Supplemental Information for "Passive sampling coupled to UV irradiation: a useful analytical approach for studying oxygenated polycyclic aromatic hydrocarbon formation in bioavailable mixtures"

Norman D. Forsberg[†], Steven G. O'Connell[†], Sarah E. Allan[†], Kim A. Anderson*[†]

†Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR, USA

*To whom correspondence may be addressed (Kim.Anderson@oregonstate.edu).

Reagents and chemicals:

Passive sampling device extracts and standard PAH solutions were prepared in high purity GC resolve and Optima grade *n*-hexane, respectively (Fisher Scientific). PAHs were obtained from AccuStandard Inc. and CIL, Inc., while OPAHs were from a variety of sources, including Chiron, Fluka, NCI, Sigma Aldrich, and CDN Isotopes. All standards were guaranteed to be greater than 97% pure.

PSD samples:

PSD samples were collected from aqueous environments that display a wide range of PAH concentrations and profiles. In the Portland Harbor, samples were obtained from the west (w) bank of the Willamette River at river miles (RM) 6.5 and 7. These sites are located near remediation operations that have been ongoing, most notably at the McCormick and Baxter Superfund site, located on the east bank of river mile 7 within the larger Portland Harbor Superfund, and the GASCO site at RM 6.3 west (w). In the Gulf of Mexico, samples were obtained from Grand Isle, LA and Gulf Breeze, FL. The LA site had little natural or human devised physical protection from the influence of Gulf waters during the oil spill. The FL site was located at the mouth of Pensacola Bay and was more protected from direct oiling compared to the LA site owing to the natural peninsula that delimits the bay. Further details on all aspects of sampling are presented in Allan et al. [2012a, b].

UV exposure and sample preparation:

UVB exposure doses were much milder than conditions present during the Deepwater Horizon oil disaster. For instance, 4 hours of UVB irradiance on the Gulf shores of Alabama results in an exposure dose 20 times greater than those delivered in this study [Peachy, 2005]. Exposure doses represent the product of the UV irradiation level and the exposure duration. Following UV exposure, samples were transferred using *n*-hexane from watch glasses to 8 mL conical glass centrifuge tubes that had been solvent rinsed and dried. Samples were volume-reduced under a gentle stream of N_2, transferred to glass chromatography vials fitted with a small volume insert, spiked with OPAH and PAH internal standards, and immediately analyzed by GC-MS.

Chemical analysis by GC-MS:

GC-MS conditions for OPAH analysis were optimized from a previous study [Layshock et al., 2009] using an Agilent 7890A gas chromatograph coupled to a 5975C mass spectrometer under electron impact ionization (70eV). Briefly, a DB5-MS (30 m length, 0.25 µm film thickness, 0.32 mm inner diameter, Agilent) was used to separate OPAHs, with a flow rate of 1 mL min^{-1} using helium (>99.99%) as a carrier gas. MS temperatures were set at 280°C for the thermal auxiliary, 230 °C for the source, and the quadrapole was set at 150 °C. Injection volume was 1 µL. Oven temperatures and pulsed splitless injection parameters can be found in O'Connell et al. [2013]. Two qualification ions and one quantification ion were used along with retention time to identify each OPAH as shown in Table S1. Retention times listed in Table S1 are from the calibration check standard analyzed immediately prior to batch results described in the text. Instrumental limits of detection were also determined by O'Connell et al. [2013] and determined from the standard deviation of the lowest calibration standard that resulted in a signal to noise ratio of 3:1, as described in U.S. EPA method detection limit procedure in Title 40 Code of Federal Regulations (40 CFR 136, Appendix B, revision 1.11). A detailed description of GC-MS parameters used for PAH analysis is described by Forsberg, et al. [2011].

Quality assurance/quality control:

Analytical batches contained approximately 40% quality control samples, including solvent blanks, continuing calibration verifications (CCV), and negative controls. Solvent blanks were free of PAHs and OPAHs, and relative standard deviations (RSD) for internal standard area counts were less than 10%. PAH CCVs were within ± 20% of expected values. RSDs were less than 5% for 85% of the target PAH analyte list and were less than 12% overall. OPAH CCVs were within ± 30% of true values, except for 5,12-naphthacenequinone, which was within ± 37%, with all corresponding RSDs less than 16%. Levels of low-molecular weight PAHs were reduced 55 to 65% in foil covered non-UV exposed controls, where the magnitude of loss was consistent with routine losses incurred during sample concentration. Non-UV exposed priority pollutant PAH standards contained low levels of 9-fluorenone, 9,10-anthraquinone, and 4H-cyclopenta[def]phenanthrene-4-one that were consistent with levels measured in negative control solutions, which were covered with foil and placed under UV bulbs for the duration of each

67 experiment. This indicates that these low level OPAHs were likely impurities in purchased PAH
68 stock solutions.

Table S1. Retention times, monitored quantitation and qualitative ions, and instrument detection limits used to identify OPAHs by GC-MS.

OPAHs	t_R (min)	Quant. ion (m/z)	Qual. ions (m/z)	IDLs[a] (ng/mL)
9-Fluorenone	16.159	180	152, 151	0.20
1,4-Phenanthrenedione	19.671	208	152, 126	0.86
9,10-Anthraquinone	19.736	208	180, 152	6.9
4H-Cyclopenta[def]phenantrenone	20.900	204	176, 205	0.21
Benzo[a]fluorenone	25.777	230	200, 231	0.45
Aceanthracenequinone	28.473	204	176, 232	27
7,12-Benz[a]anthracenequinone	29.395	202	258, 200	0.85
5,12-Naphthacenequinone	30.609	258	202, 230	1.3

[a]IDLs, instrument detection limits – calculated as described in U.S. EPA 40 CFR 136, and listed in O'Connell etl al. [2013].

Abbreviations: OPAH = oxygenated polycyclic aromatic hydrocarbon, GC-MS = gas chromatography mass spectrometry, Quant. = quantification, Qual. = qualification, IDL = instrument detection limit.

Table S2. Percent change (% Δ) in PAH concentrations observed for Superfund and Gulf of Mexico PSD extracts after 30 min of laboratory UVB exposure.

PAH	PAH concentrations change in UVB exposed PSD extracts[a]				
	Superfund[b]			Gulf of Mexico[c]	
	7w-1	7w-2	6.5w	LA	FL
Phenanthrene	-28	-30	-52	-11	-21
Anthracene	-33	-46	-78	ND[d]	ND
Fluoranthene	-25	-24	-42	-23	NC[e]
Pyrene	-31	-31	-48	NC	NC
Benz[*a*]anthracene	-30	-35	-52	ND	DP-UV[f]
Chrysene	-62	-15	-44	NC	NC
Benzo[*b*]fluoranthene	-32	-22	-28	-96	-100
Benzo[*k*]fluoranthene	-26	-19	-36	DP-UV	ND
Benzo[*e*]pyrene	-32	-28	-27	NC	ND
Benzo[*a*]pyrene	-36	-53	-61	DP-UV	ND
Indeno[1,2,3-*cd*]pyrene	-46	-79	NC	31	ND
Benzo[*ghi*]perylene	-50	-30	20	-99	ND

[a]PAHs in PSD extracts represent the freely dissolved fraction of chemical, C_{free}.

[b]Samples collected from Portland Harbor Superfund mega-site at river miles 7w and 6.5w; 7w-1 and 2 are irradiation duplicates.

[c]Samples collected from Louisiana and Florida coastal waters during the Deepwater Horizon oil spill.

[d]'ND' = not detected in pre- or post-UV irradiated extracts.

[e]'NC' = change was less than 10% between C_0 and C_{30}.

[f]DP-UV' = detected post-UV irradiation.

Abbreviations: PAH = polycyclic aromatic hydrocarbon, PSD = passive sampling device, UVB = ultraviolet B

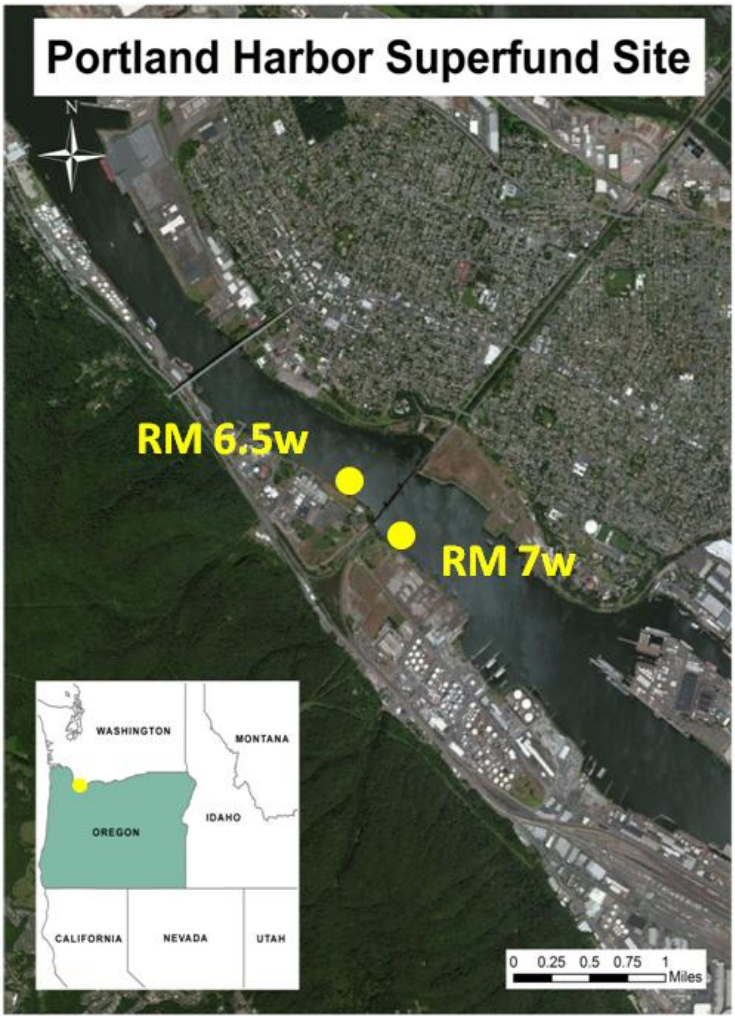

Figure S1. Sampling locations in the Superfund designated reach of the lower Willamette River. Sites are indicated by yellow dots. River mile (RM) 6.5w and 7w were sampled in September, 2010 and October, 2009, respectively [Allan et al., 2012a].

Figure S2. Approximate sampling locations in Grand Isle, Louisiana and Gulf Breeze, Florida within the Gulf of Mexico. Yellow dots indicate sampling sites. Grand Isle, LA and Gulf Breeze, FL samples were collected in June, 2010 and April, 2011, respectively [Allan et al., 2012b].

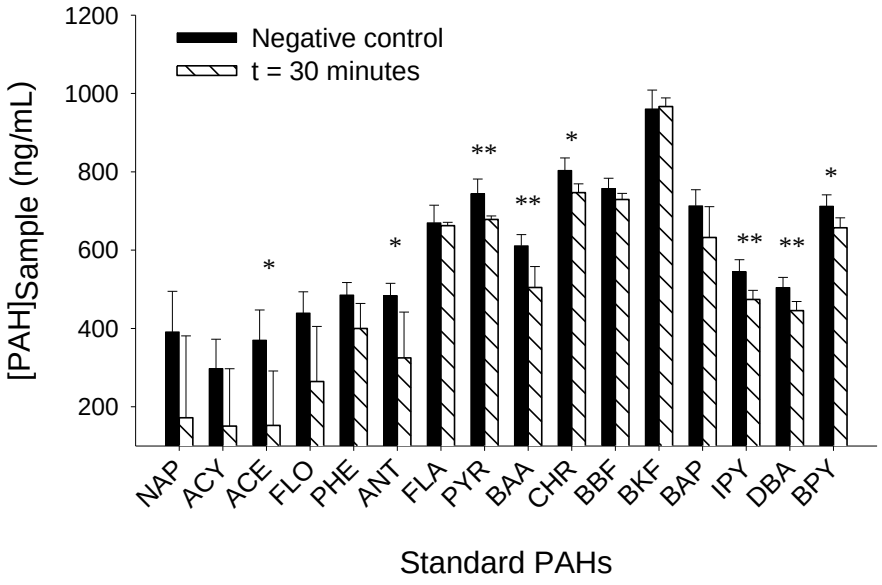

Figure S3. Degradation of PAHs in standard solutions after 30 min of UVB irradiation at an irradiance of 230 μW cm^{-2}. Bars represent the mean of triplicate experiments with error bars at ±1 SD. Differences relative to negative controls are indicated with asterisks, '*' = $p > 0.05$ and < 0.1, and '**' = $p < 0.05$. NAP, naphthalene; ACY, acenaphthylene; ACE, acenaphthene; FLO, fluorene; PHE, phenanthrene; ANT, anthracene; FLA, fluoranthene; PYR, pyrene; BAA, benz[*a*]anthracene; CHR, chrysene; BBF, benzo[*b*]fluoranthene; BKF, benzo[*k*]fluoranthene; BAP, benzo[*a*]pyrene; IPY, indeno[*1,2,3-cd*]pyrene; DBA, dibenz[*ah*]anthracene; BPY, benzo[*ghi*]perylene.

REFERENCES

Allan SE, Smith BW, Tanguay RL, Anderson KA. 2012a. Bridging environmental mixtures and toxic effects. *Environ Toxicol Chem*. 31:2877-2887.

Allan SE, Smith BW, Anderson KA. 2012b. Impact of the Deepwater Horizon oil spill on bioavailable polycyclic aromatic hydrocarbons in Gulf of Mexico coastal waters. *Environ Sci Technol*. 46:2033-2039.

Forsberg ND, Wilson GR, Anderson KA. 2011. Determination of parent and substituted polycyclic aromatic hydrocarbons in high-fat salmon using a modified QuEChERS extraction, dispersive SPE and GC–MS. *J Agric Food Chem*. 59:8108-8116.

Layshock JA, Wilson G, Anderson KA. 2010. Ketone and quinone-substituted polycyclic aromatic hydrocarbons in mussel tissue, sediment, urban dust, and diesel particulate matrices. *Environ Toxicol Chem*. 29:2450-2460.

O'Connell SG, Haigh T, Wilson G, Anderson KA. 2013. An analytical investigation of 24 oxygenated-PAHs (OPAHs) using liquid and gas chromatography-mass spectrometry. *Anal. Bioanal. Chem.* 1-12.

Peachey RBJ. 2005. The synergism between hydrocarbon pollutants and UV radiation: A potential link between coastal pollution and larval mortality. *J Exp Mar Biol Ecol*. 315:103-114.