Environmental Science & Technology Letters
Letter
pubs.acs.org/journal/estlcu

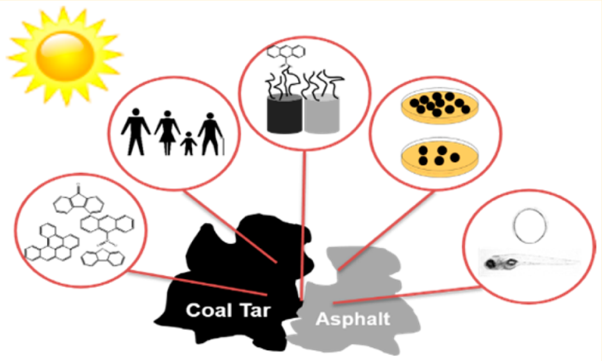
Identification and Toxicological Evaluation of Unsubstituted PAHs and Novel PAH Derivatives in Pavement Sealcoat Products

Ivan A. Titaley,[†] Anna Chlebowski,[‡] Lisa Truong,[‡] Robert L. Tanguay,[‡] and Staci L. Massey Simonich*,[†,‡]

[†]Department of Chemistry, Oregon State University, Corvallis, Oregon 97331, United States
[‡]Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, Oregon 97331, United States

Supporting Information

ABSTRACT: Pavement sealcoat products contain high concentrations of unsubstituted polycyclic aromatic hydrocarbons (PAHs), but the assessment of the potential toxicological impact is limited without the inclusion of PAH derivatives. This study determined the concentrations of 23 unsubstituted PAHs, 11 high molecular weight PAHs (MW302-PAHs), and 56 PAH derivatives, including 10 methyl-PAHs (MPAHs), 10 heterocyclic PAHs (Hetero-PAHs), 26 nitrated PAHs (NPAHs), and 10 oxygenated PAHs (OPAHs) in coal tar- and asphalt-based sealcoat products and time point scrapes. Inclusion of MW302-PAHs resulted in an increase of 4.1%−38.7% in calculated benzo[*a*]pyrene carcinogenic equivalent (B[*a*]P_{eq}) concentrations for the coal tar-based products. Increases in some NPAH and OPAH concentrations were measured after application, suggesting the possibility of photochemical transformation of unsubstituted PAHs. The Ames assay indicated that the asphalt-based product was not mutagenic, but the coal tar-based sealcoat products were. The zebrafish developmental toxicity tests suggested that fractions where NPAHs and OPAHs eluted have the most significant adverse effects.



■ INTRODUCTION

Sealcoat is a black liquid often sprayed or brushed on the asphalt pavement of driveways and parking lots. Recent research has demonstrated that some sealcoat products, in particular, those that are coal tar emulsion, contain high concentrations of unsubstituted polycyclic aromatic hydrocarbons (PAHs),[1−3] some of which are known or suspected to be carcinogenic.[4] Coal tar is a byproduct of the coal coking process and contains a myriad of PAHs and PAH derivatives. PAHs originating from sealcoat have been measured in the air[5,6] and may have adverse health effects on the aquatic environment.[7−9] In addition, incidental ingestion of settled house dust contaminated with PAHs from coal tar-based sealcoated pavement may be hazardous to human health.[6,10]

In the United States, asphalt- and coal tar-based sealcoat are the most common types of sealcoat products. Research by Mahler et al.[6] has shown that PAH concentrations measured in the coal tar-based products were 3 orders of magnitude higher than in the asphalt-based products. Geographically, the coal tar-based product was more commonly found east of the United States continental divide, while the asphalt-based product was more common to the west of the divide.[11] Pavement sealcoat products are also used in Canada.[6,12] Bans on coal tar-based sealcoat products have been enacted, or are being considered, in multiple United States municipalities and states,[6,13] while coal tar-based sealcoat use in the European Union is limited or banned.[14]

Research on PAHs released from sealcoat products has primarily focused on unsubstituted PAHs (PAHs) and methylated-PAHs (MPAHs), commonly known as alkylated-PAHs.[5,6,12,15] However, there are other PAH groups that have not been studied in sealcoat products. Heterocylic-PAHs (Hetero-PAHs) are a group of PAH derivatives where one of the carbons within the ring system is replaced by nitrogen, oxygen, or sulfur. Recent research has indicated that some Hetero-PAHs show estrogenic activity[16] and ecotoxicity.[17] Nitro-PAHs (NPAHs) and oxygenated-PAHs (OPAHs) have been detected in the environment,[18−20] and some are classified as known mutagens and/or possible or probable human carcinogens (group A2 or B2, respectively).[4,19,21] In addition, some high molecular weight PAHs (MW302-PAHs) are classified as possible/probable carcinogens and have some of the highest draft U.S. EPA Relative Potency Factors (RPFs) for PAHs.[4,22] Although MW302-PAHs have been previously detected in coal tar[23,24] and in the environment,[25] analysis of this group of PAH derivatives in sealcoat has not been previously reported.

Given the current regulatory evaluation of sealcoat products and their use, the purpose of this study was to investigate the presence of PAHs, MPAHs, MW302-PAHs, Hetero-PAHs,

Received: March 26, 2016
Revised: April 18, 2016
Accepted: April 19, 2016

DOI: 10.1021/acs.estlett.6b00116

Table 1. List of Target and Surrogate PAHs, MPAHs, and MW302-PAHs Analyzed in This Study and Their Abbreviations[a]

No.	Compound	Abbreviation	No.	Compound	Abbreviation
PAHs			**MW302-PAHs**		
1	Naphthalene	NAP	1	Dibenzo[*a*,*e*] + [*b*,*k*]fluoranthene	DBae+bkF
2	Acenaphthylene	ACY	2	Naphtho[2,3-*b*] fluoranthene	N23bF
3	Acenaphthene	ACE	3	Dibenzo[*a*,*k*]fluoranthene	DBakF
4	Fluorene	FLO	4	Dibenzo[*j*,*l*]fluoranthene	DBjlF
5	Dibenzothiophene	DBT	5	Dibenzo[*a*,*l*]pyrene	DBalP
6	Phenanthrene	PHE	6	Naphtho[2,3-*k*] fluoranthene	N23kF
7	Anthracene	ANT	7	Naphtho[2,3-*e*]pyrene	N23eP
8	Fluoranthene	FLA	8	Dibenzo[*a*,*e*]pyrene	DBaeP
9	Pyrene	PYR	9	Coronene	COR
10	Retene	RET	10	Dibenzo[*a*,*i*]pyrene	DBaiP
11	Benzo[*c*]fluorene	BcFlo	11	Dibenzo[*a*,*h*]pyrene	DBahP
12	Cyclopenta[*cd*]pyrene	CPP	**Hetero-PAHs**		
13	Benzo[*a*]anthracene	BaA	1	2-Methylbenzofuran	2-MBF
14	Chrysene + Triphenylene	CHR+TRI	2	Thianaphthene	THI
15	Benzo[*b*]fluoranthene	BbF	3	Quinoline	QUIN
16	Benzo[*k*]fluoranthene	BkF	4	Indole	IND
17	Benz[*j*][*e*]aceanthrylene	Bj+eA	5	8-Methylquinoline	8-MQN
18	Benzo[*e*]pyrene	BeP	6	Dibenzofuran	DBF
19	Benzo[*a*]pyrene	BaP	7	Xanthene	XAN
20	Dibenz[*a*,*h*]+[*a*,*c*]anthracene	DBah+acA	8	5,6-Benzoquinoline	5,6-BQN
21	Indeno[1,2,3-*cd*]pyrene	IcdPY	9	Acridine	ACR
22	Benzo[*ghi*]perylene	BghiP	10	Carbazole	CARB
23	Anthanthrene	ANH	**Surrogate Standards**		**PAH group analyzed**
MPAHs			1	d_{10}-fluorene	PAHs, MPAHs, and Hetero-PAHs
1	2-Methylnaphthalene	2-MNAP	2	d_{10}-phenanthrene	
2	1-Methylnaphthalene	1-MNAP	3	d_{10}-pyrene	
3	2,6-Dimethylnaphthalene	2,6-DMNAP	4	d_{12}-triphenylene	
4	1,3-Dimethylnaphthalene	1,3-DMNAP	5	d_{12}-benzo[a]pyrene	
5	2-Methylphenanthrene	2-MPHE	6	d_{12}-benzo[*ghi*]perylene	
6	2-Methylanthracene	2-MANT	1	d_{14}-dibenzo[*a*,*i*]pyrene	MW302-PAHs
7	1-Methylphenanthrene	1-MPHE			
8	3,6-Dimethylphenanthrene	3,6-DMPHE			
9	1-Methylpyrene	1-MPYR			
10	6-Methylchrysene	6-MCHR			

[a] PAH and MPAH standards were purchased from AccuStandard (New Haven, CT) and Chem Service (West Chester, PA); MW302-PAH standards were purchased from Chiron AS (Trondheim, Norway) and AccuStandard. Surrogate standards were purchased from CDN Isotopes (Quebec, Canada) and Cambridge Isotope Laboratories (Andover, MA).

NPAHs, and OPAHs in sealcoat products and time point scrapings after application, as well as to evaluate their toxicological relevance in a real-world complex mixture. Gas chromatography coupled with mass spectrometry (GC/MS) was used for comprehensive measurement of a wide range of compounds in the sealcoat products and time point scrapes. The Ames assay and the zebrafish (*Danio rerio*) toxicity test were used to evaluate the mutagenicity and developmental toxicity of the samples, respectively.

■ EXPERIMENTAL METHODS

Chemicals and Materials. All 23 PAHs, 10 MPAHs, 11 MW302-PAHs, 10 Hetero-PAHs, 26 NPAHs, and 10 OPAHs measured in this study, along with their abbreviations, are listed in Tables 1 and 2. Hexane, ethyl acetate, dichloromethane, and dimethyl sulfoxide (DMSO) were purchased from Fisher Scientific (Santa Clara, CA) and Sigma-Aldrich (St. Louis, MO). All materials used in this study were precleaned and baked prior to use.

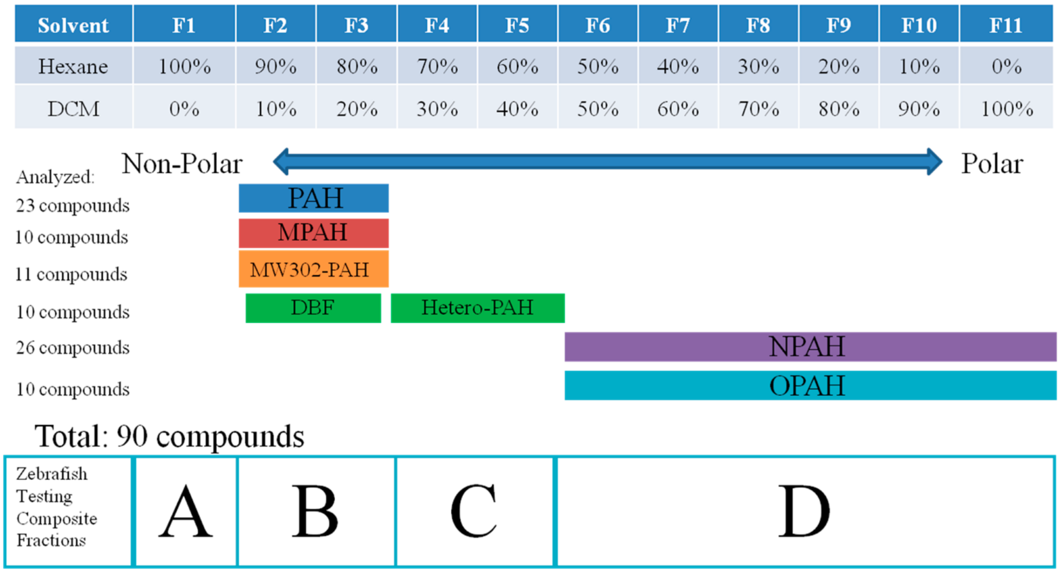
Sample Collection. The samples were collected through collaboration with the United States Geological Survey (USGS) in Austin, TX and were previously used in a study.[5] Two coal tar-based sealcoat products, Tarconite Neyra Industries, Inc. (CT-1) and Gulf Seal No. 253 Henry Company (CT-2), and one asphalt-based sealcoat product, Henry Seal No. 532, Henry Company (AS), were applied directly from the container onto separate acrylic glass sheets.[5,26] The glass sheets were kept in a dark room under ambient temperatures. The dried product from each sheet was scraped off after 3 days, sealed in precleaned Teflon-lined lid amber glass containers, and refrigerated (4 °C) until analysis. More information on sample collection is available in the Supporting Information.

To determine the change in PAH composition over time, the CT-1 product was applied on a pavement in Austin, TX, as described previously.[5] Briefly, a commercial applicator applied the CT-1 product to a test plot at the J. J. Pickle Research Campus, University of Texas. After a curing period of 24 h, the test plot was opened for parking and traffic. Four time point scrapes of the CT-1 product were taken post application at 1.6 h, 1 d, 45 d, and 149 d.[5] Care was taken during scraping to sample the CT-1 sealant layer and not the underlying asphalt.[5] Each scrape contained a composite from five locations within the test

Table 2. List of Target and Surrogate NPAHs and OPAHs Analyzed in This Study and Their Abbreviations[a]

No.	Compond	Abbreviation	No.	Compound	Abbreviation
NPAHs			**OPAHs**		
1	1-Nitronaphthalene	1-NN	1	9-Fluorenone	9-FLU
2	2-Nitronaphthalene	2-NN	2	Phenanthrene-1,4-dione	PHD
3	2-Nitrobiphenyl	2-NBP	3	9,10-Anthraquinone	ANQ
4	3-Nitrobiphenyl	3-NBP	4	2-Methyl-9,10-anthraquinone	2-MANQ
5	4-Nitrobiphenyl	4-NBP	5	Benzo[*a*]fluorenone	BFLN
6	3-Nitrodibenzofuran	3-NBF	6	Benzanthrone	BEN
7	5-Nitroacenaphthene	5-NAC	7	Aceanthrenequinone	ACQN
8	2-Nitrofluorene	2-NFL	8	7,12-Benz[*a*] anthracenquinone+Benzo[*c*] phenanthrene-[1,4]quinone	BQN+PQN
9	9-Nitroanthracene	9-NAN	9	Benzo[*cd*]pyrenone	BPYN
10	9-Nitrophenanthrene	9-NPH	10	1,6-Benzo[*a*]pyrene quinone	1,6-BPQN
11	2-Nitrodibenzothiophene	2-NBT	**Surrogate Standards**		**PAH group analyzed**
12	3-Nitrophenanthrene	3-NPH	1	d_7-1-nitronaphthalene	NPAHs
13	2-Nitroanthracene	2-NAN	2	d_9-5-nitroacenaphthene	
14	2+3-Nitrofluoranthene	2+3-NF	3	d_9-9-nitroanthracene	
15	1-Nitropyrene	1-NP	4	d_9-3-nitrofluoranthene	
16	2-Nitropyrene	2-NP	5	d_9-1-nitropyrene	
17	2,8-Dinitrodibenzothiophene	2,8-DNDB	6	d_{11}-6-nitrochrysene	
18	7-Nitrobenz[*a*]anthracene	7-NBaA	1	d_8-anthraquinone	OPAHs
19	1-Nitrotriphenylene	1-NTR			
20	6-Nitrochyrsene	6-NCH			
21	3-Nitrobenzanthrone	3-NBENZ			
22	2-Nitrotriphenylene	2-NTR			
23	1,3-Dinitropyrene	1,3-DNP			
24	1,6-Dinitropyrene	1,6-DNP			
25	1,8-Dinitropyrene	1,8-DNP			
26	6-Nitrobenzo[*a*]pyrene	6-NBaP			

[a] NPAH and OPAH standards were purchased from Chiron AS (Trondheim, Norway), AccuStandard (New Haven, CT), Chem Service (West Chester, PA), and Sigma-Aldrich (St. Louis, MO). Surrogate standards were purchased from CDN Isotopes (Quebec, Canada) and Cambridge Isotope Laboratories (Andover, MA).



Solvent	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Hexane	100%	90%	80%	70%	60%	50%	40%	30%	20%	10%	0%
DCM	0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%

Figure 1. Solvent fractionation through the second 20 g of silica SPE column. F1 is 100 mL of hexane and F11 is 100 mL of DCM; 10% increments correspond to 10 mL. Composite fractions for the zebrafish test were labeled A−D, and the observed orders of elution for PAH and PAH derivatives are outlined above.

plot, and samples were stored in precleaned Teflon-lined lid amber glass containers and refrigerated (4 °C) until analysis.

Sample Extraction and Analysis. Approximately 0.1 g of each product and time point scrape was extracted using sonication with ∼7 mL of dichloromethane (DCM) for 5 min, followed by solvent exchange to hexane. Two rounds of 20 g of silica solid phase extraction (SPE) were required to remove high levels of impurities in the extracts. In the first silica SPE cleanup, the order of solvent elution was 50 mL of hexane, 100 mL of DCM, and 50 mL of ethyl acetate. The DCM extract was solvent

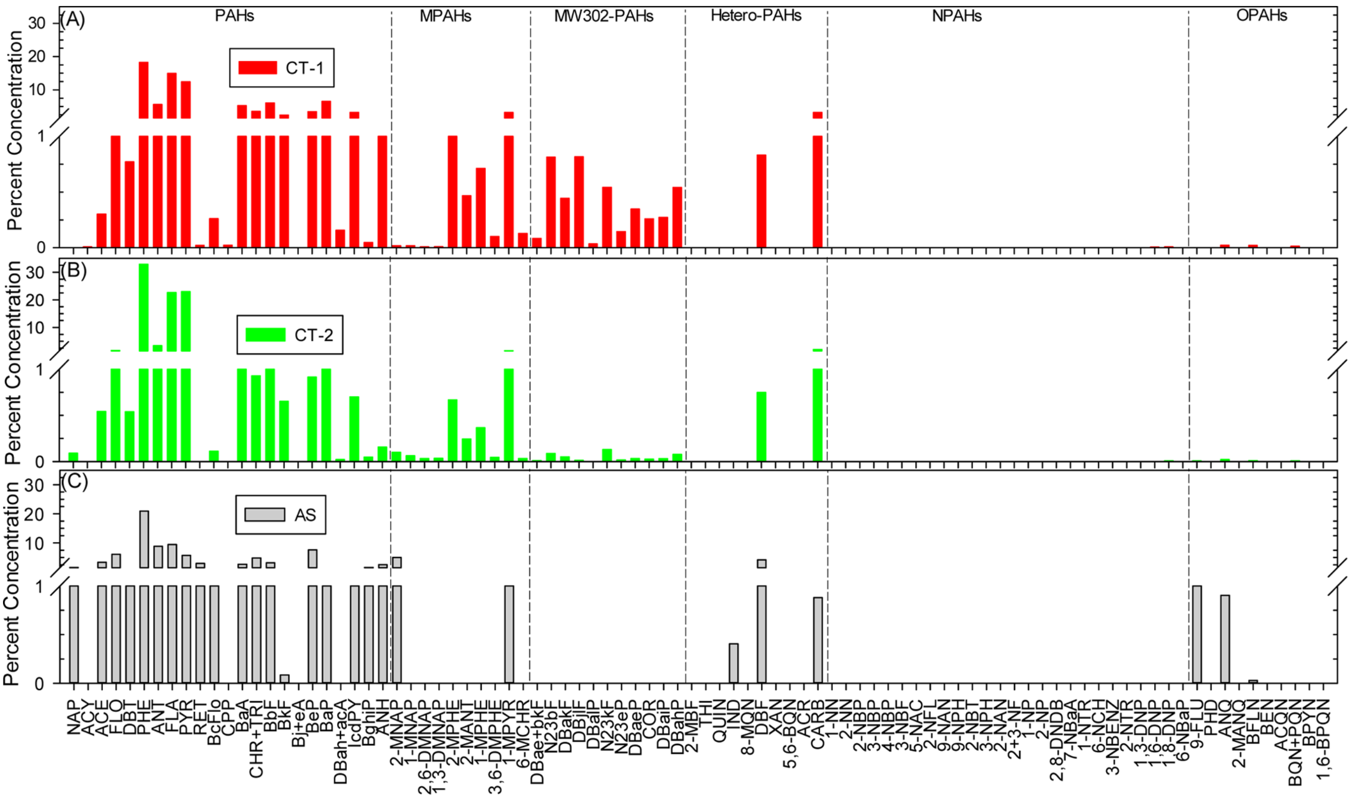


Figure 2. Profile of PAHs and PAH derivatives in (A) CT-1, (B) CT-2, and (C) AS sealcoat products.

exchanged to hexane, reduced to ~1 mL, and further purified with 20 g of silica SPE using 11 100 mL fractions with the following solvent elution (% hexane:% DCM): 100:0 (F1), 90:10 (F2), 80:20 (F3), 70:30 (F4), 60:40 (F5), 50:50 (F6), 40:60 (F7), 30:70 (F8), 20:80 (F9), 10:90 (F10), and 0:100 (F11) (Figure 1).

Each of the 11 fractions was reduced to ~500 μL, solvent exchanged to DCM, and stored at −4 °C until analysis. Aliquots of the 11 fractions were removed and set aside for the mutagenicity (100 μL) and toxicity (250 μL) tests, and the remaining analytical chemistry portion of the fractions were spiked with isotopically labeled surrogate standards. Each aliquot was stored separately in a GC vial with silicone caps and refrigerated (−4 °C) until analysis. A laboratory blank sample underwent the same handling procedures. Details on the sealcoat extract analysis, including GC/MS conditions, are given in the Supporting Information. All concentrations are reported on a dry weight basis. Figure 2 provides the percent concentration for each compound analyzed in this study.

Benzo[*a*]pyrene Carcinogenic Equivalent Concentration. In this approach, concentrations of individual carcinogenic PAHs and MW302-PAHs (C_{PAHi}) were multiplied by their corresponding draft U.S. EPA relative potency factor (RPF_i)[22] and summed to obtain a B[*a*]Pe$_{eq}$ concentration for each sealcoat sample (eq 1):

$$B[a]P_{eq} = \sum_{i=1}^{n} (C_{PAH_i} \times RPF_i) \tag{1}$$

Salmonella Mutagenicity Assay. All extract fractions were assessed for mutagenicity using the Ames assay.[27] *Salmonella* strain TA98 (Xenometrix, Inc., Allschwil, Switzerland) was used in combination with the rat S9 mix to test the mutagenicity with and without metabolic activation as previously described.[19,28,29] 2-Aminoanthracene and 4-nitro-1,2-phenylenediamine were used as positive controls for with and without the rat S9 mix, respectively. DMSO was used as the negative control for both experimental conditions. Each sealcoat fraction was tested in triplicate for each Ames endpoint. A sealcoat fraction was determined to be significantly mutagenic if the number of bacteria revertants per plate count was two standard deviations higher than its respective negative control. All samples were tested at 1:100 dilutions.

Zebrafish Developmental Toxicity Test. We combined the 11 original silica fractions (F1−F11) into four groups (A−D) according to the order of elution in order to reduce the number of zebrafish embryos used in the test (Figure 1). Fraction A (F1 only) did not contain any PAHs. Fraction B (F2 + F3) contained PAHs, MPAHs, MW302-PAHs, and DBF. Fraction C (F4 + F5) containedthe remaining Hetero-PAHs. Fraction D (F6−F11) contained NPAHs and OPAHs.

Each of the combined fractions was solvent exchanged to DMSO. Samples were stored at −20 °C until 1 h prior to zebrafish exposure. Zebrafish embryos were spawned at the OSU Sinnhuber Aquatic Research Laboratory. Embryos were dechorionated (i.e., we removed the shell-like membrane complex), and each embryo was placed into individual wells in 96-well plates with 100 μL of embryo media.[30] The sealcoat fractions were diluted 10,000-, 1000-, 400-, 200-, and 100-fold and directly dispensed using the HP D300 digital dispenser (Männerdorf, Switzerland) at 6 h post fertilization (hpf) into 16 wells in two plates, giving $n = 32$ embryos per diluted concentration at ambient temperature. Immediately after chemical dispensing, the exposed plates were placed on an orbital shaker and shaken overnight at 235 rpm. The concentration of DMSO in the extracts and negative controls was 1%. The embryos were assessed for morphological

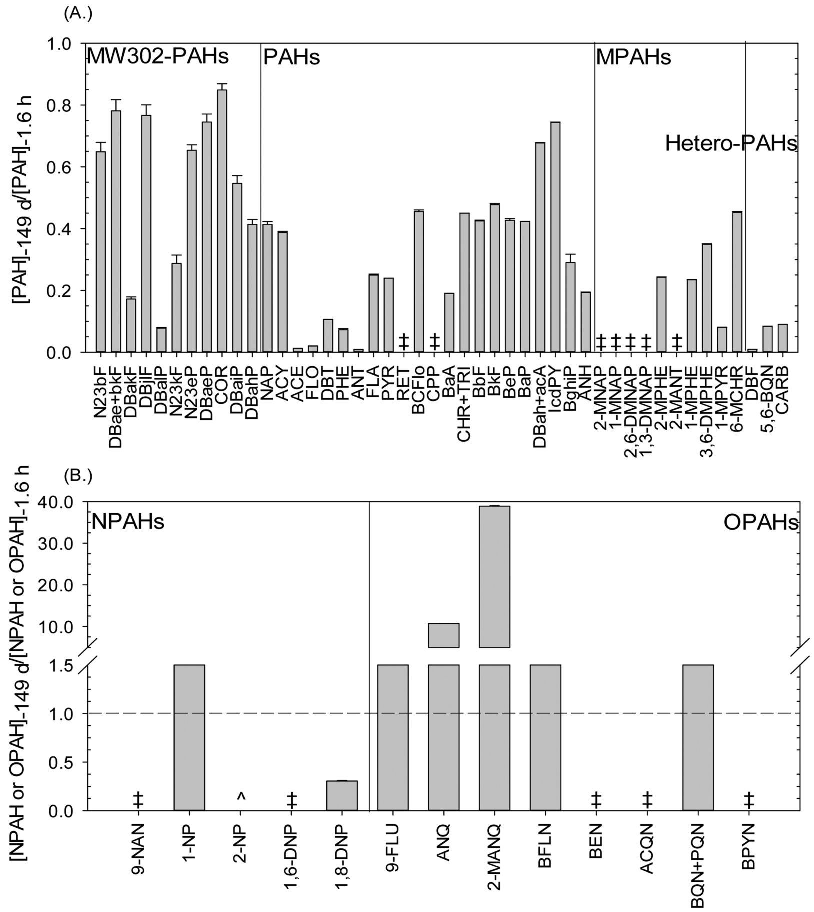


Figure 3. (A) Ratio of PAH concentrations at $t = 149$ days to 1.6 h (error bar represents ratio error propagation), indicating net losses of PAHs, MPAHs, MW302-PAHs, and Hetero-PAHs. (B) Ratio of NPAH or OPAH concentrations at $t = 149$ days to 1.6 h (error bar represents ratio error propagation), indicating net formation of some NPAHs and OPAHs. The symbol ‡ denotes compounds that were detected at 1.6 , but not at 149 days, while the symbol ∧ denotes compounds that were detected at 149 days but not at 1.6 h.

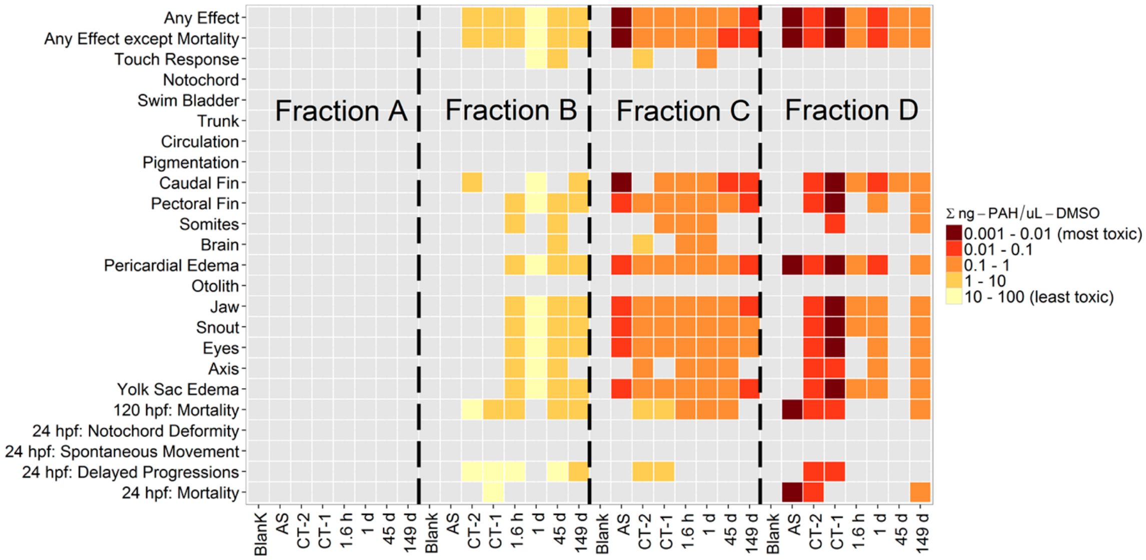


Figure 4. Heat map of lowest effect levels (LELs) of acute endpoints in the zebrafish (*Danio rerio*) developmental toxicity test. Gray color indicates that no statistically significant effect was observed. Samples analyzed included both CT products, the AS product, and the five time point scrapes of CT-1 post application that were taken at the following time points: 1.6 h (h), 1 day (d), 45 d, and 149 d. Units are given in $\sum$ ng PAH/μL DMSO, where $\sum$ ng PAH is equal to summed PAH and PAH derivatives in each fraction.

malformations at both 24 and 120 hpf as previously described.[31,32]

Statistical Analysis. Microsoft Excel 2010, JMP (Statistical Discovery from SAS), and SigmaPlot version 13 were used for statistical analyses. Error propagation in Figure 3 was calculated by multiplying the concentration ratio with the squared root of the sum of the standard error and concentration ratio of each time point scrape squared (eq 2):[33]

$$SE_{\text{error prop}} = \left(\frac{[\text{PAH}]_{149\text{d}}}{[\text{PAH}]_{1.6\text{h}}}\right) \times \sqrt{\left(\frac{SE[\text{PAH}]_{149\text{d}}}{[\text{PAH}]_{149\text{d}}}\right)^2 + \left(\frac{SE[\text{PAH}]_{1.6\text{h}}}{[\text{PAH}]_{1.6\text{h}}}\right)^2} \quad (2)$$

Custom R script[34] was used to generate the heat map in Figure 4 and to calculate EC_{50} calculations and lowest effect levels (LEL). To compute EC_{50} values for the "any effect" endpoint, a four-parameter logistic regression was fit to the data.[31]

■ RESULTS AND DISCUSSION

PAH and PAH Derivatives Measured in Sealcoat Products. Unsubstituted PAHs made up the largest percentage, by mass, of all compounds detected, regardless of whether the sealcoat product was CT or AS (Table 3). Both CT products had total PAH concentrations 600−1900-fold higher than the AS sealcoat product ($p < 0.05$, one-sided t test), consistent with previous studies.[11,12] As illustrated in Table S1, PAH concentrations ranged from 0.04 mg/kg (BkF in AS) to 45,000 mg/kg (PHE in CT-2), and MPAH concentrations ranged from 0.5 mg/kg (1-MPYR, 2,6-DMNAP, and 1,3-DMNAP in AS) to 2100 mg/kg (1-MPYR in CT-2). All 23 PAHs and 10 MPAHs analyzed in this study were detected in both CT-based products. In the AS product, 19 of 23 PAHs and 9 of 10 MPAHs were detected. Figure 2 shows the relative PAH profile for the CT and AS sealcoat products.

Table 3. Summed Concentrations (n = 3) of Various PAH Groups Measured in the CT and AS Sealcoat Products[a]

PAH group	CT-1 (mg/kg dry wt)	CT-2 (mg/kg dry wt)	AS (mg/kg dry wt)
∑PAH	39,000 (86%)	129,000 (94%)	50 (65%)
∑MPAH	2700 (6%)	4300 (3%)	20 (26%)
∑MW302-PAH	2000 (4%)	650 (0.5%)	<LOD (0%)
∑Hetero-PAH	1800 (4%)	3800 (3%)	5 (6%)
∑NPAH	8 (<0.5%)	10 (<0.5%)	<LOD (0%)
∑OPAH	32 (<0.5%)	70 (<0.5%)	2 (3%)
TOTAL	45,500	138,800	77

[a] The concentrations of each PAH group in the AS sample were significantly lower ($p < 0.05$) than in either of the CT samples.

Van Metre et al.[5] reported similar concentrations for 18 PAHs, 4 MPAHs, 1 Hetero-PAH, and 1 OPAH for CT-1 (Figure S1), with the exception of NAP and 2,6-DMNAP, which had 3−7 times higher concentrations in the Van Metre et al. paper.[5] This may be due to the loss of these volatile compounds from the sample over time. This may suggest that the Van Metre et al.[5] reported concentrations for NAP and 2,6-DMNAP are more representative for a freshly applied CT sealcoat product, and the concentrations measured in this study may be more representative of an aged CT sealcoat product.

MW302-PAHs were detected in both CT products but not in the AS product (Figure 2). N23bF, DBae+bkF, DBakF, DBjlF, DBalP, N23kF, N23eP, DBaeP, COR, DBaiP, and DBahP were measured in both CT products. The highest concentration of MW302-PAHs was in CT-1 (370 mg/kg for DBjlF and DBae+bkF), and the lowest concentration was in CT-2 (7 mg/kg for DBalP). Asphalt originates from a petrogenic source containing lower molecular weight PAHs, while CT sealcoat originates from a pyrogenic source (coal) containing higher molecular weight PAHs.[35] Although the MW302-PAHs may be less bioavailable in aquatic ecosystems following runoff from pavement sealcoat surfaces, they may be present in ambient and indoor air due to abrasion of sealcoat surfaces and tracking of sealcoat dust indoors.[6,12] Furthermore, the presence of MW302-PAHs in the CT pavement sealcoat product, and their absence from the AS pavement sealcoat product, may make MW302-PAHs a unique molecular marker for CT pavement sealcoat product use in the urban environment where coal is no longer burned.

Of the 10 Hetero-PAHs, only three were detected in CT-1 and AS (IND, DBF, and CARB), while six were detected in CT-2 (THI, IND, DBF, 5,6-BQN, ACR, CARB). Hetero-PAH concentrations ranged from 0.3 mg/kg (IND in AS) to 2800 mg/kg (CARB in CT-2). CARB has previously been measured at high concentrations in the runoff from coal tar-based sealcoated surfaces.[2] DBF has not been previously measured in sealcoat. However, it has been measured in sediment samples that are in close proximity to coal tar-sealed pavement,[36] and our analyses revealed that DBF was present in both CT and AS products.

NPAHs were not detected in the AS pavement sealcoat product. However, eight NPAHs were measured in CT samples (9-NAN, 2-NBT, 1,6-DNP, and 1,8-DNP in CT-1; 1-NN, 2-NN, 3-NPH, 1,3-DNP, and 1,8-DNP in CT-2). For OPAHs, 9-FLU, PHD, ANQ, 2-MANQ, BFLN, BEN, and BQN+PQN were measured in theCT-1 product. CT-2 contained all of the same compounds, with the addition of BPYN. Only 9-FLU, ANQ, and BFLN were measured in the AS product. The lowest measured OPAHs concentration was 0.025 mg/kg (BFLN in AS), while the highest was 31 mg/kg (ANQ in CT-2). The detection of these NPAH and OPAH compounds in both CT and AS pavement sealcoat products suggests that they are contained in the products in addition to their potential photochemical formation following sealcoat application. Given their polar characteristics, and likely increased bioavailability relative to unsubstituted PAHs, accounting for the increased toxicity due to substituted PAHs may be necessary in the future.

Benzo[*a*]pyrene Carcinogenic Equivalent Concentration in Sealcoat. To examine the potential relevance of these findings in a human health context, we calculated the benzo[*a*]pyrene carcinogenic equivalent ($B[a]P_{eq}$) concentrations of the sealcoat products using eq 1. Tables S2 and S3 show the concentrations and draft U.S. EPA RPF values used.[22] Without the inclusion of MW302-PAHs, the $B[a]P_{eq}$ concentrations in the CT products were up to 3 orders of magnitude higher than the AS product (Table S2). When MW302-PAH concentrations were included in the assessment, there was a 4.1% and 10.7% increase in the $B[a]P_{eq}$ concentrations for CT-1 and CT-2, respectively, even though MW302-PAHs comprised only 0.5% and 4% of the total PAH concentration.

Because pavement sealcoat is used in both the United States and Canada, the Health Canada[37] potency equivalence factor (PEF) values were also used to calculate the $B[a]P_{eq}$ concentrations for comparison to the draft U.S. EPA RPF approach. When MW302-PAH concentrations were included in the assessment, there was a 26.6% and 38.6% increase in the $B[a]P_{eq}$ concentrations for CT-1 and CT-2, respectively, using the Health Canada approach (Table S2). The discrepancy between the U.S. EPA and Health Canada $B[a]P_{eq}$ concentrations can be attributed to the higher PEF values for the MW302-PAHs used in the Canadian approach. As expected, in both approaches, there was no increase in B[*a*]P_{eq} concentration in the AS product when MW302-PAHs were included in the calculation because they were not detected in the AS product. The increase in B[a]P_{eq} concentrations, resulting from the inclusion of MW302-PAHs, suggests a greater potential risk to human health from coal tar-sealed surfaces than previously determined because of the presence of MW302-PAHs.[10,38,39]

PAH Losses from Sealcoated Surfaces. Time point scrapes of the CT-1 sealcoated pavement (1.6 h, 1 d, 45 d, and 149 d after application) were used to study PAH losses from a sealcoated surface. Prior studies have determined various routes of PAH loss from sealcoated pavement, including volatilization,[5,40] runoff,[36] and abrasion,[3] among others.[6]

A potential route of PAH loss from sealcoated surfaces that should be considered is photodegradation. Net losses of all PAHs, MPAHs, MW302-PAHs, and Hetero-PAHs on the sealcoat surface from 1.6 h to 149 d after application were measured (Figure 3A). On the basis of a prior study,[5] losses due to volatilization occur primarily up until 45 days post application. Photodegradation may explain some of the net loss of PAHs after the initial 45 days. By 149 d post application, the concentrations of ANT, FLO, 2-MANT, and PYR on the sealcoated surface decreased by 76%−100% relative to those 1.6 h post application (Figure 3A).

In addition, phototransformation of unsubstituted PAHs on pavement sealcoated surfaces may result in the net formation of some NPAHs and OPAHs on the same surface (Figure S2). Here, 1-NP, 9-FLU, ANQ, and 2-MANQ concentrations increased 200%−4000% on the sealcoated surface by 149 d (Figure 3B and Figure S2). Jariyasopit et al. previously showed that 1-NP was formed on ambient particulate matter after direct exposure to NO_3/N_2O_5 and the OH radical.[21,29] OPAHs, such as 9-FLU and ANQ, have also been observed to form on ambient particulate matter as the product of atmospheric reaction.[41−43] This suggests the increased concentrations of some NPAHs and OPAHs on the sealcoated surface over time may have originated, in part, from phototransformation of unsubstituted PAHs and MPAHs[19,44,45] on the sealcoated surface.

Mutagenicity Assessment. The Ames assay indicated that F2 of the CT-1 product and F2 of the CT-1 time point scrapes, as well as F3 of the CT-2 product, had indirect-acting mutagenicity two standard deviations above the negative control (Figure S3). However, none of the AS fractions were mutagenic (Figure S3). This suggests that indirect-acting mutagens in F2 and F3 of the CT samples, including PAHs, MPAHs, and MW302-PAHs[46] (Figure 1), were responsible for the mutagenicity in the CT fractions.

Developmental Toxicity Study. The sealcoat products and time point scrapes were also tested using the embryonic zebrafish developmental toxicity test, which has been previously used to assess toxicity of environmental samples,[8,18,32] including that of OPAHs.[31] The main advantage of this test is its high throughput capacity that allows for noninvasive observation of 20 acute endpoints in addition to mortality. The rapid development, coupled with 70% genetic homology to humans,[47] makes zebrafish a useful model to assess potential human health hazards.

The heat map of lowest effect levels (LELs) (Figure 4) shows there were significant developmental toxicity effects, as characterized by the same 14 acute endpoints, for all pavement sealcoat products tested. Fraction A, which did not contain any of the PAH compounds measured in this study (Figure 1) was not toxic in either the CT products or the AS product. Exposure to fraction B of both CT products induced toxicity, but no toxicity was observed in fraction B of the AS product. Toxicity was observed in fraction C of all three products with the highest toxicity observed in the AS product. Fraction D was the most toxic fraction for both the CT products and the AS product, as evidenced by high toxicity in both "any effect" and "any effect except mortality" endpoints. Fraction C contained Hetero-PAHs, and fraction D contained the NPAHs and OPAHs (Figure 1). Generally, on the basis of the same developmental endpoints as above, the toxicities of the product fractions were D > C > B > A (Figure 4). Figure S5 shows that on the basis of EC_{50}s the toxicities of the product fractions were also D > C > B > A. This suggests that the higher concentrations of PAHs, MPAHs, and MW302-PAHs measured in fraction B were less acutely toxic relative to the lower concentrations of Hetero-PAHs, NPAHs, and OPAHs measured in fractions C and D.

With regard to the time point scrapes of CT-1, the developmental toxicity did not appear to increase or decrease with time (Figure 4). However, fractions C and D showed more developmental toxicity than fractions A and B. Figure S6 shows that fractions C and D of the time point scrapes of CT-1 also had the lowest EC_{50} values. This is consistent with our conclusion above that Hetero-PAHs, NPAHs, and OPAHs contribute to the developmental toxicity of pavement sealcoat.

We used the zebrafish test as a screen test for potential toxicological impacts from pavement sealcoat exposure and to make a relative comparison between coal tar- and asphalt-based products. However, these results also suggest that there is the potential for impact to aquatic organisms living downstream from pavement sealcoat.[8]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.estlett.6b00116.

Sample collection, sample analysis, quality assurance/quality control (QA/QC), Tables S1−S3, and Figures S1−S6. (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: staci.simonich@oregonstate.edu. Phone: (541) 737-9194. Fax: (541) 737-0497.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This publication was made possible in part by Grant P30ES00210 from the National Institute of Environmental Health Sciences (NIEHS) and NIEHS Grant P42ES016465, the National Institutes of Health (NIH), and the National Science Fundation Grant AGS-11411214. Its contents are solely the responsibility of the authors and do not necessarily represent the official view of NIEHS and NIH. We thank Dr. Barbara Mahler and Dr. Peter C. Van Metre of the U.S. Geological Survey for the samples; Dr. David Yu of OSU Linus Pauling Institute for the Ames test; the toxicity screening team of OSU Sinnhuber Aquatic Research Laboratory for the Zebrafish developmental toxicity test; and OSU SRP Core D for some standards.

REFERENCES

(1) Mahler, B. J.; Van Metre, P. C.; Bashara, T. J.; Wilson, J. T.; Johns, D. A. Parking Lot Sealcoat: An Unrecognized Source of Urban Polycyclic Aromatic Hydrocarbons. *Environ. Sci. Technol.* **2005**, *39*, 5560−5566.

(2) Mahler, B. J.; Van Metre, P. C.; Foreman, W. T. Concentrations of Polycyclic Aromatic Hydrocarbons (PAHs) and Azaarenes in Runoff from Coal-Tar- and Asphalt-Sealcoated Pavement. *Environ. Pollut.* **2014**, *188*, 81−87.

(3) Scoggins, M.; Ennis, T.; Parker, N.; Herrington, C. A Photographic Method for Estimating Wear of Coal Tar Sealcoat from Parking Lots. *Environ. Sci. Technol.* **2009**, *43*, 4909−4914.

(4) List of Classification, Volumes 1−115.International Agency for Research on Cancer. http://monographs.iarc.fr/ENG/Classification/latest_classif.php (accessed February 21, 2016).

(5) Van Metre, P. C.; Majewski, M. S.; Mahler, B. J.; Foreman, W. T.; Braun, C. L.; Wilson, J. T.; Burbank, T. L. PAH Volatilization Following Application of Coal-Tar-Based Pavement Sealant. *Atmos. Environ.* **2012**, *51*, 108−115.

(6) Mahler, B. J.; Van Metre, P. C.; Crane, J. L.; Watts, A. W.; Scoggins, M.; Williams, E. S. Coal-Tar-Based Pavement Sealcoat and PAHs: Implications for the Environment, Human Health, and Stormwater Management. *Environ. Sci. Technol.* **2012**, *46*, 3039−3045.

(7) Kienzler, A.; Mahler, B. J.; Van Metre, P. C.; Schweigert, N.; Devaux, A.; Bony, S. Exposure to Runoff from Coal-Tar-Sealed Pavement Induces Genotoxicity and Impairment of DNA Repair Capacity in the RTL-W1 Fish Liver Cell Line. *Sci. Total Environ.* **2015**, *520*, 73−80.

(8) McIntyre, J.; Edmunds, R.; Anulacion, B.; Davis, J.; Incardona, J. P.; Stark, J. D.; Scholz, N. Severe Coal Tar Sealcoat Runoff Toxicity to Fish is Prevented by Bioretention Filtration. *Environ. Sci. Technol.* **2016**, *50*, 1570−1578.

(9) Bommarito, T.; Sparling, D. W.; Halbrook, R. S. Toxicity of Coal—Tar and Asphalt Sealants to Eastern Newts, *Notophthalmus viridescens*. *Chemosphere* **2010**, *81*, 187−193.

(10) Williams, E. S.; Mahler, B. J.; Van Metre, P. C. Cancer Risk from Incidental Ingestion Exposures to PAHs Associated with Coal-Tar-Sealed Pavement. *Environ. Sci. Technol.* **2013**, *47*, 1101−1109.

(11) Van Metre, P. C.; Mahler, B. J.; Wilson, J. T. PAHs Underfoot: Contaminated Dust from Coal-Tar Sealcoated Pavement is Widespread in the United States. *Environ. Sci. Technol.* **2009**, *43*, 20−25.

(12) Mahler, B. J.; Van Metre, P. C.; Wilson, J. T.; Musgrove, M.; Burbank, T. L.; Ennis, T. E.; Bashara, T. J. Coal-Tar-Based Parking Lot Sealcoat: An Unrecognized Source of PAH to Settled House Dust. *Environ. Sci. Technol.* **2010**, *44*, 894−900.

(13) Crane, J. L. Source Apportionment and Distribution of Polycyclic Aromatic Hydrocarbons, Risk Considerations, and Management Implications for Urban Stormwater Pond Sediments in Minnesota, USA. *Arch. Environ. Contam. Toxicol.* **2014**, *66*, 176−200.

(14) Polycyclic Aromatic Hydrocarbons - Harmful to the Environment! Toxic! Inevitable?, 2012. German Federal Environmental Agency. http://www.umweltbundesamt.de/en/publikationen/polycyclic-aromatic-hydrocarbons (accessed September 30, 2015).

(15) Van Metre, P. C.; Mahler, B. J. PAH Concentrations in Lake Sediment Decline Following Ban on Coal-Tar-Based Pavement Sealants in Austin, Texas. *Environ. Sci. Technol.* **2014**, *48*, 7222−7228.

(16) Brinkmann, M.; Maletz, S.; Krauss, M.; Bluhm, K.; Schiwy, S.; Kuckelkorn, J.; Tiehm, A.; Brack, W.; Hollert, H. Heterocyclic Aromatic Hydrocarbons Show Estrogenic Activity upon Metabolization in a Recombinant Transactivation Assay. *Environ. Sci. Technol.* **2014**, *48*, 5892−5901.

(17) Eisentraeger, A.; Brinkmann, C.; Hollert, H.; Sagner, A.; Tiehm, A.; Neuwoehner, J. Heterocyclic Compounds: Toxic Effects Using Algae, Daphnids, and the *Salmonella*/Microsome Test Taking Methodical Quantitative Aspects into Account. *Environ. Toxicol. Chem.* **2008**, *27*, 1590−1596.

(18) Chibwe, L.; Geier, M. C.; Nakamura, J.; Tanguay, R. L.; Aitken, M. D.; Simonich, S. L. M. Aerobic Bioremediation of PAH Contaminated Soil Results in Increased Genotoxicity and Developmental Toxicity. *Environ. Sci. Technol.* **2015**, *49*, 13889−13898.

(19) Wang, W.; Jariyasopit, N.; Schrlau, J.; Jia, Y.; Tao, S.; Yu, T.-W.; Dashwood, R. H.; Zhang, W.; Wang, X.; Simonich, S. L. M. Concentration and Photochemistry of PAHs, NPAHs, and OPAHs and Toxicity of PM$_{2.5}$ During the Beijing Olympic games. *Environ. Sci. Technol.* **2011**, *45*, 6887−6895.

(20) Witter, A. E.; Nguyen, M. H. Determination of Oxygen, Nitrogen, and Sulfur-Containing Polycyclic Aromatic Hydrocarbons (PAHs) in Urban Stream Sediments. *Environ. Pollut.* **2016**, *209*, 186−196.

(21) Jariyasopit, N.; Zimmermann, K.; Schrlau, J.; Arey, J.; Atkinson, R.; Yu, T.-W.; Dashwood, R. H.; Tao, S.; Simonich, S. L. M. Heterogeneous Reactions of Particulate Matter-Bound PAHs and NPAHs with NO$_3$/N$_2$O$_5$, OH Radicals, and O$_3$ Under Simulated Long-Range Atmospheric Transport Conditions: Reactivity and Mutagenicity. *Environ. Sci. Technol.* **2014**, *48*, 10155−10164.

(22) Integrated Risk Information System. Development of a Relative Potency Factor (RPF) Approach for Polycyclic Aromatic Hydrocarbon (PAH) Mixtures: In Support of Summary Information on the Integrated Risk Information System (IRIS); RPA/635/R-08/012A; U.S. EPA: Washington, DC, 2010. http://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=194584 (accessed June 1, 2015).

(23) Schubert, P.; Schantz, M. M.; Sander, L. C.; Wise, S. A. Determination of Polycyclic Aromatic Hydrocarbons with Molecular Weight 300 and 302 in Environmental-Matrix Standard Reference Materials by Gas Chromatography/Mass Spectrometry. *Anal. Chem.* **2003**, *75*, 234−246.

(24) Fetzer, J. C.; Kershaw, J. R. Identification of Large Polycyclic Aromatic Hydrocarbons in a Coal Tar Pitch. *Fuel* **1995**, *74*, 1533−1536.

(25) Jia, Y.; Stone, D.; Wang, W.; Schrlau, J.; Tao, S.; Simonich, S. L. M. Estimated Reduction in Cancer Risk Due to PAH Exposures if Source Control Measures During the 2008 Beijing Olympics were Sustained. *Environ. Health Perspect.* **2011**, *119*, 815−820.

(26) PAHs in Austin, Texas: Sediments and Coal-Tar Based Pavement Sealants, Polycyclic Aromatic Hydrocarbons; City of Austin, Watershed Protection and Development Review Department: Austin, TX, 2005. https://www.austintexas.gov/sites/default/files/files/Watershed/coaltar/PAHs_in_Austin_2005_final.pdf (accessed September 30, 2015).

(27) Maron, D. M.; Ames, B. N. Revised Methods for the *Salmonella* Mutagenicity Test. *Mutat. Res. Mutagen. Relat. Subj.* **1983**, *113*, 173−215.

(28) Lafontaine, S.; Schrlau, J.; Butler, J.; Jia, Y.; Harper, B.; Harris, S.; Bramer, L. M.; Waters, K. M.; Harding, A.; Simonich, S. L. M. Relative Influence of Trans-Pacific and Regional Atmospheric Transport of PAHs in the Pacific Northwest, U.S. *Environ. Sci. Technol.* **2015**, *49*, 13807−13816.

(29) Jariyasopit, N.; McIntosh, M.; Zimmermann, K.; Arey, J.; Atkinson, R.; Cheong, P. H.-Y.; Carter, R. G.; Yu, T.-W.; Dashwood, R. H.; Massey Simonich, S. L. Novel Nitro-PAH Formation from Heterogeneous Reactions of PAHs with NO$_2$, NO$_3$/N$_2$O$_5$, and OH Radicals: Prediction, Laboratory Studies, and Mutagenicity. *Environ. Sci. Technol.* **2014**, *48*, 412−419.

(30) Nüsslein-Volhard, C.; Dahm, R. *Zebrafish: A Practical Approach*; Oxford University Press: New York, 2002.

(31) Knecht, A. L.; Goodale, B. C.; Truong, L.; Simonich, M. T.; Swanson, A. J.; Matzke, M. M.; Anderson, K. A.; Waters, K. M.; Tanguay, R. L. Comparative Developmental Toxicity of Environmentally Relevant Oxygenated PAHs. *Toxicol. Appl. Pharmacol.* **2013**, *271*, 266−275.

(32) Truong, L.; Reif, D. M.; St Mary, L.; Geier, M. C.; Truong, H. D.; Tanguay, R. L. Multidimensional *In Vivo* Hazard Assessment Using Zebrafish. *Toxicol. Sci.* **2014**, *137*, 212−233.

(33) Harris, D. C. *Quantitative Chemical Analysis*, 7th ed.; Freeman: New York, 2007.

(34) R: The R Project for Statistical Computing. https://www.r-project.org/ (accessed January 25, 2016).

(35) Neff, J. M.; Stout, S. A.; Gunster, D. G. Ecological Risk Assessment of Polycyclic Aromatic Hydrocarbons in Sediments:

Identifying Sources and Ecological Hazard. *Integr. Environ. Assess. Manage.* **2005**, *1*, 22−33.

(36) Watts, A. W.; Ballestero, T. P.; Roseen, R. M.; Houle, J. P. Polycyclic Aromatic Hydrocarbons in Stormwater Runoff from Sealcoated Pavements. *Environ. Sci. Technol.* **2010**, *44*, 8849−8854.

(37) Federal Contaminated Site Risk Assessment in Canada, Part I: Guidance on Human Health Preliminary Quantitative Risk Assessment (PQRA), version 2.0; Contaminated Sites, Reports and Publications; H128-1/11-632E-PDF; Health Canada, Minister of Health, Government of Canada: Ottawa, Ontario, Canada, 2005. http://publications.gc.ca/collections/collection_2012/sc-hc/H128-1-11-632-eng.pdf (accessed February 19, 2016).

(38) Maertens, R. M.; Yang, X.; Zhu, J.; Gagne, R. W.; Douglas, G. R.; White, P. A. Mutagenic and Carcinogenic Hazards of Settled House Dust I: Polycyclic Aromatic Hydrocarbon Content and Excess Lifetime Cancer Risk from Preschool Exposure. *Environ. Sci. Technol.* **2008**, *42*, 1747−1753.

(39) Williams, E. S.; Mahler, B. J.; Van Metre, P. C. Coal-Tar Pavement Sealants Might Substantially Increase Children's PAH Exposures. *Environ. Pollut.* **2012**, *164*, 40−41.

(40) Van Metre, P. C.; Majewski, M. S.; Mahler, B. J.; Foreman, W. T.; Braun, C. L.; Wilson, J. T.; Burbank, T. L. Volatilization of Polycyclic Aromatic Hydrocarbons from Coal-Tar-Sealed Pavement. *Chemosphere* **2012**, *88*, 1−7.

(41) Souza, K. F.; Carvalho, L. R. F.; Allen, A. G.; Cardoso, A. A. Diurnal and Nocturnal Measurements of PAH, Nitro-PAH, and Oxy-PAH Compounds in Atmospheric Particulate Matter of a Sugar Cane Burning Region. *Atmos. Environ.* **2014**, *83*, 193−201.

(42) Albinet, A.; Leoz-Garziandia, E.; Budzinski, H.; Villenave, E.; Jaffrezo, J.-L. Nitrated and Oxygenated Derivatives of Polycyclic Aromatic Hydrocarbons in the Ambient Air of Two French Alpine Valleys: Part 1: Concentrations, Sources and Gas/Particle Partitioning. *Atmos. Environ.* **2008**, *42*, 43−54.

(43) Kojima, Y.; Inazu, K.; Hisamatsu, Y.; Okochi, H.; Baba, T.; Nagoya, T. Comparison of PAHs, Nitro-PAHs and Oxy-PAHs Associated with Airborne Particulate Matter at Roadside and Urban Background Sites in Downtown Tokyo, Japan. *Polycycl. Aromat. Compd.* **2010**, *30*, 321−333.

(44) Pagni, R. M.; Sigman, M. E. The Photochemistry of PAHs and PCBs in Water and on Solids. In *Environmental Photochemistry*; Boule, D. P., Ed.; The Handbook of Environmental Chemistry; Springer: Berlin Heidelberg, 1999; pp 139−179.

(45) Yu, H. Environmental Carcinogenic Polycyclic Aromatic Hydrocarbons: Photochemistry and Phototoxicity. *J. Environ. Sci. Health Part C Environ. Carcinog. Ecotoxicol. Rev.* **2002**, *20*, 149−183.

(46) Wei, E. T.; Wang, Y. Y.; Rappaport, S. M. Diesel Emissions and the Ames Test: A Commentary. *J. Air Pollut. Control Assoc.* **1980**, *30*, 267−271.

(47) Bugel, S. M.; Tanguay, R. L.; Planchart, A. Zebrafish: A Marvel of High-Throughput Biology for 21st Century Toxicology. *Curr. Environ. Health Rep.* **2014**, *1*, 341−352.