Environmental Science & Technology

Article

Cite This: Environ. Sci. Technol. 2018, 52, 10317−10327

pubs.acs.org/est

Using High-Resolution Mass Spectrometry to Identify Organic Contaminants Linked to Urban Stormwater Mortality Syndrome in Coho Salmon

Katherine T. Peter,*[,†,‡] Zhenyu Tian,[†,‡] Christopher Wu,[‡] Peter Lin,[‡] Sarah White,[‡] Bowen Du,[∥] Jenifer K. McIntyre,[⊥] Nathaniel L. Scholz,[#] and Edward P. Kolodziej[†,‡,§]

[†]Center for Urban Waters, Tacoma, Washington 98421, United States

[‡]Interdisciplinary Arts and Sciences, University of Washington Tacoma, Tacoma, Washington 98421, United States

[§]Department of Civil and Environmental Engineering, University of Washington, Seattle, Washington 98195, United States

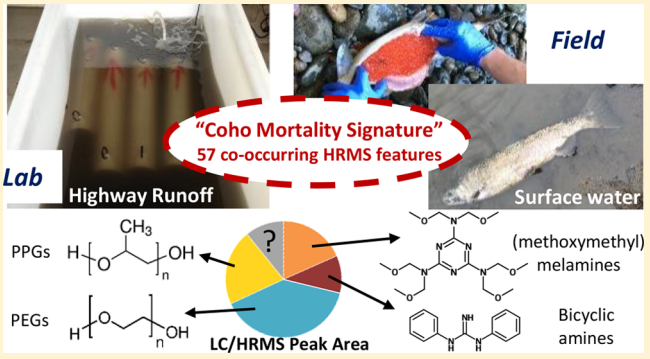
[∥]Southern California Coastal Water Research Project, Costa Mesa, California 92626, United States

[⊥]School of the Environment, Washington State University, Puyallup, Washington 98371, United States

[#]Environmental and Fisheries Science Division, Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 2725 Montlake Blvd. E., Seattle, Washington 98112, United States

Supporting Information

ABSTRACT: Urban stormwater is a major threat to ecological health, causing a range of adverse, mostly sublethal effects. In western North America, urban runoff is acutely lethal to adult coho salmon (*Oncorhynchus kisutch*) that spawn each fall in freshwater creeks. Although the mortality syndrome is correlated to urbanization and attributed to road runoff contaminant(s), the causal agent(s) remain unknown. We applied high-resolution mass spectrometry to isolate a coho mortality chemical signature: a list of nontarget and identified features that co-occurred in waters lethal to coho spawners (road runoff from controlled exposures and urban receiving waters from two field observations of symptomatic coho). Hierarchical cluster analysis indicated that tire wear particle (TWP) leachates were most chemically similar to the waters with observed toxicity, relative to other vehicle-derived sources. Prominent road runoff contaminants in the signature included two groups of nitrogen-containing compounds derived from TWP, polyethylene glycols, octylphenol ethoxylates, and polypropylene glycols. A (methoxymethyl)-melamine compound family, previously unreported in North America, was detected in road runoff and urban creeks at concentrations up to ∼9 and ∼0.3 μg/L, respectively. The results indicate TWPs are an under-appreciated contaminant source in urban watersheds and should be prioritized for fate and toxicity assessment.



INTRODUCTION

Urban stormwater is a major source of chemical pollution to aquatic habitats and a significant threat to the ecological integrity of urban receiving waters.[1−3] In western North America, an acute mortality syndrome occurs when adult coho salmon (*Oncorhynchus kisutch*), a sentinel species for degraded water quality, return to spawn in urban freshwater creeks during the fall rainy season. Systematic field assessments have shown that premature spawner mortality often reaches 50−90% of an entire run.[4,5] Landscape modeling linked coho mortality with impervious surface area in a watershed[6] and, more specifically, motor vehicle traffic density.[5] The high mortality rates pose a significant threat to long-term conservation of wild coho populations,[7] including those protected under the U.S. Endangered Species Act.[8] In addition to the adult mortality syndrome, as-yet unidentified toxicants in untreated urban stormwater are lethal to juvenile coho[9] and cause a range of sublethal effects in other fish (e.g., embryonic developmental defects, cardiovascular abnormalities, and reduced growth)[2,10−13] and stream macroinvertebrates.[9]

The available evidence suggests that exposure to one or more toxic chemical contaminants in urban stormwater causes coho mortality. Previous assessments discounted the physical condition of spawners, disease, and conventional water quality parameters (e.g., temperature and dissolved oxygen) as causal factors.[4] The mortality syndrome symptoms can be reproduced in controlled exposures using runoff from a high-traffic arterial

Received: June 15, 2018
Revised: August 22, 2018
Accepted: August 28, 2018
Published: September 7, 2018

DOI: 10.1021/acs.est.8b03287
Environ. Sci. Technol. 2018, 52, 10317−10327

roadway.[14,15] However, synthetic stormwater (e.g., mixtures of common metals and polycyclic aromatic hydrocarbons (PAHs)) did not evoke the symptoms,[14] and the causal toxicant (or toxicant mixture) remains unidentified. Given the thousands of distinct contaminants in urban runoff, this issue poses an exceptional forensic challenge.[16]

Previously, we reported that high-resolution mass spectrometry (HRMS) screening of paired water-tissue samples of symptomatic coho can prioritize toxicant candidates for targeted screening.[16] While toxicant identification efforts are ongoing, to date there exists limited chemical characterization of urban stormwater in general and, more specifically, water samples that induce acute coho mortality. Given the complexity of urban stormwater,[16−18] water quality analyses are critical to evaluate correlative links between urbanization, road runoff, and occurrence of the mortality syndrome in receiving waters impacted by multiple sources. Further, suites of surrogate and indicator chemicals, often identified from prior knowledge, are commonly used to assess the efficiency of treatment technologies,[19,20] identify source contributions to receiving waters,[21−26] and understand physical and chemical processes.[27] However, indicator compounds that could be used to assess risk and optimize stormwater treatment technologies are lacking. In the absence of known indicator(s), nontarget or suspect screening analyses that use HRMS can be used to develop unique signatures for sources and/or observed water quality impacts[28] and prioritize chemical features for identification efforts and environmental forensics.[29−33] For example, data clustering techniques (e.g., hierarchical cluster analysis) that classify samples and identify correlations in chemical composition[34] can be applied to HRMS data to evaluate similarity between potential sources, waters with an observed toxicological impact, and receiving waters in which risk is unknown.[35,36]

Our objective was to use HRMS analytical methods[16] to develop a chemical signature for the coho mortality syndrome and translate findings into a broader investigation of urban stormwater composition. Here, we identified those chemicals and unidentified HRMS features (exact mass-retention time pairs) that co-occurred in waters known to be lethal to coho. Such identifications represent both potential toxicant candidates and surrogates for risk assessment; the resulting "coho mortality signature" can be applied to evaluate potential toxicant sources and treatment efficacy. We screened road runoff from controlled exposures in conjunction with water samples from two Seattle, WA-area urban waterways where symptomatic (dying) coho spawners were present. This contaminant signature was compared to corresponding profiles for several motor vehicle-derived chemical sources such as transmission fluid, antifreeze, and tire wear particles. We prioritized high peak area features for identification and reported a suite of chemicals in Seattle-area, coho mortality-linked urban stormwater, including previously unrecognized, pervasive (methoxymethyl)melamine contaminants.

MATERIALS AND METHODS

Chemicals. A complete list is provided in the Supporting Information (SI).

Water Samples. Stormwater runoff from a high traffic density urban arterial (westbound onramp to State Route 520 in Seattle, WA, USA; 47°38′38"N, 122°18′25"W) was collected in a precleaned 1900 L stainless steel tote (Custom Metalcraft; Springfield, MO, USA) as previously described.[14] Under controlled exposure conditions, runoff from five storms (Oct. 19, 26, and 30, 2015; Nov. 13, 2015; and Oct. 25, 2016) rapidly and consistently produced the mortality syndrome symptoms (e.g., gaping, surface swimming, and loss of equilibrium), while controls (coho exposed to groundwater) survived.[14,15]

In the field, surface water grab samples were collected from two Seattle-area waterways, concurrent with observations of female coho spawners exhibiting the mortality syndrome symptoms. Samples were collected from Miller Creek (October 18, 2016, Burien, WA; 47°26′39"N, 122°21′21"W) ∼300 m upstream of the estuary, which drains into Puget Sound, and the Lower Duwamish Waterway (October 21, 2017, Seattle, WA; 47°33′35" N, 122°21′1"W) in a tidally influenced reach (map of sampling locations provided in Figure S1). Notably, these field samples were very difficult to collect, requiring a network of 50−150 citizen scientist volunteers to identify symptomatic fish prior to death. Both drainages receive runoff from >700 ha of a relatively urban landscape (∼45% medium to high-intensity developed),[37] and modest rainfall (<0.29 in) occurred in the preceding 12 h. Moderate but clear (low turbidity) flow was observed in both waterways, typical of receiving water conditions during many symptomatic coho documentations. In Miller Creek, two samples were collected ∼15 min after coho mortality: at the location of mortality ("fish site") and ∼100 m downstream ("downstream site"; represents older water). In the Lower Duwamish, a water sample was collected adjacent to the symptomatic coho while it was still alive.

To evaluate broader occurrence of stormwater-derived contaminants, grab samples were collected in Fall 2017 during baseflow conditions and two storm events from each of three Seattle-area streams where recurring seasonal coho mortality events occur:[4,5] Miller Creek (same location as above), Longfellow Creek (Seattle, WA; 47°33′14"N, 122°21′60"W), and Thornton Creek (Seattle, WA; 47°42′4"N, 122°18′25"W). All samples were collected in 4 L precleaned amber glass bottles without headspace and transported on ice to the Center for Urban Waters.

Motor Vehicle-Derived Chemical Mixtures. To compare sample chemical composition (for road runoff and creek samples) to potential motor vehicle-derived contaminant sources, we collected fluid and tire samples from local automotive shops (representing up to 3 brands/types per source). Tire wear particles (TWP) were generated by hand-abrading used tire treads with a fine metal rasp. Aqueous solutions were made by diluting (windshield wiper fluid, antifreeze) or leaching (used motor oil, used gear oil, power steering fluid, automatic transmission fluid, and TWP) 1000 μL (fluid) or 1000 mg (TWP) into 1 L of DI water, followed by gentle mixing for 48 h (20 °C). For nonaqueous phase mixtures, the aqueous fraction was isolated with a separatory funnel.

Spike and Recovery Experiments. For hexa(methoxymethyl)melamine (HMMM) quantification, in-matrix calibration curves were made using water collected during baseflow conditions from Swan Creek (nearby urban watershed; Tacoma, WA; 47°13′37" N, 122°23′35"W). Spiked (20, 200, 2000, and 10 000 ng/L; stock solution 1 mg/mL HMMM in methanol) and unspiked controls were processed in triplicate (parallel extraction of 1 L replicates, single injection per extract). Matrix-matched calibration standards (used to calculate absolute recovery) were produced by spiking SPE

DOI: 10.1021/acs.est.8b03287
Environ. Sci. Technol. 2018, 52, 10317−10327

extracts of unspiked samples; SPE extracts were diluted if samples were above (>100 μg/L) the observed linear range.

Sample Processing and Analysis. Samples were processed and analyzed by established methods.[16] SPE cartridges (3 mL, 100 mg Infinity C18 SPE cartridges; ABS Materials, Wooster, OH, USA) were preconditioned with 3 mL of 50% (v/v) methanol/deionized water followed by deionized water (25 mL), and then water samples (1 L) were loaded without prefiltration (5−10 mL/min).[16] Cartridges were rinsed with deionized water (10 mL), nitrogen-dried (15 min), and eluted (2.5 mL of methanol, 2×), and extracts were concentrated with nitrogen to 1 mL. All samples were extracted within 24 h of collection. For all road runoff, Lower Duwamish, and Fall 2017 surface water samples, four (1 L) field replicates were extracted in parallel, with one analytical injection per final extract. For the Miller Creek mortality event sample and motor vehicle sources, replicates are from a triplicate injection (only one (1 L) sample was extracted).

Analysis used an Agilent 1290 Infinity UHPLC (Santa Clara, CA, USA) for separation and an Agilent 6530 Quadrupole Time-of-Flight (QTOF) HRMS with electrospray Jet Stream Technology for detection. Chromatography used a reversed-phase C18 analytical column (Agilent ZORBAX Eclipse Plus 2.1 × 100 mm, 1.8 μm particle size) and C18 guard column at 45 °C, injection volume 5 μL, flow rate 0.4 mL/min, and binary gradient 5 mM ammonium acetate plus 0.1% acetic acid in each of water (A) and methanol (B): [5% B 0−1 min, 50% B 4 min, 100% B 17−20 min, 5% B 20.1 min; stop time 22.5 min]. HRMS spectra were acquired across 100−1700 m/z (MS) and 50−1700 m/z (MS/MS) in 2 GHz Extended Dynamic Range mode (collision-induced dissociation; data-dependent acquisition).[16] We focused efforts on ESI+ detections, as we typically detect more chemical features and peak area via ESI+ relative to ESI- (e.g., six times more features in road runoff via ESI+).

For quality assurance and quality control (QA/QC), we monitored detector performance by checking mass accuracy and retuning if mass error exceeded 2 ppm. No column carryover was detected in solvent (MeOH) blanks (analyzed every 8−12 samples). A mixture of external reference standards containing cotinine (retention time (RT) 3.4 min; 120 ng/mL), carbamazepine (RT 6.5 min; 50 ng/mL), and prometryn (RT 9.5 min; 50 ng/mL) was analyzed every 8−12 samples. The instrument was retuned and samples reanalyzed if mass accuracy was >5 ppm or area counts were >20% of initial within-batch sensitivity. Relative standard deviation of area counts in the reference standard were <15% within an analytical batch and <40% across all analytical batches (>1 year). Method blanks (DI water through SPE) were analyzed alongside samples, and fold change analyses (see below) were used to exclude signals detected in blanks. Additional instrumental parameters and QA/QC procedures are described in Du et al.[16]

Data Reduction and Analysis. Data extraction used Agilent software packages and published methods.[16] Nontarget feature extraction, adduct detection/grouping, and alignment was performed in MassHunter Profinder (B.08.00). Features (exact mass-retention time pairs) were imported into Mass Profiler Professional (B.13.00; MPP) for subsequent filtering and analysis. Complete extraction and alignment parameters are in Table S1. For each sample, features present in ≥75% of replicates (3 of 3 analytical replicates; 3 of 4 field replicates), with peak area both ≥5000 and ≥5-fold greater than the peak area in any method or solvent blanks were retained.[38−40] For road runoff, features were retained if they were also 5-fold above peak areas in groundwater controls. Hierarchical cluster analysis was performed in MPP, where Euclidean distance between samples was calculated from log-normalized peak areas, and then Ward's method was applied to cluster samples and features such that total within-cluster variance was minimized. Suspect screening and identification occurred in MassHunter ID Browser (B.07.00) and MassHunter Qualitative Analysis (B.08.00). Suspect screening used custom databases containing ∼930 stormwater-associated compounds and ∼280 vehicle/roadway-associated compounds. After suspect screening, structure elucidation was performed for as many unidentified features as possible. Identification confidence was assigned according to criteria proposed by Schymanski et al., where the highest confidence (S1) is achieved when retention time and MS/MS fragments match standards. The second tier (S2) is achieved when MS/MS fragmentation patterns match library database information (S2a) or diagnostic information indicates no other possible structure (S2b).[41] Lower confidence levels describe tentative candidates based on MS/MS fragmentation (S3), unequivocal molecular formula (S4), or exact mass (S5).[41]

RESULTS AND DISCUSSION

Coho Mortality Signature. To characterize stormwater quality and prioritize feature identifications relevant to the mortality syndrome, we developed a "coho mortality signature" by isolating all features that co-occurred in (1) road runoff (features in ≥4 of 5 samples, to minimize false negatives), (2) the Lower Duwamish, and (3) at least one of the Miller Creek fish or downstream sites (again to minimize false negatives). Because this signature grouped co-occurring features from all waters that induced mortality syndrome symptoms in coho spawners, we anticipate that signature strength (feature peak area) and composition (number of features) correlate to the potential for these waters to induce acute mortality. Conceptually, this approach assumes that any sources contributing the causal toxicant(s) to receiving waters also contain many other co-occurring chemicals detectable via HRMS. By monitoring and tracking these co-occurring features, we can develop a diagnostic and detailed suite of chemical indicators for the dynamics and risk of the mortality phenomenon in watersheds that support coho spawning habitats. Importantly, the signature may or may not include the actual causal toxicant(s). While chemicals within the coho mortality signature are logical candidates for targeted toxicity screening, our sample processing and analytical methodology may exclude one or more causal agents (e.g., C18 SPE cartridges/LC separation may exclude more polar compounds), and fractionation-based approaches for toxicant identification are beyond the scope of this study. More broadly, this approach prioritizes co-occurring chemical identifications as potential surrogate and indicator compounds for adverse biological outcomes that are linked to water quality impairment, but for which the causal agent(s) are unknown. These indicators can evaluate similarities in chemical composition between key sources, unknown water samples, and waters with a known ecotoxicological risk (e.g., those inducing acute coho mortality).

Signature development used two very different samples: (1) road runoff from controlled exposures and (2) field samples from urban receiving waters (Miller Creek, Lower Duwamish)

DOI: 10.1021/acs.est.8b03287
Environ. Sci. Technol. 2018, 52, 10317−10327

concurrent with observations of symptomatic coho spawners. Notably, road runoff consistently contained >2400 chemical features in total and an average total peak area of ~2.4 billion. In comparison to these high concentrations of runoff-derived contaminants, surface water samples from Miller Creek and the Lower Duwamish contained ~950 features (total peak area ~140 million) and 616 features (total peak area ~54 million), respectively. Concentrated road runoff produces the mortality syndrome symptomology[15] and is strongly correlated to the acute toxicity phenomenon via occurrence patterns and landscape modeling.[5] However, a coho mortality signature developed from road runoff alone is prohibitively large to track (>1400 features) and likely lacks specificity both to the coho mortality syndrome and the original toxicant source. However, including surface water samples reflecting field observations of spawner mortality greatly enhanced signature selectivity, reducing the number of common features by an order of magnitude. This also ensured these surrogates reflect chemical conditions in receiving waters during actual mortality events. For example, 1482 features were consistently detected in ≥4 of 5 road runoff samples, of which 306 were also present in the 2016 Miller Creek sample (Figure 1). Including the 2017 Lower Duwamish mortality event further refined the signature to 103 features. Subsequent manual data reduction and filtering isolated 57 unique chemical detections (see "Identifications"). We anticipate that additional field observations of symptomatic/dying coho will further enhance signature specificity.

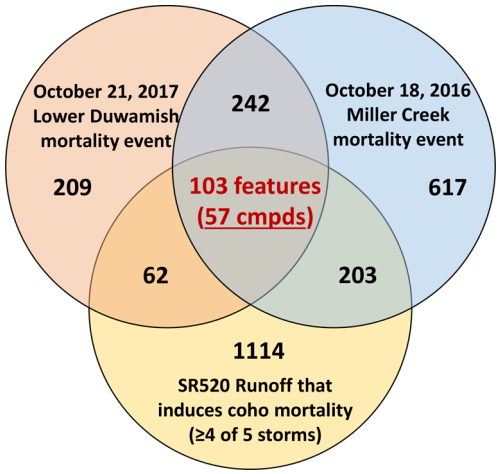


Figure 1. Venn diagram demonstrating the coho mortality signature (highlighted in red at center), showing overlap of HRMS features detected in arterial road runoff that was lethal to adult coho spawners (features in ≥4 of 5 storms), in Miller Creek on October 18, 2016 concurrent with observation of coho spawner mortality (features detected at either the site of observed mortality or ~100 m downstream), and in the Lower Duwamish on October 21, 2018, also concurrent with observation of symptomatic coho.

Evaluating Sources Relevant to Coho Mortality. Land use analyses recently implicated vehicle traffic density in the coho mortality syndrome.[5] Therefore, we used the signature to evaluate relative contributions of vehicle-related contaminant sources (antifreeze, used motor oil, used gear oil, power steering fluid, automatic transmission fluid, windshield wiper fluid, TWP) to receiving waters exhibiting coho mortality. For this analysis, nontarget data from these sources were aligned with the road runoff exposure and field mortality event samples that were used to construct the coho mortality signature.

First, to understand the relative overall contribution of each source to the road runoff and surface water samples, we evaluated the number of nontarget features in each water sample that were attributable to an individual vehicle source, using sets of source-specific (i.e., observed in only one of seven analyzed sources), yet unidentified, nontarget features. All source dilutions/leachates were prepared at high concentrations relative to anticipated environmental levels to maximize the potential for observing overlapping features. In both road runoff and surface water, <1.8% of all detected features (i.e., <52 of ~2800 total features in road runoff, <14 of ~950 in Miller Creek, <12 of ~620 in the Lower Duwamish) were attributed to any of the windshield wiper fluid, power steering fluid, or automatic transmission fluid. Likewise, <4.8% and <3.4% of features in any water sample were attributed to antifreeze or used gear oil, respectively. In contrast, used motor oil accounted for 6.7%, 7.5%, and 3% of all features and TWP leachate accounted for 14.8%, 7.5%, and 7.4% of all features in road runoff, Miller Creek, and the Lower Duwamish, respectively. These data indicated the importance of used motor oil and TWP leachate as major, chemically complex contaminant sources to road runoff and urban waterways.[42]

Next, hierarchical cluster analysis (HCA) was performed on the nontarget features in the mortality signature to determine the degree of alignment between the vehicle-derived sources and the waters linked to observations of symptomatic coho (Figure 2). The resulting dendrogram visualizes ensembles of nontarget features with the most similar abundance profiles across the various samples (feature clusters), and samples with the most similar chemical profiles (sample clusters).[34,43,44] We note that when different sample sets (e.g., different sources, receiving waters) were aligned with the waters that were toxic to adult coho spawners, the resulting list of features in the mortality signature varied slightly as an outcome of iterative software processing (i.e., n = 78 in Figure 2 *vs.* 103 above). However, because the HCA used the entire signature (rather than specific identified chemicals) and only compared aligned samples, this approach was appropriate for determining relative sample similarity and prioritizing sources for more detailed investigation of possible links to the toxicity phenomenon.

The HCA divided the water and vehicle source samples into two main clusters and several subclusters. One group (upper cluster in Figure 2) contained all of the vehicle fluids, while the lower group contained TWP leachate and the waters lethal to coho. Furthermore, the upper group was divided into two subsets: (1) windshield wiper fluid, ATF, and power steering fluid and (2) motor oil, antifreeze, and gear oil. Within the lower group, the road runoff formed one subcluster, while TWP leachate and the field mortality event waters comprised a second. Notably, many features in the mortality signature were also detected in motor oil, antifreeze, and gear oil. This observation underscores the value of a broader, nontarget approach to define chemical composition in high priority samples, rather than relying solely on predefined targets. The similarity between TWP leachate, road runoff, and surface water composition indicates that tire wear particles are a major source of dissolved phase contaminants in urban runoff that subsequently induces acute coho spawner mortality. Notably, >50% of the chemical features (n = 30) in the mortality signature, representing >60% of the total detected peak area during field mortality events, were also detected in TWP

DOI: 10.1021/acs.est.8b03287
Environ. Sci. Technol. 2018, 52, 10317−10327

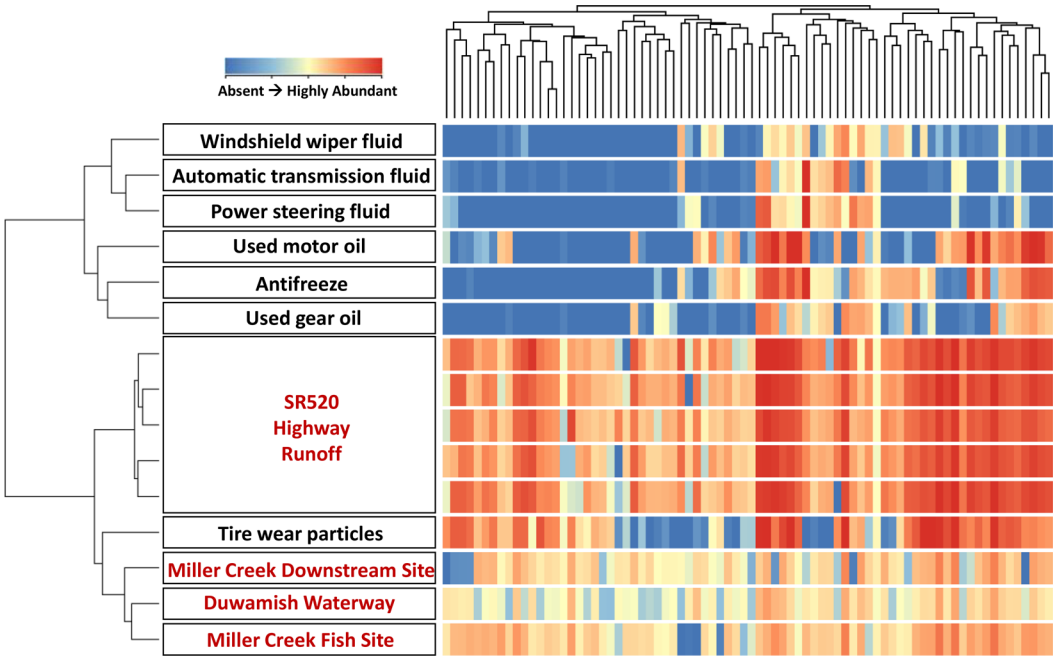


Figure 2. Hierarchical cluster analysis of waters associated with adult coho spawner mortality (red text) and vehicle-related sources (black text), for the features that comprise the coho mortality signature (n = 78 here, due to iterative software processing of different sample sets). Each row in the diagram represents an average of 3 or 4 replicate samples, each vertical line represents an individual feature, and the color of each bar represents the peak area (absent = dark blue, increasing to light blue, light yellow, peach, and dark red with increasing peak area, up to the maximum observed peak area for all data shown).

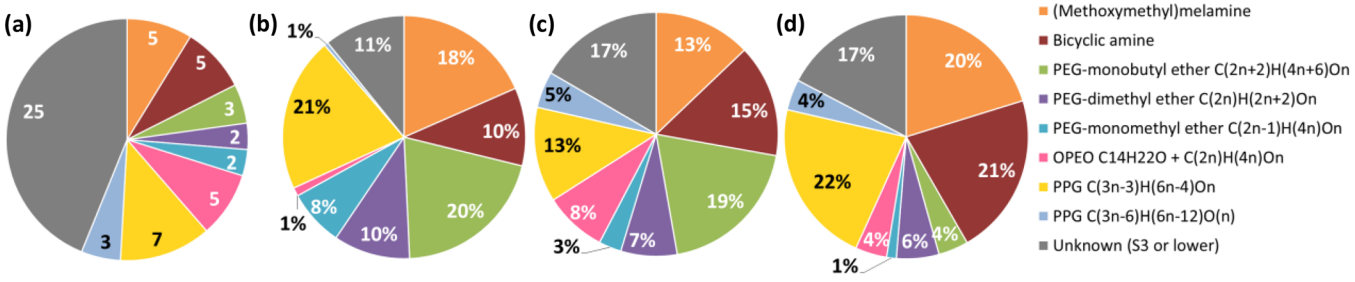


Figure 3. Observed distribution of 57 nonduplicated features in the coho mortality signature among identified compound groups for (a) number of features, (b) peak area in road runoff from controlled exposures (demonstrated to induce the syndrome), (c) peak area in Lower Duwamish field sample, and (d) peak area in Miller Creek field sample. For (c) and (d), symptomatic coho spawners were present at the time of sample collection.

leachate, indicating that TWP should be a priority for future toxicity assessments.[42]

Identification of Chemicals in the Mortality Signature. Next, we identified as many features in the coho mortality signature as possible. We observed significant feature duplication as an artifact of the feature extraction/alignment process, likely due to software settings intended to minimize false negatives (e.g., tight retention time and m/z windows and low peak height criteria). By manually inspecting exact mass, retention time, and isotope patterns, we eliminated 30 features (5 adduct mis-assignments and 25 duplicates). The highest scoring feature (molecular feature extraction score reported in Profinder via recursive feature extraction; Table S1) was retained for identification efforts. Additionally, 14 duplicate features were attributed to in-source fragmentation of identified compounds (Table S2). Thus, ~40% of the original 103 features represented data processing artifacts,[45] and the final coho mortality signature was comprised of the remaining 57 features.

Of these 57 features, identities were assigned to 21 at level S1, 10 at S2a-S2b, and 26 remained at S4−S5 (Figure 3a and Table 1). Peak area data across individual samples, observed adducts, SMILES, and primary MS/MS fragments are available in Tables S3−S4. The 31 compounds identified at levels ≥S2 (and one S4 compound with tentatively identified structural units) largely belong to five structure groups: (methoxymethyl)melamine compounds, bicyclic amines, polyethylene glycols (PEGs), octylphenol ethoxylates (OPEOs), and polypropylene glycols (PPGs). The PEGs are composed of three groups with molecular formulas $C_{2n+2}H_{4n+6}O_n$ (PEG monobutyl ether, S2b; n = 5−7), $C_{2n}H_{4n+2}O_n$ (PEG dimethyl ether, S1; n = 5−6), and $C_{2n-1}H_{4n}O_n$ (PEG monomethyl ether, S1; n = 5−6), and the OPEOs (S1) have formulas $C_{14}H_{22}O$ + $C_{2n}H_{4n}O_n$ (n = 7, 9−12). We identified one group of saturated PPGs ($C_{3n-3}H_{6n-4}O_n$, S1; n = 7, 9−14) and one group of monounsaturated PPGs ($C_{3n-6}H_{6n-12}O_n$, S2b; n = 8,10−11). These families were identifiable by $[M+NH_4]$ adducts and repeating MS/MS losses of m/z 44.0262 (C_2H_4O; PEGs and OPEOs) and m/z 58.0419 (C_3H_6O; PPGs).[46] Likewise,

DOI: 10.1021/acs.est.8b03287
Environ. Sci. Technol. 2018, 52, 10317−10327

Table 1. Compounds Identified within the Coho Mortality Signature by HRMS

compound group	compound	formula	theoretical mass [M]	CAS no.	RT (min)	detection confidence	mass error in highway runoff (ppm)[a]
(methoxymethyl) melamines	tetra(methoxymethyl)melamine (TMMM)	$C_{11}H_{22}N_6O_4$	302.1703	50322-79-7	5.00	S2b	2.1
	penta(methoxymethyl)melamine (PMMM)	$C_{13}H_{26}N_6O_5$	346.1965		5.70	S2b	1.6
	hexamethylolmelamine pentamethyl ether (HMPE)	$C_{14}H_{28}N_6O_6$	376.2070		5.75	S2a	1.7
	hexa(methoxymethyl)melamine (HMMM)	$C_{15}H_{30}N_6O_6$	390.2227	3089-11-0	6.80	S1	0.79
	diformylated HMMM	$C_{16}H_{32}N_6O_7$	420.2332		7.14	S2a	0.8
bicyclic amines	1,3-diphenylguanidine	$C_{13}H_{13}N_3$	211.1109	102-06-7	4.30	S1	0.9
	1,3-dicyclohexylurea	$C_{13}H_{24}N_2O$	224.1889	2387-23-7	8.56	S1	0.68
	1-cyclohexyl-3-phenylurea	$C_{13}H_{18}N_2O$	218.1419	886-59-9	7.70	S1	2.5
	N-methyl-dicyclohexylamine	$C_{13}H_{25}N$	195.1987	7560-83-0	4.65	S1	−2.0
	unknown	$C_{18}H_{28}N_4O_2$	332.2212		10.48	S4	−1.0
PEG series $C_{2n+2}H_{4n+6}O_n$	tetraethylene glycol monobutyl ether	$C_{12}H_{26}O_5$	250.1780	1559-34-8	6.00	S2b	1.4
	pentaethylene glycol monobutyl ether	$C_{14}H_{30}O_6$	294.2042	23601-39-0	6.23	S2b	1.1
	hexaethylene glycol monobutyl ether	$C_{16}H_{34}O_7$	338.2305		6.48	S2b	0.0
PEG series $C_{2n}H_{4n+2}O_n$	tetraethylene glycol dimethyl ether (tetraglyme)	$C_{10}H_{22}O_5$	222.1467	143-24-8	4.32	S1	1.0
	pentaethylene glycol dimethyl ether (pentaglyme)	$C_{12}H_{26}O_6$	266.1729	1191-87-3	4.55	S1	0.8
PEG series $C_{2n-1}H_{4n}O_n$	tetraethylene glycol monomethyl ether	$C_9H_{20}O_5$	208.1311	23783-42-8	3.73	S1	0.5
	pentaethylene glycol monomethyl ether	$C_{11}H_{24}O_6$	252.1573	23778-52-1	4.05	S1	0.9
OPEO series $C_{14}H_{22}O + C_{2n}H_{4n}O_n$	OPEO-6	$C_{26}H_{46}O_7$	470.3244		13.50	S1	1.5
	OPEO-8	$C_{30}H_{54}O_9$	558.3768		13.59	S1	0.9
	OPEO-9	$C_{32}H_{58}O_{10}$	602.4030		13.63	S1	−0.1
	OPEO-10	$C_{34}H_{62}O_{11}$	646.4292		13.67	S1	−2.1
	OPEO-11	$C_{36}H_{66}O_{12}$	690.4554		13.71	S1	−4.0
PPG series $C_{3n-3}H_{6n-4}O_n$	heptapropylene glycol	$C_{21}H_{44}O_8$	424.3036		8.59	S1	−0.8
	nonapropylene glycol	$C_{27}H_{56}O_{10}$	540.3873		10.73	S1	−0.1
	decapropylene glycol	$C_{30}H_{62}O_{11}$	598.4292		11.61	S1	1.07
	undecapropylene glycol	$C_{33}H_{68}O_{12}$	656.4711		12.36	S1	−0.56
	dodecapropylene glycol	$C_{36}H_{74}O_{13}$	714.5129		13.02	S1	−1.14
	tridecapropylene glycol	$C_{39}H_{80}O_{14}$	772.5548		13.60	S1	−5.02
	tetradecapropylene glycol	$C_{42}H_{86}O_{15}$	830.5967		14.10	S1	−0.67
PPG series $C_{3n-6}H_{6n-12}O_n$	PPG ($C_{3n-6}H_{6n-12}O_n$), n = 8	$C_{18}H_{36}O_8$	380.241		6.26	S2b	−3.26
	PPG ($C_{3n-6}H_{6n-12}O_n$), n = 10	$C_{24}H_{48}O_{10}$	496.3247		8.45	S2b	0.35
	PPG ($C_{3n-6}H_{6n-12}O_n$), n = 11	$C_{27}H_{54}O_{11}$	554.3666		9.50	S2b	−3.84

[a]Mass error is defined as the difference between the observed and theoretical mass.

(methoxymethyl)melamine compounds were identified by characteristic losses of 30.0106 (CH_2O) and 32.0262 (CH_4O).[47,48]

Notably, the 32 identified features account for 83−89% of the total peak area in any of the water samples associated with coho mortality (Figure 3b-d). Compounds with CHO-based molecular formulas (PEGs, OPEOs, and PPGs) account for 41−60% of the total identified peak area, while CHNO-based compounds account for 28−42%. The relative peak area distribution across compound groups is most similar between the road runoff and the Lower Duwamish site, while the Miller Creek sample had proportionally less PEG monobutyl ethers and proportionally more CHNO compounds.

PEGs, OPEOs, and PPGs. Long-chain glycols and ethoxylates are widely used in commercial and automotive products such as antifreeze, personal care products, surfactants, and polyurethane coatings.[49,50] PEGs and PPGs are also the primary biodegradation products of nonionic surfactants (e.g., alcohol/alkylphenol ethoxylates)[51,52] and are commonly detected in municipal wastewater[53] and waters from hydraulic fracturing operations.[46] There are large airports in both the Miller Creek (SeaTac International) and Lower Duwamish (Boeing Field) watersheds that use de/anti-icing fluids; however, these are primarily composed of propylene and ethylene glycol, rather than the long-chain glycols observed here. Additionally, the high PEG and PPG peak areas observed in arterial road runoff (no direct airport influence) indicate that vehicle-related sources are important contributors of the observed PEGs and PPGs. However, the aquatic toxicity of long-chain glycols is considered low,[54] and due to the ubiquity of PEGs, OPEOs, and PPGs in manufactured products and environmental matrices, they lack source-specificity.

Bicyclic Amines. In both field mortality event samples, 1,3-diphenylguanidine (DPG, S1), a vulcanization accelerator

DOI: 10.1021/acs.est.8b03287
Environ. Sci. Technol. 2018, 52, 10317−10327

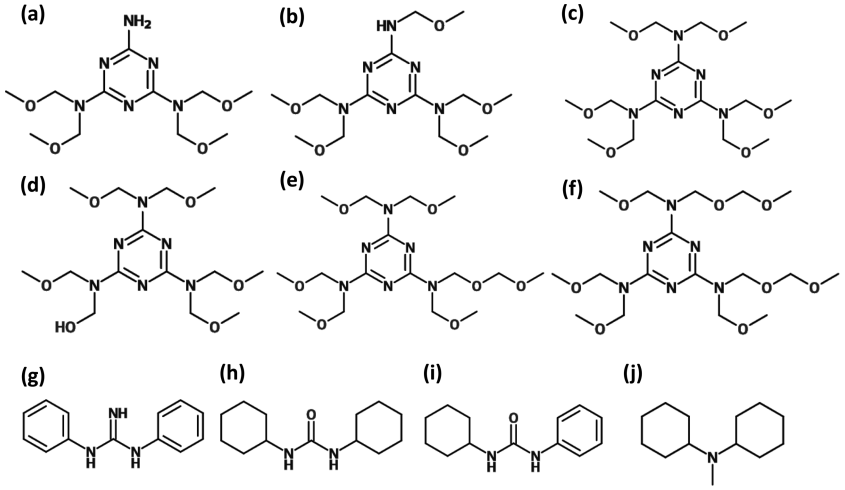


Figure 4. Structures of select compounds identified within the coho mortality signature: (a–f) (methoxymethyl)melamine and (g–j) bicyclic amine groups, including (a) TMMM, (b) PMMM, (c) HMMM, (d) HMPE, (e) diformylated HMMM, (f) double diformylated HMMM, (g) DPG, (h) 1,3-dicyclohexylurea, (i) 1-cyclohexyl-3-phenylurea, and (j) *N*-methyl-dicyclohexylamine.

widely used in tire manufacturing, was one of the top three largest peak area features. DPG was also consistently among the top ∼10 peak area compounds in road runoff. We estimated DPG concentrations of ∼1800 ng/L in road runoff and ∼20 ng/L in surface water via 6-point calibration (0.5–100 μg/L; extracts diluted into linear range). We note that of the samples analyzed in this study, only the Lower Duwamish sample was spiked with an internal standard mixture (12 of 12 detected; average peak area response 96 ± 15% relative to the internal standard control). In a representative road runoff sample (same source, collected in 2017), we detected 10 of 12 internal standards with an average peak area response 47 ± 28% relative to the control. Thus, concentrations reported here should be considered semiquantitative estimates that do not account for potential matrix effects.

During tire wear and aging, up to 14% of DPG used during manufacturing can be released as the parent DPG or its major reaction product from the curing process, aniline (a metabolic poison).[55,56] DPG is a clear example of an ubiquitous dissolved organic contaminant from TWP, although its aquatic toxicity remains unknown. Identified compounds that are structurally similar to DPG included 1,3-dicyclohexylurea (S1; ∼300 ng/L in road runoff, ∼10 ng/L in surface water via single-point calibration), *N*-methyl-dicyclohexylamine (S1; ∼10 ng/L in road runoff, ∼0.5 ng/L in surface water), and 1-cyclohexyl-3-phenylurea (S1; ∼350 ng/L in road runoff, ∼5 ng/L in surface water; Figure 4). The first two have been detected in TWP leachate,[57] and 1,3-dicyclohexylurea is a known reaction byproduct during surface-grafting of polymers with amine and hydroxyl functionalities.[58–60] Additionally, an unidentified feature with molecular formula $C_{18}H_{28}N_4O_2$ was detected (S4) within the top six peak area features of both field samples. Although the structure remains unknown, its MS/MS spectra indicated C_6H_{12} and $C_2H_6N_2$ structural units (Figure S2). Based on these structural units, its large peak area, and its detection in TWP leachate, we included it alongside the other CHNO-based "bicyclic amines" (Figure S2).

(Methoxymethyl)melamine Compounds. The largest (or second largest) peak area feature within the mortality signature in every mortality-linked road runoff and surface water sample was identified (S1) as hexa(methoxymethyl)melamine (HMMM). Among all detections (not just the mortality signature), HMMM was within the top 10 peak area features in both road runoff and Miller Creek, and within the top 20 in the Lower Duwamish. HMMM was previously detected in German and Dutch rivers and has been suggested as a marker for road runoff.[61–63] Although HMMM was previously detected in TWP leachate,[57] European Union detections of HMMM are primarily attributed to its release from industrial wastewaters, coatings, and plastics, as HMMM is used as a curing agent and cross-linker.[64] Notably, we identified four additional compounds structurally similar to HMMM in the coho mortality signature [tetra(methoxymethyl)melamine (TMMM, S2b), penta(methoxymethyl)melamine (PMMM, S2b), hexamethylolmelamine pentamethyl ether (HMPE, S2a[65]), and diformylated HMMM (S2a[65])], and a fifth related compound in road runoff and surface waters (double diformylated HMMM, S2b; Figure 4; MS/MS spectra in Figure S3). PMMM, HMPE, and diformylated HMMM have been reported to co-occur with HMMM in surface waters.[61,63,65] Interestingly, two previous studies report HMMM co-occurrence in surface waters with urea compounds (3-cyclohexyl-1,1-dimethylurea[63] and *N*,*N*′-diethyl-*N*,*N*′-diphenylurea[61]) that are structurally similar to those reported here, indicating widespread co-occurrence of these structural groups in vehicle-impacted receiving waters.

Given the observed high peak areas of HMMM in both road runoff and field mortality event samples, we quantified it via a pure standard. The linear range for HMMM on the QTOF was 0.5–100 μg/L, and spike-recovery experiments (20–10 000 ng/L) achieved consistent absolute recoveries of 55–92% (average 68 ± 13%; Figure S4). Sample extracts were diluted into the linear range for quantification (Figure S5). In arterial road runoff, HMMM concentrations ranged from 2000 to 6500 ng/L. Similarly, HMMM concentrations in TWP leachates ranged from 4000 to 8800 ng/L (vs <14 ng/L in other analyzed vehicle fluids). We estimated 10 ng/L HMMM in the Lower Duwamish and 35–54 ng/L in Miller Creek concurrent with symptomatic/dying coho. In urban creeks (Miller, Longfellow, and Thornton), we detected 8–13 ng/L HMMM during baseflow conditions and 30 to ∼200 ng/L during storm events. Despite these relatively low concentrations, HMMM is a particularly good indicator compound

DOI: 10.1021/acs.est.8b03287
Environ. Sci. Technol. 2018, 52, 10317–10327

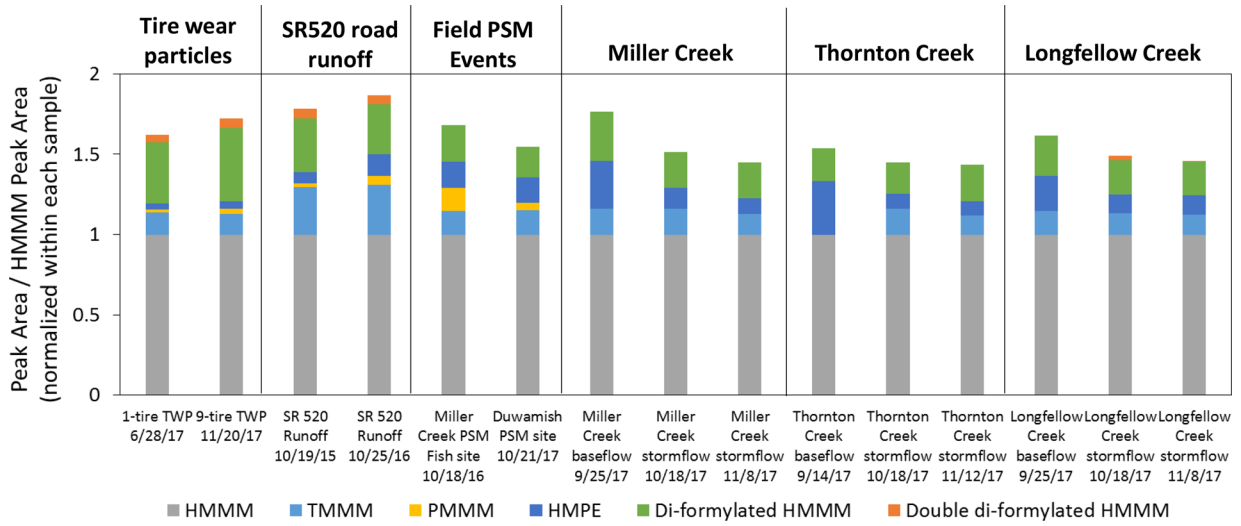


Figure 5. Normalized peak area (peak area relative to peak area of HMMM, to show road runoff and surface water on the same scale) for (methoxymethyl)melamine compounds in selected samples, including tire wear particle leachate, arterial road runoff used in controlled exposures, field observations of symptomatic coho spawners, and both baseflow and storm events in representative urban watersheds (Miller Creek, Thornton Creek, and Longfellow Creek).

due to its excellent peak area response (~10 000 peak area counts per pg on column) via ESI+ detection.

The broader HMMM family is typically detected in all of these samples (Figure 5). The total relative peak area of HMMM family members was >1.4 times that of HMMM alone. Because all HMMM family compounds exhibited similar dilution trends (Figure S5), we assumed identical peak area responses across the family and estimated 2800−9100 ng/L total concentration of HMMM and its structural analogs in road runoff. For urban surface waters, we estimated 11−18 ng/L during baseflow conditions and 42−280 ng/L during storm events. To our knowledge, this is the first report of HMMM in North American stormwater and surface waters and the first report of the entire HMMM family co-occurring in a single stormwater or surface water sample. Given their concentrations in roadway runoff, this family of chemicals may represent a significant unrecognized contributor to the pollutant load in urban watersheds and may serve as a good indicator for the coho mortality syndrome.

Environmental Implications. Using nontarget and suspect screening HRMS, we developed a chemical signature for an urban runoff coho salmon mortality syndrome via clustered observations of symptomatic coho in field events and controlled exposures. This data analysis strategy reduced the thousands of chemicals in urban road runoff to a reasonable set (57) of features and chemicals that link water chemistry to salmon mortality events. Although viable source control and management options remain uncertain until the specific toxicant(s) are identified, the signature can be applied to evaluate water quality in high-risk watersheds and to optimize stormwater treatment technologies for directed reduction in risk to coho spawners. The signature also provides surrogates and chemical indicators for investigating and quantifying road runoff and urbanization impacts on receiving waters.

Here, the signature was used to assess the potential relevance of different chemical sources to the acute mortality phenomenon. TWP leachates were most chemically similar to waters that induce the mortality syndrome, relative to other vehicular sources analyzed. Risks associated with the other sources should not be discounted, as they contain contaminants with high aquatic toxicity (e.g., PAHs).[66] It is also possible that highly toxic contaminants may arise from a single source that lacks chemical complexity, which would be difficult to identify and track using the methodologies described here.

Several groups of important urban contaminants within the mortality signature were identified, including PEGs, OPEOs, PPGs, and two groups of N-containing compounds derived from TWP. In particular, a family of (methoxymethyl)-melamine compounds, previously unreported in North American surface waters, was detected in Seattle-area road runoff and urban creeks at concentrations up to ~9 and ~0.3 μg/L, respectively. Similarly, DPG, a constituent of tire wear, was one of the largest peak area features in the water samples. While PEGs, OPEOs, and PPGs are known pervasive urban contaminants,[46,53] the prevalence of TWP-derived constituents in road runoff and surface waters (especially many compounds with limited characterization and unknown toxicity in the scientific literature) supports a growing appreciation that tire wear particles are an under-recognized source of contaminants to aquatic environments.[42] Related ongoing studies indicate that some of these chemicals are moderately to highly persistent in receiving waters, bioavailable and detected in exposed aquatic organisms, and should be prioritized for fate and risk assessments of urban surface water.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b03287.

Figures and tables with detailed data analysis parameters and supplemental analyses (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: ktpeter@uw.edu.

ORCID

Katherine T. Peter: 0000-0001-7379-265X

Zhenyu Tian: 0000-0002-7491-7028

DOI: 10.1021/acs.est.8b03287
Environ. Sci. Technol. 2018, 52, 10317−10327

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by EPA grant No. 01J18101, University of Washington (Tacoma and Seattle campuses). The authors gratefully acknowledge the outstanding citizen scientist network from Miller/Walker Community Salmon Investigation and Puget Soundkeeper (and others) for watershed monitoring efforts that enabled challenging field event collections. We thank the Suquamish Tribe, Grover's Creek Hatchery, staff from the Ecotoxicology Program at NOAA's Northwest Fisheries Science Center, Washington State University Stormwater Center, and U.S. Fish and Wildlife field biologists for their assistance with arterial runoff collection. Jessica Lundin provided a helpful and detailed review.

■ REFERENCES

(1) Walsh, C. J.; Roy, A. H.; Feminella, J. W.; Cottingham, P. D.; Groffman, P. M.; Morgan, R. P. The Urban Stream Syndrome: Current Knowledge and the Search for a Cure. *J. North Am. Benthol. Soc.* **2005**, *24* (3), 706−723.

(2) Kayhanian, M.; Stransky, C.; Bay, S.; Lau, S.-L.; Stenstrom, M. K. Toxicity of Urban Highway Runoff with Respect to Storm Duration. *Sci. Total Environ.* **2008**, *389* (2−3), 386−406.

(3) LeFevre, G. H.; Paus, K. H.; Natarajan, P.; Gulliver, J. S.; Novak, P. J.; Hozalski, R. M. Review of Dissolved Pollutants in Urban Storm Water and Their Removal and Fate in Bioretention Cells. *J. Environ. Eng.* **2015**, *141* (1), 04014050.

(4) Scholz, N. L.; Myers, M. S.; McCarthy, S. G.; Labenia, J. S.; McIntyre, J. K.; Ylitalo, G. M.; Rhodes, L. D.; Laetz, C. A.; Stehr, C. M.; French, B. L.; McMillan, B.; Wilson, D.; Reed, L.; Lynch, K. D.; Damm, S.; Davis, J. W.; Collier, T. K. Recurrent Die-Offs of Adult Coho Salmon Returning to Spawn in Puget Sound Lowland Urban Streams. *PLoS One* **2011**, *6* (12), e28013.

(5) Feist, B. E.; Buhle, E. R.; Baldwin, D. H.; Spromberg, J. A.; Damm, S. E.; Davis, J. W.; Scholz, N. L. Roads to Ruin: Conservation Threats to a Sentinel Species across an Urban Gradient. *Ecol. Appl.* **2017**, *27* (8), 2382−2396.

(6) Feist, B. E.; Buhle, E. R.; Arnold, P.; Davis, J. W.; Scholz, N. L. Landscape Ecotoxicology of Coho Salmon Spawner Mortality in Urban Streams. *PLoS One* **2011**, *6* (8), e23424.

(7) Spromberg, J. A.; Scholz, N. L. Estimating the Future Decline of Wild Coho Salmon Populations Resulting from Early Spawner Die-Offs in Urbanizing Watersheds of the Pacific Northwest, USA. *Integr. Environ. Assess. Manage.* **2011**, *7* (4), 648−656.

(8) United States; Endangered Species Act, 1973.

(9) McIntyre, J. K.; Davis, J. W.; Hinman, C.; Macneale, K. H.; Anulacion, B. F.; Scholz, N. L.; Stark, J. D. Soil Bioretention Protects Juvenile Salmon and Their Prey from the Toxic Impacts of Urban Stormwater Runoff. *Chemosphere* **2015**, *132*, 213−219.

(10) Mayer, T.; Rochfort, Q.; Marsalek, J.; Parrott, J.; Servos, M.; Baker, M.; McInnis, R.; Jurkovic, A.; Scott, I. Environmental Characterization of Surface Runoff from Three Highway Sites in Southern Ontario, Canada: 2. Toxicology. *Water Qual. Res. J. Can.* **2011**, *46* (2), 121.

(11) McIntyre, J. K.; Davis, J. W.; Incardona, J. P.; Stark, J. D.; Anulacion, B. F.; Scholz, N. L. Zebrafish and Clean Water Technology: Assessing Soil Bioretention as a Protective Treatment for Toxic Urban Runoff. *Sci. Total Environ.* **2014**, *500*−*501*, 173−180.

(12) McIntyre, J. K.; Edmunds, R. C.; Redig, M. G.; Mudrock, E. M.; Davis, J. W.; Incardona, J. P.; Stark, J. D.; Scholz, N. L. Confirmation of Stormwater Bioretention Treatment Effectiveness Using Molecular Indicators of Cardiovascular Toxicity in Developing Fish. *Environ. Sci. Technol.* **2016**, *50* (3), 1561−1569.

(13) Jezierska, B.; Ługowska, K.; Witeska, M. The Effects of Heavy Metals on Embryonic Development of Fish (a Review). *Fish Physiol. Biochem.* **2009**, *35* (4), 625−640.

(14) Spromberg, J. A.; Baldwin, D. H.; Damm, S. E.; McIntyre, J. K.; Huff, M.; Sloan, C. A.; Anulacion, B. F.; Davis, J. W.; Scholz, N. L. Coho Salmon Spawner Mortality in Western US Urban Watersheds: Bioinfiltration Prevents Lethal Storm Water Impacts. *J. Appl. Ecol.* **2016**, *53* (2), 398−407.

(15) McIntyre, J. K.; Lundin, J. I.; Cameron, J. R.; Chow, M. I.; Davis, J. W.; Incardona, J. P.; Scholz, N. L. Interspecies Variation in the Susceptibility of Adult Pacific Salmon to Toxic Urban Stormwater Runoff. *Environ. Pollut.* **2018**, *238*, 196−203.

(16) Du, B.; Lofton, J. M.; Peter, K. T.; Gipe, A. D.; James, C. A.; McIntyre, J. K.; Scholz, N. L.; Baker, J. E.; Kolodziej, E. P. Development of Suspect and Non-Target Screening Methods for Detection of Organic Contaminants in Highway Runoff and Fish Tissue with High-Resolution Time-of-Flight Mass Spectrometry. *Env. Sci. Process. Impacts* **2017**, *19*, 1185.

(17) Zgheib, S.; Moilleron, R.; Saad, M.; Chebbo, G. Partition of Pollution between Dissolved and Particulate Phases: What about Emerging Substances in Urban Stormwater Catchments? *Water Res.* **2011**, *45* (2), 913−925.

(18) Zgheib, S.; Moilleron, R.; Chebbo, G. Priority Pollutants in Urban Stormwater: Part 1 − Case of Separate Storm Sewers. *Water Res.* **2012**, *46* (20), 6683−6692.

(19) Merel, S.; Anumol, T.; Park, M.; Snyder, S. A. Application of Surrogates, Indicators, and High-Resolution Mass Spectrometry to Evaluate the Efficacy of UV Processes for Attenuation of Emerging Contaminants in Water. *J. Hazard. Mater.* **2015**, *282*, 75−85.

(20) Benotti, M. J.; Trenholm, R. A.; Vanderford, B. J.; Holady, J. C.; Stanford, B. D.; Snyder, S. A. Pharmaceuticals and Endocrine Disrupting Compounds in U.S. Drinking Water. *Environ. Sci. Technol.* **2009**, *43* (3), 597−603.

(21) Kumata, H.; Yamada, J.; Masuda, K.; Takada, H.; Sato, Y.; Sakurai, T.; Fujiwara, K. Benzothiazolamines as Tire-Derived Molecular Markers: Sorptive Behavior in Street Runoff and Application to Source Apportioning. *Environ. Sci. Technol.* **2002**, *36* (4), 702−708.

(22) Kumata, H.; Mori, M.; Takahashi, S.; Takamiya, S.; Tsuzuki, M.; Uchida, T.; Fujiwara, K. Evaluation of Hydrogenated Resin Acids as Molecular Markers for Tire-Wear Debris in Urban Environments. *Environ. Sci. Technol.* **2011**, *45* (23), 9990−9997.

(23) McKenzie, E. R.; Money, J. E.; Green, P. G.; Young, T. M. Metals Associated with Stormwater-Relevant Brake and Tire Samples. *Sci. Total Environ.* **2009**, *407* (22), 5855−5860.

(24) Pan, S.; Sun, Y.; Zhang, G.; Li, J.; Xie, Q.; Chakraborty, P. Assessment of 2-(4-Morpholinyl) Benzothiazole (24MoBT) and N-Cyclohexyl-2-Benzothiazolamine (NCBA) as Traffic Tracers in Metropolitan Cities of China and India. *Atmos. Environ.* **2012**, *56*, 246−249.

(25) Dickenson, E. R. V.; Snyder, S. A.; Sedlak, D. L.; Drewes, J. E. Indicator Compounds for Assessment of Wastewater Effluent Contributions to Flow and Water Quality. *Water Res.* **2011**, *45* (3), 1199−1212.

(26) Glassmeyer, S. T.; Furlong, E. T.; Kolpin, D. W.; Cahill, J. D.; Zaugg, S. D.; Werner, S. L.; Meyer, M. T.; Kryak, D. D. Transport of Chemical and Microbial Compounds from Known Wastewater Discharges: Potential for Use as Indicators of Human Fecal Contamination. *Environ. Sci. Technol.* **2005**, *39* (14), 5157−5169.

(27) Westerhoff, P.; Yoon, Y.; Snyder, S.; Wert, E. Fate of Endocrine-Disruptor, Pharmaceutical, and Personal Care Product Chemicals during Simulated Drinking Water Treatment Processes. *Environ. Sci. Technol.* **2005**, *39* (17), 6649−6663.

(28) Soulier, C.; Coureau, C.; Togola, A. Environmental Forensics in Groundwater Coupling Passive Sampling and High Resolution Mass Spectrometry for Screening. *Sci. Total Environ.* **2016**, *563*−*564*, 845−854.

(29) Hollender, J.; Schymanski, E. L.; Singer, H. P.; Ferguson, P. L. Nontarget Screening with High Resolution Mass Spectrometry in the

DOI: 10.1021/acs.est.8b03287
Environ. Sci. Technol. 2018, 52, 10317−10327

Environment: Ready to Go? *Environ. Sci. Technol.* **2017**, *51* (20), 11505−11512.
(30) Moschet, C.; Lew, B. M.; Hasenbein, S.; Anumol, T.; Young, T. M. LC- and GC-QTOF-MS as Complementary Tools for a Comprehensive Micropollutant Analysis in Aquatic Systems. *Environ. Sci. Technol.* **2017**, *51* (3), 1553−1561.
(31) Newton, S. R.; McMahen, R. L.; Sobus, J. R.; Mansouri, K.; Williams, A. J.; McEachran, A. D.; Strynar, M. J. Suspect Screening and Non-Targeted Analysis of Drinking Water Using Point-of-Use Filters. *Environ. Pollut.* **2018**, *234*, 297−306.
(32) Pochodylo, A. L.; Helbling, D. E. Emerging Investigators Series: Prioritization of Suspect Hits in a Sensitive Suspect Screening Workflow for Comprehensive Micropollutant Characterization in Environmental Samples. *Environ. Sci. Water Res. Technol.* **2017**, *3* (1), 54−65.
(33) Getzinger, G. J.; O'Connor, M. P.; Hoelzer, K.; Drollette, B. D.; Karatum, O.; Deshusses, M. A.; Ferguson, P. L.; Elsner, M.; Plata, D. L. Natural Gas Residual Fluids: Sources, Endpoints, and Organic Chemical Composition after Centralized Waste Treatment in Pennsylvania. *Environ. Sci. Technol.* **2015**, *49* (14), 8347−8355.
(34) Chiaia-Hernández, A. C.; Günthardt, B. F.; Frey, M. P.; Hollender, J. Unravelling Contaminants in the Anthropocene Using Statistical Analysis of Liquid Chromatography−High-Resolution Mass Spectrometry Nontarget Screening Data Recorded in Lake Sediments. *Environ. Sci. Technol.* **2017**, *51* (21), 12547−12556.
(35) Zheng, W.; Wang, X.; Tian, D.; Zhang, H.; Tian, W.; Andersen, M. E.; Zheng, Y.; Sun, X.; Jiang, S.; Cao, Z.; He, G.; Qu, W. Pollution Trees: Identifying Similarities among Complex Pollutant Mixtures in Water and Correlating Them to Mutagenicity. *Environ. Sci. Technol.* **2012**, *46* (13), 7274−7282.
(36) Teuschler, L. Deciding Which Chemical Mixtures Risk Assessment Methods Work Best for What Mixtures. *Toxicol. Appl. Pharmacol.* **2007**, *223* (2), 139−147.
(37) *Coast Change Analysis Program (C-CAP): Regional Land Cover Classification Scheme*; NOAA Office for Coastal Management, August 21, 2017.
(38) Singh, R. R.; Lester, Y.; Linden, K. G.; Love, N. G.; Atilla-Gokcumen, G. E.; Aga, D. S. Application of Metabolite Profiling Tools and Time-of-Flight Mass Spectrometry in the Identification of Transformation Products of Iopromide and Iopamidol during Advanced Oxidation. *Environ. Sci. Technol.* **2015**, *49* (5), 2983−2990.
(39) Schollée, J. E. *Data Analysis for Non-Target Screening to Prioritize, Characterize, and Identify Micropollutant Transformation Products Formed in Wastewater Treatment*; ETH Zurich: Switzerland, 2016.
(40) Knolhoff, A. M.; Zweigenbaum, J. A.; Croley, T. R. Nontargeted Screening of Food Matrices: Development of a Chemometric Software Strategy To Identify Unknowns in Liquid Chromatography−Mass Spectrometry Data. *Anal. Chem.* **2016**, *88* (7), 3617−3623.
(41) Schymanski, E. L.; Jeon, J.; Gulde, R.; Fenner, K.; Ruff, M.; Singer, H. P.; Hollender, J. Identifying Small Molecules via High Resolution Mass Spectrometry: Communicating Confidence. *Environ. Sci. Technol.* **2014**, *48* (4), 2097−2098.
(42) Wagner, S.; Hüffer, T.; Klöckner, P.; Wehrhahn, M.; Hofmann, T.; Reemtsma, T. Tire Wear Particles in the Aquatic Environment - a Review on Generation, Analysis, Occurrence, Fate and Effects. *Water Res.* **2018**, *139*, 83−100.
(43) Chen, T.-B.; Zheng, Y.-M.; Lei, M.; Huang, Z.-C.; Wu, H.-T.; Chen, H.; Fan, K.-K.; Yu, K.; Wu, X.; Tian, Q.-Z. Assessment of Heavy Metal Pollution in Surface Soils of Urban Parks in Beijing, China. *Chemosphere* **2005**, *60* (4), 542−551.
(44) Vega, M.; Pardo, R.; Barrado, E.; Debán, L. Assessment of Seasonal and Polluting Effects on the Quality of River Water by Exploratory Data Analysis. *Water Res.* **1998**, *32* (12), 3581−3592.
(45) Mahieu, N. G.; Patti, G. J. Systems-Level Annotation of Metabolomics Data Reduces 25,000 Features to Fewer than 1,000 Unique Metabolites. *Anal. Chem.* **2017**, *89* (19), 10397−10406.
(46) Thurman, E. M.; Ferrer, I.; Rosenblum, J.; Linden, K.; Ryan, J. N. Identification of Polypropylene Glycols and Polyethylene Glycol Carboxylates in Flowback and Produced Water from Hydraulic Fracturing. *J. Hazard. Mater.* **2017**, *323*, 11−17.
(47) Marcelli, A.; Favretto, D.; Traldi, P. High Performance Liquid Chromatography Combined with Electrospray Ionization Ion Trap Mass Spectrometry in the Characterization of (Methoxymethyl)-Melamine Resins. *Rapid Commun. Mass Spectrom.* **1997**, *11*, 1321−1328.
(48) Chang, T. T. Characterization of (Methoxymethyl)Melamine Resins by Liquid Chromatography/Mass Spectrometry. *Anal. Chem.* **1994**, *66* (19), 3267−2373.
(49) Luft, A.; Bröder, K.; Kunkel, U.; Schulz, M.; Dietrich, C.; Baier, R.; Heininger, P.; Ternes, T. A. Nontarget Analysis via LC-QTOF-MS to Assess the Release of Organic Substances from Polyurethane Coating. *Environ. Sci. Technol.* **2017**, *51* (17), 9979−9988.
(50) DeArmond, P. D.; DiGoregorio, A. L. Rapid Liquid Chromatography−Tandem Mass Spectrometry-Based Method for the Analysis of Alcohol Ethoxylates and Alkylphenol Ethoxylates in Environmental Samples. *J. Chromatogr. A* **2013**, *1305*, 154−163.
(51) Zgoła-Grześkowiak, A.; Grześkowiak, T.; Zembrzuska, J.; Łukaszewski, Z. Comparison of Biodegradation of Poly(Ethylene Glycol)s and Poly(Propylene Glycol)S. *Chemosphere* **2006**, *64* (5), 803−809.
(52) González, S.; Barceló, D.; Petrovic, M. Advanced Liquid Chromatography-Mass Spectrometry (LC-MS) Methods Applied to Wastewater Removal and the Fate of Surfactants in the Environment. *TrAC, Trends Anal. Chem.* **2007**, *26* (2), 116−124.
(53) Schymanski, E. L.; Singer, H. P.; Longrée, P.; Loos, M.; Ruff, M.; Stravs, M. A.; Ripollés Vidal, C.; Hollender, J. Strategies to Characterize Polar Organic Contamination in Wastewater: Exploring the Capability of High Resolution Mass Spectrometry. *Environ. Sci. Technol.* **2014**, *48* (3), 1811−1818.
(54) U.S. EPA; *Ecological Structure Activity Relationships (ECOSAR) Predictive Model*; U.S. EPA: Washington, DC, 2012.
(55) Wik, A. Toxic Components Leaching from Tire Rubber. *Bull. Environ. Contam. Toxicol.* **2007**, *79* (1), 114−119.
(56) Unice, K. M.; Bare, J. L.; Kreider, M. L.; Panko, J. M. Experimental Methodology for Assessing the Environmental Fate of Organic Chemicals in Polymer Matrices Using Column Leaching Studies and OECD 308 Water/Sediment Systems: Application to Tire and Road Wear Particles. *Sci. Total Environ.* **2015**, *533*, 476−487.
(57) Nilsson, N. H.; Malmgren-Hansen, B.; Thomsen, U. S. *Mapping, Emissions and Environmental and Health Assessment of Chemical Substances in Artificial Turf*; Danish Ministry of the Environment: Copenhagen, Denmark, 2008.
(58) Jaratrotkamjorn, R.; Nourry, A.; Pasetto, P.; Choppé, E.; Panwiriyarat, W.; Tanrattanakul, V.; Pilard, J.-F. Synthesis and Characterization of Elastomeric, Biobased, Nonisocyanate Polyurethane from Natural Rubber. *J. Appl. Polym. Sci.* **2017**, *134* (42), 45427.
(59) Takamura, M.; Yamauchi, T.; Tsubokawa, N. Grafting and Crosslinking Reaction of Carboxyl-Terminated Liquid Rubber with Silica Nanoparticles and Carbon Black in the Presence of Sc(OTf)3. *React. Funct. Polym.* **2008**, *68* (6), 1113−1118.
(60) Hall, L. M.; Hall, L. H.; Kertesz, T. M.; Hill, D. W.; Sharp, T. R.; Oblak, E. Z.; Dong, Y. W.; Wishart, D. S.; Chen, M.-H.; Grant, D. F. Development of Ecom $_{50}$ and Retention Index Models for Nontargeted Metabolomics: Identification of 1,3-Dicyclohexylurea in Human Serum by HPLC/Mass Spectrometry. *J. Chem. Inf. Model.* **2012**, *52* (5), 1222−1237.
(61) Schwarzbauer, J.; Ricking, M. Non-Target Screening Analysis of River Water as Compound-Related Base for Monitoring Measures. *Environ. Sci. Pollut. Res.* **2010**, *17* (4), 934−947.
(62) Seitz, W.; Winzenbacher, R. A Survey on Trace Organic Chemicals in a German Water Protection Area and the Proposal of Relevant Indicators for Anthropogenic Influences. *Environ. Monit. Assess.* **2017**, *189* (6), 244.

DOI: 10.1021/acs.est.8b03287
Environ. Sci. Technol. 2018, 52, 10317−10327

(63) de Hoogh, C. J.; Wagenvoort, A. J.; Jonker, F.; van Leerdam, J. A.; Hogenboom, A. C. HPLC-DAD and Q-TOF MS Techniques Identify Cause of *Daphnia* Biomonitor Alarms in the River Meuse. *Environ. Sci. Technol.* **2006**, *40* (8), 2678−2685.
(64) Dsikowitzky, L.; Schwarzbauer, J. Hexa(Methoxymethyl)-Melamine: An Emerging Contaminant in German Rivers. *Water Environ. Res.* **2015**, *87* (5), 461−469.
(65) Bobeldijk, I.; Stoks, P. G. M.; Vissers, J. P. C.; Emke, E.; van Leerdam, J. A.; Muilwijk, B.; Berbee, R.; Noij, T. H. Surface and Wastewater Quality Monitoring: Combination of Liquid Chromatography with (Geno)Toxicity Detection, Diode Array Detection and Tandem Mass Spectrometry for Identification of Pollutants. *J. Chromatogr. A* **2002**, *970* (1−2), 167−181.
(66) Incardona, J. P.; Scholz, N. L. The Influence of Heart Developmental Anatomy on Cardiotoxicity-Based Adverse Outcome Pathways in Fish. *Aquat. Toxicol.* **2016**, *177*, 515−525.

DOI: 10.1021/acs.est.8b03287
Environ. Sci. Technol. 2018, 52, 10317−10327