et al. 2014; Haggard et al. 2017).

Predicted BaP equivalents from chemical analysis

The BaP-Eq_{chem} of each PE extract was calculated based on concentrations of targeted PAHs from GC/MS analysis. No dataset for the specific cell line used here was available, so IEFs were taken from Machala et al. (2001), who measured PAH-induced AhR-mediated response in a rat hepatoma H4IIE cell line stably transfected with luciferase reporter gene. IEFs were not available for all PAHs, so calculated BaP-Eq_{chem} values are representative of only 14 compounds (Table S8). While the dataset from Machala et al. is the most applicable that could be found, these IEFs come from a cell line with a completely different time-dependent expression profile and are not directly applicable to the cell line used here. This contributes greatly to the uncertainty in the derived BaP-Eq_{chem} values, and highlights the need for more studies providing cell line-specific IEFs for a wide range of ubiquitous environmental contaminants.

BaP-Eq_{chem} values calculated using potencies from Machala et al. ranged from 1.6 to 7.9 ng/ μ L BaP, as shown in Table 3. The percent of BaP-Eq_{bio} accounted for by this BaP-Eq_{chem} is also displayed. The percent contributions of individual PAHs to the total predicted BaP-Eq_{chem} are displayed in Figure 4. Among the targeted PAHs, contributions to BaP-Eq_{chem} were dominated by high molecular weight PAHs that were present at low concentrations in the PE extracts, including dibenz(a,h)anthracene (DBA), indeno(1,2,3-c,d)pyrene (IND), benzo(b,k)fluoranthene (BBKFLRA), and chrysene (CHRY).

Potencies calculated from known chemical composition using IEFs explained only 2–23% of the AhR-mediated potency observed in bioassay experiments (Table 3), and BaP-Eq_{chem} and BaP-Eq_{bio} were not significantly correlated. This suggests that other compound groups present in the gaseous fraction of ambient air may also be contributing to BaP-Eq_{bio} of the extracts. These may include additional parent PAHs and alkyl-PAHs not measured in this study, as well as oxygenated PAHs and N- and S-heterocyclic PAHs (Larsson et al. 2014; Sun et al. 2014; Lam et al. 2018). Compounds other than PAHs may also be responsible for some of the observed AhR-mediated potency. The use of BaP-Eq_{chem} values derived from a different bioassay may also contribute to this discrepancy.

The correlation observed between concentrations of EHDPP and AhR activity suggests that this compound, or other OFRs with similar source, may be contributing to BaP-Eq_{bio} as well, though further research is needed to understand the AhR-mediated potency of OFRs.

Furthermore, a major weakness of predicting potency based on compound IEFs is that it considers only additive interactions, without taking into account synergistic and antagonistic effects, which are highly probable in complex environmental mixtures. This, along with the scarcity of IEF values for the targeted compounds, most likely contributed to the discrepancy between observed and predicted AhR-mediated potency.

CONCLUSIONS

This study demonstrated the use of PEs coupled with *in vitro* bioassays as an approach to measure cumulative biological effects of ambient gaseous air pollution. While some AhR-mediated activity was seen in the PE blank, the activity of field samples was found to be significantly elevated above blank levels, suggesting that interference from the PE matrix or typical laboratory contamination did not prohibit the use of PE extracts in bioassays for AhR activation. In future studies using this approach, a thinner PE sheet (~ 50 µm) may be preferable to avoid extra cleanup steps caused by PE precipitate in the final extract, as thinner PEs contain less PE mass and require less time for extraction. In addition, future work employing effect-directed analysis, as has been used in passive sampling studies of wastewater (Sonavane et al. 2018), could aid in identifying contaminants driving observed biological effects.

AhR-mediated potency varied significantly between different sites and was greatest in downtown Cleveland. Potency of the extracts displayed some correlation with PAHs common in the gaseous phase, as well as EHDPP, though causative links were difficult to establish. This work highlights the importance of learning more about the AhR-mediated potency of emerging contaminants that are present at elevated concentrations in urban ambient air, including OPEs and other OFRs. This study further supports previous studies suggesting that the BaP-Eq_{chem} approach underestimates risks of exposure to environmentally-relevant chemical mixtures, as AhR activation caused by organic contaminants in a mixture may be augmented by other unmonitored chemicals in the mixture and their unforeseen interactions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment:

We acknowledge funding from the US EPA Great Lakes Restoration Initiative (GLRI) GLAS #00E00597-0, project officer Todd Nettesheim. Funding was also provided by National Institute of Environmental Health Sciences (NIEHS) grant P42ES007381 (Superfund Basic Research Program at Boston University). We thank Angela Slitt (URI) for consulting on experimental design and Mike Denison (UC Davis) for providing H1G1 cells used in bioassays.

REFERENCES

Andrysík Z, Vondráček J, Marvanová S, Ciganek M, Nešpor J, Pánková K, Mahadevan B, Topinka J, Baird WM, Kozubík A, et al. 2011 Activation of the aryl hydrocarbon receptor is the major toxic mode of action of an organic extract of a reference urban dust particulate matter mixture: The role

of polycyclic aromatic hydrocarbons. *Mutat Res - Fundam Mol Mech Mutagen* 714(1–2):53–62. 10.1016/j.mrfmmm.2011.06.011.

- Barrado AI, García S, Sevillano ML, Rodríguez JA, Barrado E. 2013 Vapor-phase concentrations of PAHs and their derivatives determined in a large city: Correlations with their atmospheric aerosol concentrations. *Chemosphere* 93(9):1–7. doi: 10.1016/j.chemosphere.2013.05.031 . [accessed 2013 Jul 10]. 10.1016/j.chemosphere.2013.05.031http://www.ncbi.nlm.nih.gov/pubmed/23816454. [accessed 2013 Jul 10]. http://www.ncbi.nlm.nih.gov/pubmed/23816454 . [PubMed: 23706401]
- Boström C-E, Gerde P, Hanberg A, Jernström B, Johansson C, Kyrklund T, Rannug A, Törnqvist M, Victorin K, Westerholm R. 2002 Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. *Environ Health Perspect* 110(s3):451–489. 10.1289/ehp.02110s3451. [PubMed: 12060843]
- Brommer S, Jantunen LM, Bidleman TF, Harrad S, Diamond ML. 2014 Determination of vapor pressures for organophosphate esters. *J Chem Eng Data* 59(5):1441–1447. 10.1021/je401026a.
- California Office of Environmental and Health Hazard Assessment. 2017 Proposition 65 List [accessed 2017 Jan 1]. <https://oehha.ca.gov/proposition-65/proposition-65-list>.
- Center for International Earth Science Information Network (CIEISIN), Columbia University, International Food Policy Research Institute (IFPRI), The World Bank, Centro Internacional de Agricultura Tropical (CIAT). 2011 Global Rural-Urban Mapping Project, Version 1 (GRUMPv1): Population Count Grid
- Chen G, Bunce NJ. 2003 Polybrominated diphenyl ethers as Ah receptor agonists and antagonists. *Toxicol Sci* 76(2):310–320. 10.1093/toxsci/kfg236. [PubMed: 14514964]
- Denison MS, Soshilov AA, He G, Degroot DE, Zhao B. 2011 Exactly the same but different: Promiscuity and diversity in the molecular mechanisms of action of the aryl hydrocarbon (dioxin) receptor. *Toxicol Sci* 124(1):1–22. 10.1093/toxsci/kfr218. [PubMed: 21908767]
- Érsekóvá A, Hilscherová K, Klánová J, Giesy JP, Novák J. 2014 Effect-based assessment of passive air samples from four countries in Eastern Europe. *Environ Monit Assess* 186(6):3905–16. doi: 10.1007/s10661-014-3667-z . [accessed 2015 Jun 23]. 10.1007/s10661-014-3667-zhttp://www.ncbi.nlm.nih.gov/pubmed/24532343. [accessed 2015 Jun 23]. http://www.ncbi.nlm.nih.gov/pubmed/24532343 . [PubMed: 24532343]
- Farhat A, Buick JK, Williams A, Yauk CL, O'Brien JM, Crump D, Williams KL, Chiu S, Kennedy SW. 2014 Tris(1,3-dichloro-2-propyl) phosphate perturbs the expression of genes involved in immune response and lipid and steroid metabolism in chicken embryos. *Toxicol Appl Pharmacol* 275(2):104–12. doi: 10.1016/j.taap.2013.12.020 . [accessed 2014 Sep 11]. 10.1016/j.taap.2013.12.020http://www.ncbi.nlm.nih.gov/pubmed/24407104. [accessed 2014 Sep 11]. http://www.ncbi.nlm.nih.gov/pubmed/24407104 . [PubMed: 24407104]
- Farhat A, Crump D, Chiu S, Williams KL, Letcher RJ, Gauthier LT, Kennedy SW. 2013 In ovo effects of two organophosphate flame Retardants-TCPP and TDCPP-on pipping success, development, mRNA expression, and thyroid hormone levels in chicken embryos. *Toxicol Sci* 134(1):92–102. 10.1093/toxsci/kft100. [PubMed: 23629516]
- Gerlach CV, Das SR, Volz DC, Bisson WH, Kolluri SK, Tanguay RL. 2014 Mono-substituted isopropylated triaryl phosphate, a major component of Firemaster 550, is an AHR agonist that exhibits AHR-independent cardiotoxicity in zebrafish. *Aquat Toxicol* 154:71–79. 10.1016/j.aquatox.2014.05.007. [PubMed: 24865613]
- Gold MD, Blum A, Ames BN. 1978 Another flame retardant, tris-(1,3-dichloro-2-propyl)-phosphate, and its expected metabolites are mutagens. *Science* 200(4343):785–787. 10.1126/science.347576. [PubMed: 347576]
- Gu C, Goodarzi M, Yang X, Bian Y, Sun C, Jiang X. 2012 Predictive insight into the relationship between AhR binding property and toxicity of polybrominated diphenyl ethers by PLS-derived QSAR. *Toxicol Lett* 208(3):269–274. 10.1016/j.toxlet.2011.11.010. [PubMed: 22119921]
- Gungormus E, Tuncel S, Hakan Tecer L, Sofuoğlu SC. 2014 Inhalation and dermal exposure to atmospheric polycyclic aromatic hydrocarbons and associated carcinogenic risks in a relatively small city. *Ecotoxicol Environ Saf* 108:106–113. 10.1016/j.ecoenv.2014.06.015. [PubMed: 25046852]
- Hafner WD, Carlson DL, Hites RA. 2005 Influence of local human population on atmospheric polycyclic aromatic hydrocarbon concentrations. *Environ Sci Technol* 39(19):7374–7379. doi:

10.1021/es0508673 . [accessed 2012 Dec 3]. 10.1021/es0508673http://pubs.acs.org/doi/abs/10.1021/es0508673. [accessed 2012 Dec 3]. http://pubs.acs.org/doi/abs/10.1021/es0508673 . [PubMed: 16245804]

Haggard DE, Das SR, Tanguay RL. 2017 Comparative toxicogenomic responses to the flame retardant mTTP in developing zebrafish. *Chem Res Toxicol* 30(2):508–515. 10.1021/acs.chemrestox.6b00423. [PubMed: 27957850]

Han D, Nagy SR, Denison MS. 2004 Comparison of recombinant cell bioassays for the detection of Ah receptor agonists. *BioFactors* 20(1):11–22. 10.1002/biof.5520200102. [PubMed: 15096657]

Joyce AS, Portis LM, Parks AN, Burgess RM. 2016 Evaluating the relationship between equilibrium passive sampler uptake and aquatic organism bioaccumulation. *Environ Sci Technol* 50(21):11437–11451. 10.1021/acs.est.6b03273. [PubMed: 27680295]

Kennedy K, Macova M, Bartkow ME, Hawker DW, Zhao B, Denison MS, Mueller JF. 2010 Effect based monitoring of seasonal ambient air exposures in Australia sampled by PUF passive air samplers. *Atmos Pollut Res* 1(1):50–58. 10.1007/s10661-014-3667-z. [PubMed: 21552507]

Klein GP, Hodge EM, Diamond ML, Yip A, Dann T, Stern G, Denison MS, Harper PA. 2005 Gas-phase ambient air contaminants exhibit significant dioxin-like and estrogen-like activity in vitro. *Environ Health Perspect* 114(5):697–703. doi: 10.1289/ehp.8496 . [accessed 2012 Nov 30]. 10.1289/ehp.8496http://www.ehponline.org/ambra-doi-resolver/10.1289/ehp.8496. [accessed 2012 Nov 30]. http://www.ehponline.org/ambra-doi-resolver/10.1289/ehp.8496 .

Kobayashi R, Cahill TM, Okamoto RA, Maddalena RL, Kado NY. 2007 Controlled exposure chamber study of uptake and clearance of airborne polycyclic aromatic hydrocarbons by wheat grain. *Environ Sci Technol* 41(22):7934–7940. 10.1021/es071459x. [PubMed: 18075111]

Kobayashi R, Okamoto RA, Maddalena RL, Kado NY. 2008 Polycyclic aromatic hydrocarbons in edible grain: A pilot study of agricultural crops as a human exposure pathway for environmental contaminants using wheat as a model crop. *Environ Res* 107(2):145–151. 10.1016/j.envres.2007.11.002. [PubMed: 18295196]

Lam MM, Bülow R, Engwall M, Giesy JP, Larsson M. 2018 Methylated PACs are more potent than their parent compounds: A study of aryl hydrocarbon receptor-mediated activity, degradability, and mixture interactions in the H4IIE-luc assay. *Environ Toxicol Chem* 37(5):1409–1419. 10.1002/etc.4087. [PubMed: 29334126]

Larsson M, Hagberg J, Giesy JP, Engwall M. 2014 Time-dependent relative potency factors for polycyclic aromatic hydrocarbons and their derivatives in the H4IIE-luc bioassay. *Environ Toxicol Chem* 33(4):943–953. 10.1002/etc.2517. [PubMed: 24408822]

Liu C, Wang Q, Liang K, Liu J, Zhou B, Zhang X, Liu H, Giesy JP, Yu H. 2013 Effects of tris(1,3-dichloro-2-propyl) phosphate and triphenyl phosphate on receptor-associated mRNA expression in zebrafish embryos/larvae. *Aquat Toxicol* 128–129:147–57. doi: 10.1016/j.aquatox.2012.12.010 . [accessed 2014 Sep 18]. 10.1016/j.aquatox.2012.12.010http://www.ncbi.nlm.nih.gov/pubmed/23306105. [accessed 2014 Sep 18]. http://www.ncbi.nlm.nih.gov/pubmed/23306105 .

Machala M, Vondráček J, Bláha L, Cigánek M, Nešpor J. 2001 Aryl hydrocarbon receptor-mediated activity of mutagenic polycyclic aromatic hydrocarbons determined using in vitro reporter gene assay. *Mutat Res - Genet Toxicol Environ Mutagen* 497(1–2):49–62. 10.1016/S1383-5718(01)00240-6.

Matsumoto Y, Ide F, Kishi R, Akutagawa T, Sakai S, Nakamura M, Ishikawa T, Fujii-Kuriyama Y, Nakatsuru Y. 2007 Aryl hydrocarbon receptor plays a significant role in mediating airborne particulate-induced carcinogenesis in mice. *Environ Sci Technol* 41(10):3775–3780. 10.1021/es062793g. [PubMed: 17547212]

McDonough CA, Khairy MA, Muir DCG, Lohmann R. 2014 Significance of population centers as sources of gaseous and dissolved PAHs in the lower Great Lakes. *Environ Sci Technol* 48(14):7789–7797. 10.1021/es501074r. [PubMed: 24918966]

McDonough CA, Puggioni G, Helm PA, Muir D, Lohmann R. 2016 Spatial distribution and air-water exchange of organic flame retardants in the lower great lakes. *Environ Sci Technol* 50(17):9133–9141. 10.1021/acs.est.6b02496. [PubMed: 27458653]

Melymuk L, Robson M, Helm PA, Diamond ML. 2011 Evaluation of passive air sampler calibrations: Selection of sampling rates and implications for the measurement of persistent organic pollutants in air. *Atmos Environ* 45(10):1867–1875. doi: 10.1016/j.atmosenv.2011.01.011 . [accessed 2013

Oct 31]. 10.1016/j.atmosenv.2011.01.011<http://linkinghub.elsevier.com/retrieve/pii/S1352231011000185>. [accessed 2013 Oct 31]. <http://linkinghub.elsevier.com/retrieve/pii/S1352231011000185>.

- Melymuk L, Robson M, Helm PA, Diamond ML. 2012 PCBs, PBDEs, and PAHs in Toronto air: spatial and seasonal trends and implications for contaminant transport. *Sci Total Environ* 429:272–80. doi: 10.1016/j.scitotenv.2012.04.022. [accessed 2013 Mar 4]. 10.1016/j.scitotenv.2012.04.022<http://www.ncbi.nlm.nih.gov/pubmed/22578845>. [accessed 2013 Mar 4]. <http://www.ncbi.nlm.nih.gov/pubmed/22578845>. [PubMed: 22578845]
- Nagy SR, Sanborn JR, Hammock BD, Denison MS. 2002 Development of a green fluorescent protein-based cell bioassay for the rapid and inexpensive detection and characterization of Ah receptor agonists. *Toxicol Sci* 65(2):200–210. 10.1093/toxsci/65.2.200. [PubMed: 11812924]
- Novák J, Jálková V, Giesy JP, Hilscherová K. 2009 Pollutants in particulate and gaseous fractions of ambient air interfere with multiple signaling pathways in vitro. *Environ Int* 35(1):43–9. doi: 10.1016/j.envint.2008.06.006. [accessed 2012 Nov 21]. 10.1016/j.envint.2008.06.006<http://www.ncbi.nlm.nih.gov/pubmed/18678411>. [accessed 2012 Nov 21]. <http://www.ncbi.nlm.nih.gov/pubmed/18678411>. [PubMed: 18678411]
- O’Connell SG, Kincl LD, Anderson KA. 2014 Silicone wristbands as personal passive samplers. *Environ Sci Technol* 48(6):3327–35. 10.1021/es405022f. [PubMed: 24548134]
- Peverly AA, Ma Y, Venier M, Rodenburg Z, Spak SN, Hornbuckle KC, Hites RA. 2015 Variations of flame retardant, polycyclic aromatic hydrocarbon, and pesticide concentrations in Chicago’s atmosphere measured using passive sampling. *Environ Sci Technol* 49(9):5371–9. doi: 10.1021/acs.est.5b00216. [accessed 2016 Apr 6]. 10.1021/acs.est.5b00216<http://www.ncbi.nlm.nih.gov/pubmed/25874663>. [accessed 2016 Apr 6]. <http://www.ncbi.nlm.nih.gov/pubmed/25874663>. [PubMed: 25874663]
- Porter E, Crump D, Egloff C, Chiu S, Kennedy SW. 2014 Use of an avian hepatocyte assay and the avian Toxchip Polymerase chain reaction array for testing prioritization of 16 organic flame retardants. *Environ Toxicol Chem* 33(3):573–82. doi: 10.1002/etc.2469. [accessed 2014 Sep 9]. 10.1002/etc.2469<http://www.ncbi.nlm.nih.gov/pubmed/24273086>. [accessed 2014 Sep 9]. <http://www.ncbi.nlm.nih.gov/pubmed/24273086>. [PubMed: 24273086]
- Ramírez N, Cuadras A, Rovira E, Marcé RM, Borrull F. 2011 Risk assessment related to atmospheric polycyclic aromatic hydrocarbons in gas and particle phases near industrial sites. *Environ Health Perspect* 119(8):1110–6. doi: 10.1289/ehp.1002855. [accessed 2013 Oct 29]. 10.1289/ehp.1002855<http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3237345&tool=pmcentrez&rendertype=abstract>. [accessed 2013 Oct 29]. <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3237345&tool=pmcentrez&rendertype=abstract>. [PubMed: 21478082]
- Ritz C, Baty F, Streibig JC, Gerhard D. 2015 Dose-response analysis using R. *PLoS One* 10(12):1–13. 10.1371/journal.pone.0146021.
- Salamova A, Ma Y, Venier M, Hites RA. 2014 High levels of organophosphate flame retardants in the Great Lakes atmosphere. *Environ Sci Technol Lett* 1(1):8–14. doi: 10.1021/ez400034n. [accessed 2015 Jul 8]. 10.1021/ez400034n<http://pubs.acs.org/doi/abs/10.1021/ez400034n>. [accessed 2015 Jul 8]. <http://pubs.acs.org/doi/abs/10.1021/ez400034n>.
- Shoeib M, Ahrens L, Jantunen L, Harner T. 2014 Concentrations in air of organobromine, organochlorine and organophosphate flame retardants in Toronto, Canada. *Atmos Environ* 99:140–147. doi: 10.1016/j.atmosenv.2014.09.040. [accessed 2014 Oct 14]. 10.1016/j.atmosenv.2014.09.040<http://linkinghub.elsevier.com/retrieve/pii/S135223101400733X>. [accessed 2014 Oct 14]. <http://linkinghub.elsevier.com/retrieve/pii/S135223101400733X>.
- Sonavane M, Schollée JE, Hidasi AO, Creusot N, Brion F, Suter MJF, Hollender J, Aït-Aïssa S. 2018 An integrative approach combining passive sampling, bioassays, and effect-directed analysis to assess the impact of wastewater effluent. *Environ Toxicol Chem* 37(8):2079–2088. 10.1002/etc.4155. [PubMed: 29667746]
- Sun Y, Miller C a, Wiese TE, Blake D a. 2014 Methylated phenanthrenes are more potent than phenanthrene in a bioassay of human aryl hydrocarbon receptor (AhR) signaling. *Environ Toxicol Chem* 33(10):2363–7. doi: 10.1002/etc.2687. [accessed 2014 Oct 5]. 10.1002/etc.2687<http://www.ncbi.nlm.nih.gov/pubmed/25874663>.

www.ncbi.nlm.nih.gov/pubmed/25043914. [accessed 2014 Oct 5]. <http://www.ncbi.nlm.nih.gov/pubmed/25043914> . [PubMed: 25043914]

Weschler CJ, Nazaroff WW. 2012 SVOC exposure indoors: Fresh look at dermal pathways. *Indoor Air* 22(5):356–377. 10.1111/j.1600-0668.2012.00772.x. [PubMed: 22313149]

Wong F, Suzuki G, Michinaka C, Yuan B, Takigami H, de Wit CA. 2016 Dioxin-like activities, halogenated flame retardants, organophosphate esters and chlorinated paraffins in dust from Australia, the United Kingdom, Canada, Sweden and China. *Chemosphere* 168:1–9. 10.1016/j.chemosphere.2016.10.074. [PubMed: 27771541]

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FIGURE 1.

Concentration and composition of PAHs and OPEs in PE extracts (A and B; ng/μL) and ambient air (C and D; ng/m³). Site name abbreviations are BLK: PE Blank; CUY: Cuyahoga National Park; KENT: Kent; FHL: Fairport Harbor Lakefront; UH: University Heights; CLH: Cleveland Downtown 3; CLT: Cleveland Downtown 2; CLF: Cleveland Lakefront 1; CLE: Cleveland Lakefront 2; CLD: Cleveland Downtown 1

FIGURE 2.

Concentration-response curves for triplicate cell exposures to PE extract dilution curves, including the PE Blank. Concentrations are expressed as the mass of PE extracted per mL DMSO in each dosing solution. Activity is expressed as the ratio of the response to the PE extract as compared to the response of the positive control (120 nM BaP).

FIGURE 3.

Map of BaP-Eq_{bio}, total PAH concentrations (Σ_{40} PAH), and total OPE concentrations (Σ_{12} OPE) in PE extracts from each site. The size of each circle represents the value at each site, with the smallest and largest circles representing the minimum and maximum, of the range of values.

FIGURE 4.

Relative contribution of PAHs to BaP-EQ_{chem}, based on IEFs from Machala et al. (2001).

Compound abbreviations are FLRA: fluoranthene; PYR: pyrene; BAA: benzo[a]anthracene;

CHRY: chrysene; DIMEBAA: 7,12-dimethylbenz[a]anthracene; BBJKFLRA:

benzo[b,j,k]fluoranthene; BAP: benzo[a]pyrene; IND: indeno[1,2,3-c,d]pyrene; DIBA:

dibenz[a,h]anthracene. Place name acronyms are defined in the caption for Figure 1.

TABLE 1.

Sampling Site Characteristics

Location Name	Latitude	Longitude	Deployment Date Range	Volume Air Sampled (m ³) ^a	Site Class	Nearby Population Density ^b
Cleveland Lakefront 1	41.507	-81.703	6/30/13-9/7/13	7466	Urban	359397
Cleveland Lakefront 2	41.492	-81.733	7/11/13-9/11/13	6588	Urban	342363
Cleveland Downtown 1	41.492	-81.679	7/1/13-9/5/13	7013	Urban	453257
Cleveland Downtown 2	41.477	-81.682	7/1/13-9/5/13	5994	Semi-Urban	481527
Cleveland Downtown 3	41.447	-81.660	7/1/13-9/5/13	7023	Semi-Urban	497567
University Heights	41.488	-81.549	7/2/13-9/8/13	4938	Semi-Urban	510538
Fairport Harbor Lakefront	41.758	-81.277	7/3/13-8/29/13	4562	Residential	68591
Kent	41.164	-81.361	7/2/13-9/10/13	4934	Residential	118272
Cuyahoga National Park	41.162	-81.543	7/2/13-9/7/13	7026	Rural/Park	168225

^aVolume of air sampled calculated using the sampling rate for phenanthrene, which was estimated based on PRC loss data from co-deployed thin PEs multiplied by the deployment length.

^bPopulation density determined by calculating the total number of people within a 10 km radius using the GRUMPv1 database from Columbia University CIESIN (Center for International Earth Science Information Network (CIESIN), 2011).

TABLE 2.

Potency and Maximum Efficacy of PE Extracts

Sample	EC _{BaP50} ±STDEV (g PE/mL)	Maximum Efficacy±STDEV (% of pos. control)
Cleveland Lakefront 1	2.2±1.2	188±39
Cleveland Lakefront 2	1.9±0.2	109±4
Cleveland Downtown 1	0.5±0.1	138±39
Cleveland Downtown 2	1.6±0.2	94±13
Cleveland Downtown 3	1.1±0.3	179±55
University Heights	1.6±0.3	230±18
Fairport Harbor Lakefront	4.1±0.9	178±22
Kent	2.6±0.4	188±18
Cuyahoga National Park	6.6±1.2	110±15

EC_{BaP50} = Concentration of the sample resulting in 50% of the effect observed for the plate-specific positive control (120 nM BaP)

TABLE 3.

BaP Equivalency of PE Extracts based on Bioassay and Chemical Analysis

Sample	BaP-Eq _{bio}	BaP-Eq _{chem}	% $\frac{\text{BaP-Eq}_{\text{chem}}}{\text{BaP-Eq}_{\text{bio}}}$
Cleveland Lakefront 1	64	2.9	4%
Cleveland Lakefront 2	75	3.0	6%
Cleveland Downtown 1	283	6.0	2%
Cleveland Downtown 2	89	6.1	7%
Cleveland Downtown 3	129	5.8	2%
University Heights	89	4.7	3%
Fairport Harbor Lakefront	35	2.5	23%
Kent	54	7.9	11%
Cuyahoga National Park	21	1.6	7%

BaP-Eq_{bio} = Benzo[a]pyrene equivalents (ng/uL) based on bioassay dose-response curveBaP-Eq_{chem} = Benzo[a]pyrene equivalents (ng/uL) estimated based on chemical analysis