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

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

**Running Head:** AhR potency of mixtures from passive air samplers

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

17 **ABSTRACT**

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

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## 39 INTRODUCTION

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

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

57 In addition to PAHs, recent studies have demonstrated that many organic flame retardant  
58 compounds (OFRs) are also ubiquitous in ambient urban air, and that one particular class, the  
59 organophosphate esters (OPEs), are present at unexpectedly high levels in urban ambient air  
60 (Salamova et al. 2014; Shoeib et al. 2014). Furthermore, some currently-used chlorinated OPEs

61 are expected to be present predominantly in the gaseous phase (Brommer et al. 2014; Salamova  
62 et al. 2014; Peverly et al. 2015). O’Connell et al. used silicone wristbands as personal monitoring  
63 devices for exposure to gas-phase HOCs and frequently detected OPEs, along with several 2-3-  
64 ring PAHs (O’Connell et al. 2014).

65           Chronic exposure to gas-phase OPEs and other OFRs in ambient air is of concern  
66 because several studies have provided evidence that many OPEs, including tris(1,3-dichloro-2-  
67 propyl) phosphate (TDCIPP), tris(1-chloro-2-propyl) phosphate (TCIPP), triphenyl phosphate  
68 (TPHP), and tris(2-ethylhexyl) phosphate (TEHP), can disrupt normal development, metabolism,  
69 immune response, and hormone function (Farhat et al. 2013; Liu et al. 2013; Farhat et al. 2014;  
70 Porter et al. 2014). Studies have also indicated that TDCIPP is carcinogenic and/or mutagenic  
71 (Gold et al. 1978; Farhat et al. 2014), and, along with tris(2-chloroethyl) phosphate (TCEP), it  
72 has been designated a carcinogen under California Proposition 65 (California OEHHA, 2017).  
73 Some OFRs, including tris(methylphenyl) phosphate (meta; TmMPP) and TDCIPP, have also  
74 been associated with changes in expression of genes regulated by AhR in a few past studies,  
75 though evidence of this is sparse (Liu et al. 2013; Porter et al. 2014). Previous studies indicate  
76 that some polybrominated diphenyl ether (PBDE) congeners are also weak or moderate AhR  
77 agonists, and that binding affinity appears to depend on the degree and position of bromination  
78 (Chen and Bunce 2003; Gu et al. 2012). Recent work has also indicated that concentrations of  
79 PBDEs may be positively correlated with dioxin-like activity in dust samples, possibly due to the  
80 cooccurrence of polybrominated dioxins/furans (PBDD/Fs) (Wong et al. 2016).

81           Activation of AhR is linked to induction and repression of a large number of genes,  
82 including modulation of cell growth and proliferation, tumor promotion, immunological effects,  
83 cardiotoxicity, and endocrine disruption, with the severity and type of response dependent upon

84 the specific ligand and its binding affinity (Denison et al. 2011). Previous studies on health risks  
85 of ambient air pollution have used induction equivalency factors (IEFs) to represent the AhR-  
86 mediated potency of PAHs relative to benzo[a]pyrene (BaP) (Kennedy et al. 2010; Ramírez et al.  
87 2011). This IEF-based approach assumes an additive, rather than synergistic or antagonistic,  
88 relationship between multiple ligands. AhR is activated by binding with variable affinity to  
89 several PAHs, with 4-5-ring PAHs generally more potent than the 2-3-ring PAHs that dominate  
90 gas-phase air pollution (Boström et al. 2002). Highly potent PAHs such as (BaP) are typically  
91 present only at very low concentrations in the gas phase due to low volatility. The lower  
92 molecular weight PAHs, especially phenanthrene, fluoranthene, and the methylated  
93 phenanthrenes/anthracenes, may contribute more significantly to the potency of the gaseous  
94 fraction due to their high gas-phase concentrations (Boström et al. 2002).

95         Despite low concentrations of potent high molecular weight PAHs in the gaseous fraction  
96 of ambient air pollution, previous studies have shown that this fraction appears to be responsible  
97 for a significant portion of the AhR-mediated activity associated with ambient air. In studies of  
98 gas-phase air pollution, Ramirez et al. found that, while concentrations of PAHs known to be  
99 most potent with respect to cytochrome P450 1A1 (CYP1A1) induction were low in the gaseous  
100 fraction, this fraction was estimated to contribute 34-86% of total carcinogenicity associated with  
101 16 PAHs based on potency relative to BaP (Ramírez et al. 2011). Previous studies by Klein et al.  
102 and Novak et al. also observed significant AhR activation from the gaseous, as well as  
103 particulate, fraction of ambient air pollutants (Klein et al. 2005; Novák et al. 2009). Kennedy et  
104 al. found a statistically significant relationship between PAH concentrations and AhR activity in  
105 samples of gaseous and fine particulate contaminants, but determined that the specific PAHs  
106 targeted in the study accounted for less than 3% of the observed AhR activity (Kennedy et al.

107 2010). Similarly, Érseková et al. found that quantified PAHs accounted for only 3-33% of  
108 measured AhR activity from ambient air samples (Érseková et al. 2014). While some of these  
109 studies considered contributions of compound groups besides PAHs, including polychlorinated  
110 biphenyls (PCBs) and organochlorine pesticides, none have investigated whether OFRs may  
111 explain some fraction of AhR activity.

112 Previous studies have noted that gaseous HOCs should not be ignored in risk  
113 assessments, but all of this work was carried out using high-volume air samplers or passive  
114 polyurethane foam (PUF) samplers, which are less selective for gaseous HOCs than polyethylene  
115 passive samplers (PEs) (Melymuk et al. 2011). Studies using less selective sampling strategies  
116 could not fully rule out that some fraction of particulate-bound HOCs may have contributed to  
117 the measured AhR activity. PEs accumulate only gas-phase HOCs and have an affinity for HOCs  
118 that is similar to that of fatty tissue, so they have been used in many studies predicting the extent  
119 to which HOCs will bioaccumulate (Joyce et al. 2016). The present study is the first to our  
120 knowledge to investigate AhR activation caused by the freely gaseous fraction of HOCs taken up  
121 by a single-phase sampler (pre-cleaned polyethylene), and will help contribute to our  
122 understanding of the biological relevance of the truly gaseous fraction of ambient air in urban  
123 and residential environments.

124 PEs were deployed throughout the Cleveland (OH) area on the southern shore of Lake  
125 Erie from June to September of 2013. Extracts from PEs were analyzed by gas chromatography  
126 coupled with mass spectrometry (GC/MS) for a suite of PAHs and OFRs and were also analyzed  
127 via an *in vitro* bioassay to measure AhR activation. The objectives of this study were to (i)  
128 investigate the use of PEs as a viable vehicle for isolating gaseous HOCs for use in *in vitro*  
129 bioassays, (ii) explore whether AhR-mediated activity of PE extracts correlated significantly



130 with any PAHs or OFRs measured in the extracts, and (iii) determine what portion of AhR-  
131 mediated activity measured via *in vitro* bioassays could be predicted based on targeted chemical  
132 analysis of commonly monitored PAHs.

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

144

## 145 **MATERIALS AND METHODS**

### 146 *Passive air sampler deployment*

147 800- $\mu$ m-thick low-density polyethylene sheeting (United Plastics, Inc.) was cut into  
148 approximately 7.5 cm x 13 cm pieces and cleaned in solvent (DCM and hexane) to remove  
149 background contamination. At each of nine sampling sites throughout the Cleveland area, four  
150 PEs were fastened inside an inverted stainless steel bowl using zip-ties and the bowl was  
151 suspended so that the PEs were hanging at approximately 2 m height.

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

159 A map of the study region is shown in the Supplementary Information (SI Figure S1) and  
160 characteristics of the deployment sites are summarized in [Table 1](#). Deployments took place from  
161 June to September of 2013, with each set of PEs deployed for about 60 days. After deployment,  
162 PEs were removed from the protective bowl, wrapped in precombusted aluminum foil, and  
163 shipped on ice overnight to the University of Rhode Island Graduate School of Oceanography,  
164 where they were kept frozen until extraction.

#### 165 *Sample preparation*

166 Each 800- $\mu\text{m}$  PE was extracted twice in pentane, each time for 18-24 hours. 50- $\mu\text{m}$  PEs  
167 were extracted once for 18-24 hours in pentane. Every batch of PEs was extracted along with a  
168 laboratory blank, which was a PE that had been cleaned alongside the field samples and then  
169 stored frozen in precombusted aluminum foil. All four 800- $\mu\text{m}$  PEs deployed simultaneously at  
170 the same site were composited into one extract and concentrated to 1 mL in a warm water bath  
171 under a gentle stream of nitrogen. Extracts from 800- $\mu\text{m}$  PEs appeared to contain a white  
172 precipitate, possibly from co-extracted polyethylene material. To remove the particulate, extracts  
173 were serially frozen, causing the precipitate to solidify at the bottom of the vial, and the

174 overlying liquid was removed via Pasteur pipet and reconstituted to 1 mL with pentane. Two  
175 aliquots were removed from the 1 mL solution: one for chemical analysis and the other for  
176 biological analysis. A schematic summarizing sample preparation is shown in the  
177 Supplementary Material (Figure S2).

### 178 *Chemical analysis by GC/MS*

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

187 Extracts were also spiked with non-native polybrominated diphenyl ethers (BDEs 35, 77,  
188 128, and 183) and analyzed on an Agilent 7890 GC coupled to an Agilent 5977 MSD in negative  
189 chemical ionization (NCI) mode with methane reagent gas for 12 polybrominated diphenyl  
190 ethers (BDEs) and 8 novel halogenated flame retardants (NHFRs), as well as 3 polybrominated  
191 biphenyls (PBBs), which were used as PRCs in sampling rate determination for co-deployed thin  
192 PEs. A complete list of target compounds and abbreviations is available in the Supplementary  
193 Material (Table S1). BDEs and NHFRs were quantified using an 8-point calibration curve with  
194 linearity  $r^2 > 0.995$ .

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

#### 200 *Calculation of ambient air concentrations*

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

211 To translate concentrations within the PE to concentrations in ambient air, the volume of  
212 air sampled by each PE during deployment was estimated using data on the percent loss of  
213 labeled PRCs from co-deployed 50- $\mu$ m thick PEs. From the PRC loss data, the best-fit value for  
214 the thickness of the diffusive boundary layer (DBL) at the air-PE interface was determined.  
215 Because all PEs were deployed under the same conditions and the thickness of the PE sheet does  
216 not affect air-side resistance, the DBL thickness determined for thin sheets was then used in a

217 two-film model describing PE-side and air-side mass transfer rates to calculate the percent  
218 equilibration reached by each target compound in the 800  $\mu\text{m}$ -thick PEs. This approach for  
219 estimation of percent equilibration from PRC loss data has been described in detail in previous  
220 work (McDonough et al. 2016).

#### 221 *Biological analysis by reporter cell bioassay*

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

226 The AhR reporter cell line used was H1G1.1c3, a murine hepatoma cell line consisting of  
227 Hepa-1c1c7 cells stably transfected with AhR-responsive green fluorescent protein (GFP)  
228 reporter gene (Nagy et al. 2002). Cells were plated in 96-well plate ( $3 \times 10^5$  cells per well; Costar  
229 96-well black plate with a clear bottom) and allowed to attach overnight at  $37^\circ\text{C}$  in selective  
230 medium (Nagy et al. 2002). The medium was then changed to non-selective medium and the  
231 cells in 100  $\mu\text{L}$  of medium were treated with 1  $\mu\text{L}$  of each sample dilution for a final vehicle  
232 concentration of 1% DMSO. All wells were prepared in triplicate and incubated at  $33^\circ\text{C}$ . For  
233 each test extract, the cells in three wells were treated with 1  $\mu\text{L}$  of DMSO as a negative control,  
234 and the cells in another set of three wells were left untreated to control for any natural cell  
235 fluorescence. On each plate, three wells were treated with BaP at a final well concentration of  
236 120 nM dissolved in DMSO as a positive control. On one plate, a 10-point dilution curve was  
237 also run for BaP ( $1.2 \times 10^{-5}$  – 12000 nM), and results were normalized to the positive control 120  
238 nM BaP (Figure S3).

239 AhR-mediated activity was measured by reading the GFP fluorescence emitted by the  
240 cells at 515 nm using a Spectra Max M3 plate reader at 24 and 48 hours post dosing (hpd). The  
241 mean fluorescence value of the DMSO-treated negative control triplicate wells was subtracted  
242 from each sample's fluorescence reading, and the response was expressed as a ratio over the  
243 mean fluorescence value for the triplicate 120 nM BaP positive controls run on the same plate to  
244 control for plate-to-plate differences in cell response.

#### 245 *Calculation of extract potency*

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

$$254 \quad f(x) = \frac{d - c}{1 + \exp(b(\log(e) - \log(x)))} \quad \text{Eq 1}$$

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

260 Dosing solutions were prepared so that each sample was representative of the same  
261 amount of extracted PE to facilitate comparison with the PE blank and control for any  
262 interference caused by background contamination in the PE matrix. However, due to site-to-site  
263 variability in sampling rates, the volume of air represented by each sample differed among sites  
264 (Table 1). For this reason, after determination of  $EC_{BaP50}$  from the concentration-response curve  
265 fit,  $EC_{BaP50}$  values were normalized based on the volume of air sampled at each site. Aliquots of  
266 PE extracts used in dosing solutions were representative of 1900-3100  $m^3$  of air, and were all  
267 normalized to 2000  $m^3$ .

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

$$279 \quad BaP-Eq_{chem} = \sum(IEF_n \cdot C_n)(ng/\mu L) \quad Eq 2$$

280 For the bioassay results, the BaP equivalent of each sample extract was expressed as the  
281 amount of BaP needed to achieve the same response as the extract. The bioassay-derived toxic

282 equivalency (BaP-Eq<sub>bio</sub>) was calculated as in Equation 3 as the ratio of the amount of BaP  
283 needed to achieve a response of 50% the maximum efficacy (EC<sub>50</sub> for the BaP curve) over the  
284 volume of PE extract added to the well to achieve that same effect (the EC<sub>BaP50</sub> of the extract).

$$285 \quad BaP-Eq_{bio} = \frac{EC_{BaP50}[BaP](ng/well)}{EC_{BaP50}[Extract](\mu L/well)} \quad Eq \ 3$$

286 The degree to which chemical analysis explained observed potency (%<sub>chem</sub>) was then  
287 expressed as in Equation 4.

$$288 \quad \%_{chem} = \frac{BaP-Eq_{chem}}{BaP-Eq_{bio}} \cdot 100 \quad Eq \ 4$$

289

## 290 **RESULTS AND DISCUSSION**

### 291 *Chemical composition of passive sampler extracts*

292 Concentrations of all compounds in PE extracts are presented in the Supplementary  
293 Material for PAHs (Table S2), OPEs (Table S3), and halogenated flame retardants (HFRs; Table  
294 S4). Concentrations of PAHs and OPEs in the PE extracts are displayed in **Figure 1** (left side)  
295 along with estimated ambient air concentrations (right side). All concentrations for field samples  
296 were normalized to an air volume of 2000 m<sup>3</sup> to facilitate comparison between sites.

297 Total alkyl and parent PAHs ( $\Sigma_{40}$ PAH) in PE extracts ranged from 3.6 ng/ $\mu$ L for the  
298 extract from Cuyahoga National Park to 34 ng/ $\mu$ L for a residential suburban area in University  
299 Heights. Concentrations of PAHs were dominated by phenanthrene (0.6-16.3 ng/ $\mu$ L; 10-57%),  
300 fluoranthene (0.1-6 ng/ $\mu$ L; 1-18%), 2-methylphenanthrene (0.1-1 ng/ $\mu$ L; 1-6%), and fluorene  
301 (0.3-1 ng/ $\mu$ L; 3-9%).



302 Concentrations of OPEs were much greater than those of halogenated organic flame  
303 retardants (HFRs). Total OPEs ( $\Sigma_{12}$ OPE) ranged from 0.4 ng/ $\mu$ L for the extract from Cuyahoga  
304 National Park to 2.0 ng/ $\mu$ L for a residential area in Kent.  $\Sigma_{12}$ OPE was dominated by TPHP at all  
305 downtown Cleveland sites (0.09-0.78 ng/ $\mu$ L; 28-69%), while Cuyahoga National Park and  
306 Fairport Harbor were dominated by TEHP (0.30 – 0.57 ng/ $\mu$ L; 68%), and University Heights and  
307 Kent were dominated by tri-n-butyl phosphate (TNBP; 0.50 ng/ $\mu$ L; 59%) and TCIPP (1.60  
308 ng/ $\mu$ L; 78%), respectively. Concentrations of total BDEs ( $\Sigma_{12}$ BDE) ranged from 10 pg/ $\mu$ L in  
309 Cuyahoga National Park to 46 pg/ $\mu$ L at Downtown Cleveland Site 2, and were dominated by  
310 BDE 47 and 154. Concentrations of total NHFRs ( $\Sigma_{18}$ NHFRs) were greatest in the PE blank due  
311 to the presence of 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) and Dechlorane Plus, which  
312 were not found in any of the field sample extracts.

313 2-ring, 3-ring, and 4-ring PAHs, as well as their alkylated and substituted counterparts,  
314 were generally correlated in the different extracts ( $0.3 \leq r^2 \leq 0.9$ ), while 5-6-ring PAHs did not  
315 exhibit significant correlation among themselves or with any other group of PAHs (Table S5).  
316 Correlation among individual PAHs was expected, as they are typically emitted from the same  
317 sources. Correlation among PAHs was further confirmed by principal component analysis  
318 (PCA), which showed that 76% of variation in samples was explained by two principal  
319 components, the first with loadings primarily from 3-4-ring PAHs, and the second with loadings  
320 primarily from 2-ring and 4-5-ring PAHs (Figure S4). In contrast, individual OPEs were  
321 generally not significantly correlated, though some degree of correlation ( $r^2 \geq 0.3$ ) was observed  
322 between TDCIPP and TNBP (Table S6). Additionally, TNBP, TDCIPP, and ethylhexyl diphenyl  
323 phosphate (EHDPP) exhibited some correlation with PAHs (Table S7).

324

325 *Ambient air concentrations*

326 Ambient gaseous concentrations of  $\Sigma_{40}\text{PAH}$  ranged from 7.1 ng/m<sup>3</sup> in Cuyahoga National  
327 Park to 36.2 ng/m<sup>3</sup> at urban site Cleveland Downtown 1 and were dominated by the  
328 methylnaphthalenes (1.7-8.8 ng/m<sup>3</sup>; 18-33%), phenanthrene (0.3-9.8 ng/m<sup>3</sup>; 2-33%), and  
329 fluorene (0.5-2.6 ng/m<sup>3</sup>; 5-14%). Concentrations were similar in range to those measured by  
330 Peverly et al. in Chicago using polyurethane foam passive samplers (PUFs) in 2012 - 2014  
331 ( $\Sigma_{16}\text{PAH} = 9 - 52$  ng/m<sup>3</sup>), and by Melymuk et al. in Toronto in 2007 - 2008 ( $\Sigma_{27}\text{PAH} = 0.3 - 51$   
332 ng/m<sup>3</sup>), also using PUFs (Melymuk et al. 2012; Peverly et al. 2015). Concentrations in this study  
333 were similar but lower than previous measurements of total gaseous PAHs using PEs in the  
334 downtown Cleveland area by McDonough et al. in 2012 ( $\Sigma_{15}\text{PAH} = 23-80$  ng/m<sup>3</sup>; McDonough et  
335 al. 2014). In larger-scale regional studies, atmospheric concentrations of PAHs have often been  
336 found to correlate with population density (Hafner et al. 2005; McDonough et al. 2014), but here  
337 no significant ( $p < 0.05$ ) correlation between gaseous PAH concentrations and population density  
338 within 5-30 km was observed.

339 Gaseous concentrations of  $\Sigma_{12}\text{OPE}$  ranged from 0.01 ng/m<sup>3</sup> in Cuyahoga National Park to  
340 1.1 ng/m<sup>3</sup> in Kent. This was similar in range to measurements by Peverly et al. in Chicago using  
341 PUFs in 2012-2014 ( $\Sigma_{13}\text{OPE} = 0.5 - 1.5$  ng/m<sup>3</sup>), and slightly lower than measurements of  
342 particulate  $\Sigma_{12}\text{OPE}$  in the Cleveland area by Salamova et al. in 2012 (mean  $\Sigma_{12}\text{OPE} = 2.1 \pm 0.4$   
343 ng/m<sup>3</sup>; Salamova et al. 2014; Peverly et al. 2015). TCIPP was the most abundant OPE at all sites  
344 (0.01-1.0 ng/m<sup>3</sup>; 9-98%) except University Heights, where TNBP dominated (0.6 ng/m<sup>3</sup>; 87%).  
345 TCIPP was also found to be most abundant in Cleveland particulate  $\Sigma_{12}\text{OPE}$  in a previous study  
346 (0.85 $\pm$ 0.3 ng/m<sup>3</sup>; Salamova et al. 2014)

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

### 358 *Concentration-response curves*

359 Extracts from all PEs, including the PE Blank, induced concentration-dependent  
360 activation of AhR-dependent GFP. All concentration-response data are displayed along with  
361 curve fits and 95% confidence intervals in **Figure 2**, with response represented as a ratio  
362 compared to response elicited by the plate-specific positive control. For all extracts, an initial  
363 increase in GFP induction was seen with increasing concentration. However, there was a  
364 precipitous decline in the fluorescence for all extracts (except Cleveland Lakefront 1) at the  
365 greatest concentrations, possibly due to cytotoxicity or inhibition of fluorescence response at  
366 high concentrations of PE extract. These points were omitted during concentration-response  
367 curve fitting, as we were interested in determining only the induction potencies of the extracts.  
368 Most extracts did not exhibit a clear plateau in response, making determination of maximum  
369 efficacy, as well as EC<sub>50</sub>, somewhat uncertain. Furthermore, maximum efficacy of the samples

370 varied from 94%-230% of positive control response (Table 2). For this reason,  $EC_{BaP50}$ ,  
371 measured relative to the plate-specific positive control, was used to compare the potencies of the  
372 samples.

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

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

388 The potency and maximum efficacy of the extracts did not appear to be correlated. This is  
389 most likely due to a complex interplay between the unique composition of ligands in each  
390 sample, their affinity for the AhR, the resulting ligand-receptor complex's ability to bind other  
391 necessary transcription factors, and cytotoxicity of specific components. Response could also be

392 affected by ligands interacting with other pathways that could amplify or dampen AhR response.  
393 Klein et al. also observed a lack of correlation between potency of extracts and maximum  
394 efficacy with respect to AhR binding of gas-phase extracts from active air sampling (Klein et al.  
395 2005).

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

#### 406 *Bioassay-derived BaP equivalents for PE extracts*

407 A map of results for BaP-Eq<sub>bio</sub> is displayed alongside maps of total concentrations of  
408 PAHs and OPEs in the PE extracts ( $\Sigma_{40}$ PAH and  $\Sigma_{12}$ OPE) in [Figure 3](#). BaP-Eq<sub>bio</sub> values ranged  
409 from 21-283 ng/ $\mu$ L BaP equivalents and were generally greatest in the downtown Cleveland area  
410 and lowest at the rural/residential sites further from the city center.

411 BaP-Eq<sub>bio</sub> values were compared to concentrations of PAHs and organic flame retardants  
412 (OPEs, PBDEs, and NHFRs) in the PE extracts to determine whether there was any significant  
413 correlation between potency and chemical composition. Though some correlations were found,

414 few were likely to be driving potency. No correlations with PBDE and NHFR concentrations  
415 were observed. BaP-Eq<sub>bio</sub> weakly correlated only with 2-ring alkyl/substituted PAHs ( $r^2 = 0.42$ ;  $p$   
416  $< 0.1$ ; SE = 64; N = 9) and also displayed some correlation with EHDPP ( $r^2 = 0.66$ ;  $p < 0.01$ ; SE  
417 = 49; N = 9). Maximum efficacy of PE extracts showed some correlation with concentrations of  
418 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)  
419 parent PAHs. Correlations between BaP-Eq<sub>bio</sub> and alkyl/substituted PAHs were only investigated  
420 by grouping compounds (2-ring alkyl/substituted PAHs; 3-4-ring alkyl/substituted PAHs)  
421 because quantitative standards were not available for all alkylated PAHs. However, it is  
422 important to note that AhR-mediated potency differs greatly between PAH isomers. Because  
423 there is a high degree of correlation observed between different low molecular weight PAHs at  
424 different locations in this study (Table S5), it was expected that the composition of  
425 alkyl/substituted PAHs is most likely similar between sites, so correlations with BaP-Eq<sub>bio</sub> are  
426 likely driven by the same compounds at all sites.

427         There is little information available regarding the biological effects of alkylated PAHs.  
428 Recent studies using a yeast reporter assay system and a H4IIE-*luc* reporter-gene assay suggest  
429 that methyl- and dimethyl-substituted phenanthrenes are in some cases more potent with respect  
430 to AhR activation than their unsubstituted counterparts (Sun et al. 2014; Lam et al. 2018). The  
431 statistically significant correlation between BaP-Eq<sub>bio</sub> and EHDPP suggests that this compound,  
432 or unmonitored compounds with which it covaries spatially, could be contributing to AhR  
433 activity. As no compelling evidence is available for EHDPP as an AhR activator, the presence of  
434 other AhR activators that covary with EHDPP is somewhat more likely. Previous studies have  
435 shown that levels of OPEs and other OFRs can correlate in air due to their historical use in the  
436 same formulations (Salamova et al. 2014). Additionally, some OPEs that were not targeted in

437 this study, including mono-substituted isopropyl triaryl phosphate (mITP), have been shown to  
438 have relatively strong AhR activity (Gerlach et al. 2014; Haggard et al. 2017).

#### 439 *Predicted BaP equivalents from chemical analysis*

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

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

457 Potencies calculated from known chemical composition using IEFs explained only 2-23%  
458 of the AhR-mediated potency observed in bioassay experiments (Table 3), and BaP-Eq<sub>chem</sub> and

459 BaP-Eq<sub>bio</sub> were not significantly correlated. This suggests that other compound groups present in  
460 the gaseous fraction of ambient air may also be contributing to BaP-Eq<sub>bio</sub> of the extracts. These  
461 may include additional parent PAHs and alkyl-PAHs not measured in this study, as well as  
462 oxygenated PAHs and N- and S-heterocyclic PAHs (Larsson et al. 2014; Sun et al. 2014; Lam et  
463 al. 2018). Compounds other than PAHs may also be responsible for some of the observed AhR-  
464 mediated potency. The use of BaP-Eq<sub>chem</sub> values derived from a different bioassay may also  
465 contribute to this discrepancy.

466 The correlation observed between concentrations of EHDPP and AhR activity suggests  
467 that this compound, or other OFRs with similar source, may be contributing to BaP-Eq<sub>bio</sub> as well,  
468 though further research is needed to understand the AhR-mediated potency of OFRs.  
469 Furthermore, a major weakness of predicting potency based on compound IEFs is that it  
470 considers only additive interactions, without taking into account synergistic and antagonistic  
471 effects, which are highly probable in complex environmental mixtures. This, along with the  
472 scarcity of IEF values for the targeted compounds, most likely contributed to the discrepancy  
473 between observed and predicted AhR-mediated potency.

474

## 475 **CONCLUSIONS**

476 This study demonstrated the use of PEs coupled with *in vitro* bioassays as an approach to  
477 measure cumulative biological effects of ambient gaseous air pollution. While some AhR-  
478 mediated activity was seen in the PE blank, the activity of field samples was found to be  
479 significantly elevated above blank levels, suggesting that interference from the PE matrix or  
480 typical laboratory contamination did not prohibit the use of PE extracts in bioassays for AhR



481 activation. In future studies using this approach, a thinner PE sheet (~ 50  $\mu\text{m}$ ) may be preferable  
482 to avoid extra cleanup steps caused by PE precipitate in the final extract, as thinner PEs contain  
483 less PE mass and require less time for extraction. In addition, future work employing effect-  
484 directed analysis, as has been used in passive sampling studies of wastewater (Sonavane et al.  
485 2018), could aid in identifying contaminants driving observed biological effects.

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

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

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505

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684 **TABLES AND FIGURES**

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686 **TABLE 1.** Sampling Site Characteristics

Location Name	Latitude	Longitude	Deployment Date Range	Volume Air Sampled (m <sup>3</sup> ) <sup>a</sup>	Site Class	Nearby Population Density <sup>b</sup>
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

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688 <sup>a</sup> Volume of air sampled calculated using the sampling rate for phenanthrene, which was  
689 estimated based on PRC loss data from co-deployed thin PEs multiplied by the deployment  
690 length.

691 <sup>b</sup> Population density determined by calculating the total number of people within a 10 km radius  
692 using the GRUMPv1 database from Columbia University CIESIN (Center for International Earth  
693 Science Information Network (CIEISIN), 2011).

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701 **TABLE 2.** Potency and Maximum Efficacy of PE Extracts

Sample	EC <sub>BaP50</sub> ±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

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703 EC<sub>BaP50</sub> = Concentration of the sample resulting in 50% of the effect observed for the plate-  
 704 specific positive control (120 nM BaP)

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720 **TABLE 3.** BaP Equivalency of PE Extracts based on Bioassay and Chemical Analysis

Sample	BaP-Eq <sub>bio</sub>	BaP-Eq <sub>chem</sub>	% $\frac{\text{BaP-Eq}_{\text{chem}}}{\text{BaP-Eq}_{\text{bio}}}$
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%

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722 BaP-Eq<sub>bio</sub> = Benzo[a]pyrene equivalents (ng/uL) based on bioassay dose-response curve

723 BaP-Eq<sub>chem</sub> = Benzo[a]pyrene equivalents (ng/uL) estimated based on chemical analysis

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735 **FIGURE LEGENDS**

736 **FIGURE 1.** Concentration and composition of PAHs and OPEs in PE extracts (A and B; ng/ $\mu$ L)  
737 and ambient air (C and D; ng/m<sup>3</sup>). Site name abbreviations are BLK: PE Blank; CUY: Cuyahoga  
738 National Park; KENT: Kent; FHL: Fairport Harbor Lakefront; UH: University Heights; CLH:  
739 Cleveland Downtown 3; CLT: Cleveland Downtown 2; CLF: Cleveland Lakefront 1; CLE:  
740 Cleveland Lakefront 2; CLD: Cleveland Downtown 1

741 **FIGURE 2.** Concentration-response curves for triplicate cell exposures to PE extract dilution  
742 curves, including the PE Blank. Concentrations are expressed as the mass of PE extracted per  
743 mL DMSO in each dosing solution. Activity is expressed as the ratio of the response to the PE  
744 extract as compared to the response of the positive control (120 nM BaP).

745 **FIGURE 3.** Map of BaP-Eq<sub>bio</sub>, total PAH concentrations ( $\Sigma_{40}$ PAH), and total OPE  
746 concentrations ( $\Sigma_{12}$ OPE) in PE extracts from each site. The size of each circle represents the  
747 value at each site, with the smallest and largest circles representing the minimum and maximum,  
748 of the range of values.

749 **FIGURE 4.** Relative contribution of PAHs to BaP-EQ<sub>chem</sub>, based on IEFs from Machala et al.  
750 (2001). Compound abbreviations are FLRA: fluoranthene; PYR: pyrene; BAA:  
751 benzo[a]anthracene; CHRY: chrysene; DIMEBAA: 7,12-dimethylbenz[a]anthracene;  
752 BBJKFLRA: benzo[b,j,k]fluoranthene; BAP: benzo[a]pyrene; IND: indeno[1,2,3-c,d]pyrene;  
753 DIBA: dibenz[a,h]anthracene. Place name acronyms are defined in the caption for Figure 1.

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