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Article

Treading Water: Tire Wear Particle Leachate Recreates an Urban Runoff Mortality Syndrome in Coho but Not Chum Salmon

Jenifer K. McIntyre,* Jasmine Prat, James Cameron, Jillian Wetzel, Emma Mudrock, Katherine T. Peter, Zhenyu Tian, Cailin Mackenzie, Jessica Lundin, John D. Stark, Kennith King, Jay W. Davis, Edward P. Kolodziej, and Nathaniel L. Scholz

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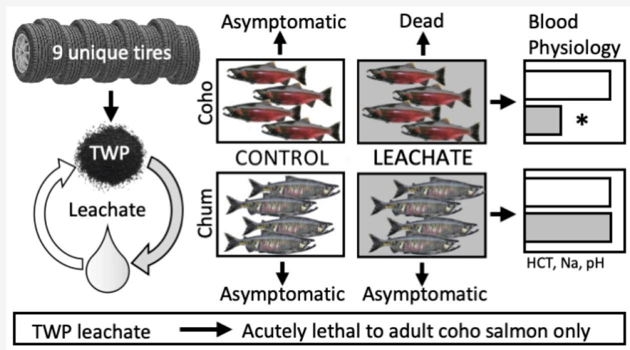

ABSTRACT: Tire tread wear particles (TWP) are increasingly recognized as a global pollutant of surface waters, but their impact on biota in receiving waters is rarely addressed. In the developed U.S. Pacific Northwest, acute mortality of adult coho salmon (*Oncorhynchus kisutch*) follows rain events and is correlated with roadway density. Roadway runoff experimentally triggers behavioral symptoms and associated changes in blood indicative of cardiorespiratory distress prior to death. Closely related chum salmon (*O. keta*) lack an equivalent response. Acute mortality of juvenile coho was recently experimentally linked to a transformation product of a tire-derived chemical. We evaluated whether TWP leachate is sufficient to trigger the acute mortality syndrome in adult coho salmon. We characterized the acute response of adult coho and chum salmon to TWP leachate (survival, behavior, blood physiology) and compared it with that caused by roadway runoff. TWP leachate was acutely lethal to coho at concentrations similar to roadway runoff, with the same behaviors and blood parameters impacted. As with runoff, chum salmon appeared insensitive to TWP leachate at concentrations lethal to coho. Our results confirm that environmentally relevant TWP exposures cause acute mortalities of a keystone aquatic species.

KEYWORDS: *urbanization, stormwater, endangered species, water quality, tire tread, Oncorhynchus, microplastics, 6PPD-quinone*

■ INTRODUCTION

Urban stormwater runoff is widely recognized as a major source of degradation of quality to surface waters in the United States[1] and elsewhere.[2,3] Among contributing sources, runoff from roads can be particularly toxic to aquatic animals, altering species abundance and assemblages in receiving waters,[4] with effects on individuals ranging from reproductive impairment to mortality.[5−11] In the Pacific Northwest of the United States, coho salmon (*Oncorhynchus kisutch*) returning annually from the ocean to spawn in urban and urbanizing watersheds die at high rates from exposure to stormwater runoff;[12,13] mortality rates are approximately 40−100% for typical urban stream networks. The acute mortality syndrome is typified by changes in behavior and blood parameters consistent with cardio-respiratory distress, including surface swimming and gaping corresponding with increased hematocrit and decreased plasma pH and ions in both adult[14] and juvenile[15,16] coho salmon. Notably, chum salmon (*O. keta*) coexposed to urban stormwater runoff in the field[12] and roadway runoff under controlled conditions[14] appear unaffected, by both behavioral and physiological metrics.

Roadways appear to be the source of contaminants causing the documented mortality syndrome. In a land-use analysis of 51 watersheds with coho salmon, higher rates of mortality occurred in watersheds containing more and busier roads.[13] Roadway runoff is a highly complex mixture containing well-documented contaminants like metals and polycyclic aromatic hydrocarbons (PAHs) with known toxicological effects.[17,18] Roadway runoff also contains thousands of mostly unidentified and/or toxicologically uncharacterized organic chemicals.[19,20] Chemical sources include vehicle fluids (e.g., motor oil, engine coolant, windshield fluid), particulates from vehicle use (e.g., combustion engine exhaust, brake friction materials, tire tread abrasion), and roadway maintenance chemicals such as herbicides.[21] Among vehicle sources, particles worn from tires are a particularly rich source of contaminants. For example, chemicals leaching from tire tread wear particles (TWP) accounted for 15% of all chemical detections in a

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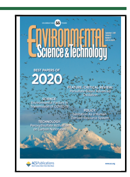

Table 1. Estimated Equivalent Concentrations of Tire Wear Particle (TWP) and Observed Concentrations of 6PPD-Quinone in Exposure Waters for Experiments with Coexposed Coho and Chum Salmon[f]

		Equivalent [TWP] from Estimation Methods (mg/L)							
		Method 1	Method 2						
		Nontarget HRMS data[a]	HMMM	DCU	DPG				
Experiment: Date	Nominal [TWP] (mg/L)		23 μg/g TWP[b]	18 μg/g TWP[b]	101 μg/g TWP[b]	94 μg/g TWP[c]	16.2 μg/g TWP[c]	Median Equivalent [TWP] (mg/L)[d]	[6PPD-Q] (μg/L)[e]
Acute Mortality: 11/20/2017	320	210	140	180	56	60	350	180	1.8
Acute Mortality: 11/21/2017	320	140	120	130	51	54	320	130	1.3
Sublethal: 11/27/2017	320	250	150	200	68	73	430	200	2.1
Sublethal: 11/28/2017	320	300	180	250	70	76	440	250	2.4

[a]Derived from abundance of dissolved TWP-derived chemicals detected by nontarget HRMS, via source estimation methods described in Peter et al. 2019. [b]Based on per-mass loads of HMMM (hexa(methoxymethyl)melamine), DCU (1,3-dicyclohexylurea), and DPG (1,3-diphenylguanidine) in TWP from Peter et al. 2020. [c]Minimum and maximum per-mass loads in TWP from Unice et al. 2015. [d]Median of all individual equivalent estimates (values for DPG at 101 ug/g and 94 ug/g were first averaged). [e]Semiquantitative concentrations of 6PPD-Q (2-anilino-5-[(4-methylpentan-2-yl)amino]cyclohexa-2,5-diene-1,4-dione) determined as in Tian et al. 2020. [f]Estimates reflect the quantity of TWP needed to explain observed abundances of dissolved TWP-derived chemicals under efficient leaching conditions. More details in Supporting Information.

recent study of roadway runoff.[22] Using chemical tracers, TWP in snow, surface runoff, and aquatic habitats were estimated at 0.5−563 mg/L.[23] In the Pacific Northwest, a recent assessment of urban streams during rain events detected tire-derived chemicals at concentrations equivalent of up to 150 mg/L TWP.[24]

We recently showed that TWP leachate can cause acute mortality of juvenile coho salmon.[25] We subsequently identified the primary causal toxicant as 6PPD-quinone, a transformation product of the antioxidant 6PPD ubiquitously added to tire formulations to protect rubber polymers against ozone.[25] Although 6PPD-quinone was detected in TWP leachate, roadway runoff, and some surface waters near or above concentrations lethal to juvenile coho in the laboratory,[25] we had yet to confirm the relevance for adults: the life stage for which the acute mortality syndrome has been documented in the field. The objective of the current study was to determine whether environmentally relevant concentrations of TWP are sufficient to recreate the acute mortality syndrome of adult coho salmon exposed to urban runoff, including differences in species sensitivity between coho and chum salmon. We compared survival, behavior, and blood physiology of prespawning adult coho and chum salmon exposed to a TWP leachate. We additionally compared the effect of TWP leachate on coho blood physiology with that of coho exposed to roadway runoff from a previous study using identical methods.[14] We used high resolution mass spectrometry (HRMS) to confirm the presence of 6PPD-quinone and chemically characterize the TWP exposures.

■ METHODS

Generation of Tire Wear Particles (TWP) and Leachate. We generated TWP from nine unique tires (two new and seven used) by previously reported methods (Supporting Information; Table S1). New tires were one popular high-end and one popular low-end tire purchased from a tire retailer in Tacoma, WA. Used tires were a variety of popular brands acquired from various retailers. TWP were stored at room temperature in dedicated food grade high density polyethylene (HDPE) containers. Particle sizes were determined by 2D image analysis using ImageJ[26] (function: particle analysis), averaged across two photographs (12 megapixel) of 3 g of TWP on a white background. Particles were slightly larger overall ($\bar{x}$ ± SD: 310 ± 306 μm; range: 0.011−3000 μm) than those created on road surfaces.[27,28] We estimate that >86% of TWP were >100 μm. For each exposure, an equal-weight mixture of TWP was divided among two 100-μm polypropylene felt filter bags (15 cm × 51 cm; Filter Specialists Inc. (FSI), X01). Each bag was placed in a polypropylene filter housing (FSI Model X-100) attached to a magnetic drive pump (Pentair WD40HDX) recirculating well water (9.7 °C, pH 8.0, 345 μS/cm) from a 1345-L stainless-steel tote (Custom Metalcraft, Springfield, MO, USA) at 20 L/min. The pump, filter housing, and tote were connected by 2.54-cm PVC tubing, with air evacuated through a bleed valve. TWP were leached (1000 mg/L or 320 mg/L) at ambient outdoor temperatures (0−11 °C) for 22 h immediately preceding each exposure and used directly or diluted further with well water (see below). While it is possible that particles <100 μm passed through the filter bags, none were visible in the final TWP leachate.

Coho and Chum Salmon Exposures. Prespawn adult coho and chum salmon were collected in freshwater during November 2017 at the Suquamish Tribe Grovers Creek Hatchery (Poulsbo, WA) upon return from marine waters. Individual fish were seined by hatchery personnel and placed in PVC tubes with perforations at each end (Figure S1) to reduce handling stress as previously described.[14] Coho length and weight were 583 ± 63 mm ($\bar{x}$ ± SD) and 2.4 ± 0.9 kg, respectively; chum were 634 ± 61 mm and 3.0 ± 1.0 kg. The ratio of fish length to tube length was 52−77%. Following a 3-h acclimation in a raceway fed by Grovers Creek, tubes were lifted out of the raceway and transported 200 m in 500 L of creek water in a HDPE tote to nearby exposure tanks.

Four salmon in individual tubes were placed in each HDPE tank containing 400 L of exposure water as previously described.[8,14] A submerged pump circulated aerated water directly into each PVC tube via a hose inserted into the anterior gate. Water quality (temperature, dissolved oxygen, conductivity, pH) was monitored before, during, and after

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exposures and was within normal ranges for salmon husbandry ($\overline{x}$ ± SD: 9.4 ± 0.5 °C, 8.1 ± 1.1 mg/L DO, 358 ± 20 μS/cm, pH 7.8 ± 0.3). At the end of exposure, fish were euthanized by a blow to the head following protocols approved by the Institutional Animal Care and Use Committee of Washington State University (ASAF#0504−003). Only apparently healthy fish were targeted for use in the experiments; however, upon inspection one female control coho was found to have spawned (i.e., senescent) and another control coho was accidentally caught in the posterior assembly of the PVC tube and consequently died. These two fish (4%) were excluded from subsequent analyses. There were no mortalities among chum salmon.

Experiments with TWP Leachate. *Acute mortality experiments with coho and chum salmon.* Four individual coho salmon in each of four tanks were exposed to well water (controls) or one of three concentrations of TWP leachate: 1000 mg/L (made by leaching from 600 g TWP in 600 L of well water) or leachate diluted in well water to 320 or 100 mg/L. Survival was determined at 24 h by releasing each fish into an observation tank with 1000 L of well water. This experiment was conducted twice (Figure S2). For the replicated exposure, survival was evaluated by conducting spot checks at 3 and 6 h, in addition to evaluation at 24 h. To determine if chum salmon were also sensitive to TWP leachate, two adult coho and two adult chum salmon in each of four HDPE tanks were coexposed to clean water or a lethal concentration of TWP leachate. Two tanks contained well water and two tanks contained 320 mg/L TWP leachate (270 g TWP in 841 L well water). Survival was determined at 24 h. This experiment was conducted three times (Figure S2), with water chemistry analysis for the final two replications (Table 1).

Sublethal symptomology of coexposed coho and chum salmon. In previous studies, coho exposed to roadway runoff for a few hours showed significant changes in blood parameters that coincided with behavioral changes symptomatic of the mortality syndrome.[14,15] To evaluate whether short-term exposures to TWP leachate produce similar changes in behavior and blood parameters, we coexposed coho and chum salmon as above (320 mg/L TWP leachate), but after 3 h, individuals were transferred to an observation tank. Behavior was scored on an ordinal scale (Table S2) from 0 (asymptomatic) to 5 (immobile on side) similar to previous methods,[14,15] followed by euthanasia as described above. This experiment was conducted three times (Figure S2), with water chemistry analyzed for the first two replications.

As previously described,[14] arterial blood was drawn immediately after euthanasia from the dorsal aorta through the dorsal palate into a 3 mL syringe containing 7 IU/mL ion-balanced lyophilized lithium heparin (Portex LineDraw) fitted with a 22-gauge 25-mm needle (BD PrecisionGlide). Following blood draw, the syringe was gently rolled horizontally for 1 min to dissolve and mix the anticoagulant before analysis; the total time between euthanasia and blood analysis was 3−5 min. After discarding the first drop, approximately 100 μL of blood was loaded into a cartridge (EC8+; ABAXIS) and inserted into a hand-held *VetScan iSTAT 1* blood analyzer (ABAXIS). Only measured metrics on the EC8+ cartridge were used for the study; partial pressure of CO_2 (pCO_2), pH, hematocrit, glucose, Na and Cl. Plasma K was also measured, but values were previously determined to decline rapidly over time[14] and were therefore not analyzed. Hematocrit (HCT) measurement by the *iSTAT* is conductometric, which underestimates HCT in fish relative to the volumetric approach used in the conventional microhematocrit method.[14,29] We therefore concurrently used the microhematocrit method[30] to determine HCT (Figure S3) and leukocrit. Temperature-dependent parameters (pH and pCO_2) were corrected for the colder temperature of the exposure water using manufacturer equations.[31] The length and weight of each fish used for behavior and blood analysis are in Table S3.

Assessment of Blood Parameters. Blood parameters in coho and chum sublethally exposed to TWP leachate were compared with those of control fish using a linear mixed model with treatment (TWP leachate vs clean water) as the independent variable, plasma Na, Cl, glucose, pH, partial pressure of CO_2 (pCO_2), hematocrit, and leukocrit as dependent variables, and tank number and experiment date as a random factors. Separate models were run for coho and chum salmon using the *lmerTest* function in R (version 3.6.1). Correction of the critical p-value for multiple comparisons was conducted by a modified false discovery method.[32] For comparison with blood parameters in coho and chum sublethally exposed to roadway runoff,[14] a principal component analysis (PCA) was conducted for each species on the six blood metrics in common with the previous study on runoff (plasma Na, Cl, glucose, hematocrit, pH, and pCO_2). We excluded fish with missing values for any metric, resulting in a total of 58 coho (TWP leachate: 9 control +11 exposed; runoff: 21 control +17 exposed;) and 56 chum (TWP leachate: 11 control +12 exposed; runoff: 15 control +18 exposed). The PCA was conducted in R (version 3.6.1) using the *prcomp* function after centering and scaling the data. A parallel analysis was conducted to assess the number of principle components (PC) to retain in the PCA model.[33] Eigenvalues for each PC were compared to randomly generated eigenvalues for the size of the data set (e.g., 6 factors x 58 coho; 1000 iterations, 95th percentile). A PC was retained if the eigenvalue from the PCA was greater than that randomly generated by the parallel analysis. For retained PCs, a varimax rotation was used to compute factor loadings, which were retained if ≥ |0.25|. Finally, a multivariate general linear model (SPSS version 26; IBM Corp) was used to test whether PC scores were significantly associated with treatment (exposure to test water vs control water) or experiment (control vs runoff or control vs leachate).

Water Analysis by LC-HRMS. Water samples were collected from four of the six coexposures of coho and chum (nominal 320 mg/L TWP) prior to the introduction of fish. For each, 2 L was collected from each of the four exposure tanks. Samples from the two tanks containing TWP leachate were composited in 4-L precleaned amber glass bottles, as were samples from the two tanks containing control water. Composites were transported on ice to the Center for Urban Waters (Tacoma, WA, USA) and extracted within 24 h by established methods;[22] see Supporting Information.

Concentration Analysis of TWP Leachate. Two methods were applied to estimate the 'equivalent concentration' of TWPs in exposure waters, reflecting the mass of TWP needed to explain the observed abundance of dissolved TWP-derived organic contaminants detected by HRMS. The first method used nontarget HRMS features (accurate mass−retention time pairs with grouped isotopes/adducts) that were extracted and aligned with Agilent software (Profinder B.08.00; Mass Profiler Professional B.13.00), using previously reported

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settings to filter the data and select features amenable to source estimation.[34] Following previously described source estimation methods,[35] 551−593 HRMS features (i.e., unique chemicals, including both known and unidentified compounds) in each exposure water sample were used to estimate the TWP concentration. The second method to estimate equivalent TWP concentration used measured concentrations of three tire-derived chemicals [1,3-diphenylguanidine (DPG), hexa-(methoxymethyl)melamine (HMMM), and 1,3-dicyclohexylurea (DCU)] in the exposure waters (details in Supporting Information).

Retrospective Analysis of 6PPD-Quinone in TWP Leachate. In addition to measurements used to assess equivalent TWP concentration, we confirmed the presence of 2-anilino-5-[(4-methylpentan-2-yl)amino]cyclohexa-2,5-diene-1,4-dione (6PPD-quinone) in exposure waters using archived extracts and an analytical calibration curve as reported by Tian et al.[25] Detector saturation and/or matrix effects likely impacted observed peak areas; therefore, concentrations of 6PPD-quinone by HRMS analysis of archived extracts are considered semiquantitative. Ongoing research using a commercial 6PPD-quinone standard and an optimized analytical method is expected to refine concentration estimates.

RESULTS

Concentrations of TWP-Derived Chemicals in TWP Leachate. High concentrations of 6PPD-quinone were present in the nominal 320 mg/L TWP leachate (Table 1). Concentrations were approximately twice the median lethal concentration (LC_{50}) established using juvenile coho salmon.[25] The chemical estimation methods resulted in equivalent concentrations of TWP in exposure waters of 130−250 mg/L (Table 1), which were 41−77% of nominal concentrations. Differences from nominal concentrations and among experiment days are attributed to the low leaching temperatures (≤10 °C) and the large mass of TWP in each filter bag, resulting in mass transfer limitations during leaching. Because chemical exposures were lower than expected based on nominal concentrations, toxicology results are described in terms of nominal TWP concentrations as the more conservative approach to toxicity assessment.

Acute Mortality of Adult Coho Salmon from TWP Leachate. The lowest nominal concentration of TWP leachate (100 mg/L) killed 25−50% of adult coho salmon during the 24-h exposure (1 of 4 in replication 1; 2 of 4 in replication 2). In comparison, there were no survivors in the intermediate (320 mg/L) or high (1000 mg/L) TWP leachate treatments (Figure S4). While mortality was tabulated at 24-h, spot checks revealed that most mortality occurred within the first 6 h (100% of mortality for the high concentration and 67% for the intermediate concentration).

No Effects on Adult Chum Salmon from TWP Leachate. During a 24-h coexposure to 320 mg/L TWP leachate, all coho salmon died but no chum salmon died (Figure 1). In sublethal coexposures, we scored the behavior of individual coho and chum salmon after 3 h. In control water, two of 12 coho exposed to control water were mildly lethargic, whereas the remaining control coho were asymptomatic (Table 2). In TWP leachate, coho were severely symptomatic (Table 2), with a modal score of 5 on a distress scale from 1 (mildly lethargic) to 5 (immobile on side) (Table S2). Of the 12 chum salmon exposed to control water, 11 were asymptomatic and one mildly lethargic. Chum salmon exposed

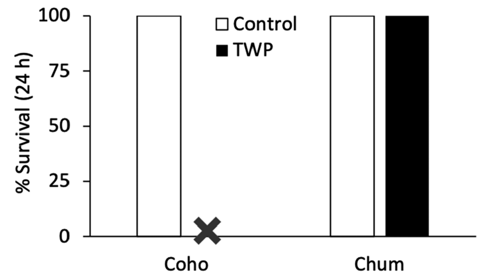

Figure 1. Survival of coho and chum salmon exposed to control water or tire wear particle (TWP) leachate for 24 h at a nominal concentration of 320 mg/L. Averages are for three replicates of four fish for each species and treatment. The " × " denotes no survivors for coho exposed to TWP leachate. There was no variability among replicates.

Table 2. Individual Behavior Scores for Each of the Co-Exposed Coho and Chum Salmon (n = 4) after Exposure (3−3.5 h) to Tire Wear Particle (TWP) Leachate (320 mg/L) or Control Water for Three Replicated Exposures[b]

	Coho		Chum	
Exposure	TWP	Control	TWP	Control
Nov 27	2/2/3/3[a]	0/0/0/1	0/0/0/1	0/0/0/0
Nov 28	0/5/5/5	0/0/0/0	0/0/0/0	0/0/0/0
Nov 30	1/2/5/5	0/0/0/1	0/0/0/0	0/0/0/1

[a]Behavior score for each of four fish exposed to each treatment. [b]Behavior scores range from 0 (asymptomatic) to 5 (immobile), with complete descriptions in Table S2.

to TWP leachate received the same score distribution as control-exposed chum (11 scores of 0, one score of 1), showing that they were not behaviorally affected by exposure to TWP leachate.

Blood Changes in Coho Salmon from TWP Leachate Are Similar to Roadway Runoff. Coho salmon exposed to TWP leachate (320 mg/L) showed a significant increase in hematocrit and decreases in plasma Na and pH compared with controls (Table 3). Leukocrit, pCO_2, plasma chloride, and glucose were not significantly affected. In chum salmon, no blood parameters were significantly altered by TWP leachate exposure. In a comparison of the effects of TWP leachate with those of roadway runoff on blood parameters that are common between the current and previous study,[14] three principal components (PCs) were retained from the PCA for each species. These PCs explained 83% of the variance in the blood data for coho (PC1 = 41%; PC2 = 24%; PC3 = 18%) and 79% for chum (PC1 = 34%; PC2 = 23%; PC3 = 22%). For chum, treatment (control vs exposed) did not affect any PC (p = 0.095−0.795), nor was there an interaction between treatment and experiment type for any PC (p = 0.172−0.330). In contrast, for coho, treatment was significantly associated with PC1 ($F(1,58) = 64.214$, $p < 0.001$), but not PC2 ($F(1,58) = 0.079$, p = 0.780) or PC3 ($F(1,58) = 0.880$, $p = 0.352$) (Figure 2A). This effect did not depend on whether the exposure water was TWP leachate or roadway runoff (i.e., no interaction between treatment and experiment: $F(1,58) = 0.745$, p = 0.392). Factor loading showed that variation in PC1 was driven strongly by an increase in hematocrit and a decrease in plasma pH, Na, and Cl relative to controls (Figure 2B; Table S4). Therefore, the effect of exposure on blood parameters in coho was the same for roadway runoff and for TWP leachate;

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Table 3. Blood Parameters for Coho and Chum Salmon Exposed to Control Water or Tire Wear Particle Leachate (TWP)[c]

Species		Coho			Chum		
Treatment	Units	Control	TWP	*p*	Control	TWP	*p*
Na	mM	140 (1.3)	132 (3.8)[a]	0.032	145 (1.3)	147 (1.1)	0.239
Cl	mM	117 (1.7)	110 (3.4)	0.132	121 (2)	120 (0.8)	0.676
Glucose	mM	116 (12.7)	118 (11.7)	0.506	130 (5)	125 (5.8)	0.483
pH	std	7.72 (0.03)	7.51 (0.07)[a]	<0.001	7.59 (0.06)	7.59 (0.04)	0.944
pCO_2	mmHg	6.2 (0.3)	7.2 (1.1)	0.279	7.1 (0.5)	8.4 (0.7)	0.095
Hematocrit	%	37 (1.1)	56 (2.2)[a]	<0.001	34 (1.4)	34 (1)	0.688
Leukocrit[b]	%	0.91 (0.07)	0.93 (0.12)	0.638	0.7 (0.06)	0.66 (0.07)	0.685

[a]Significant difference from control within species for linear mixed model of treatment effect. [b]Determined by microcapillary method. [c]Values are averages (one standard error of the mean).

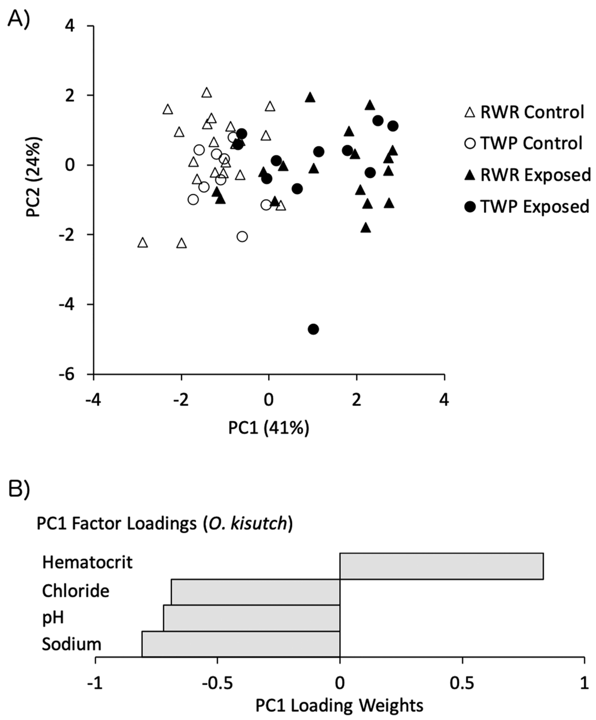

Figure 2. (A) Principle component (PC) scores along PC1 and PC2 for coho salmon exposed to roadway runoff (RWR),[14] tire wear particle (TWP) leachate (this study), or control water from each experiment. PC1 was significantly related to treatment, with higher scores associated with exposure to roadway runoff or TWP leachate. (B) Factor loadings (>|0.5|) indicate that elevated hematocrit and reduced chloride, sodium, and pH contributed strongly to positive values along PC1.

both driven by increased hematocrit and decreased plasma pH and ions, while neither TWP leachate nor roadway runoff affected the blood parameters of chum.

■ DISCUSSION

Short-term (<24 h) exposures to TWP leachate containing 6PPD-quinone, and other TWP-derived chemicals, at environmentally relevant concentrations for roadway runoff[23,25] were consistently lethal to adult coho salmon, similar to effects previously reported for juvenile coho.[25] This species of Pacific salmon appears to be especially sensitive to chemicals derived from tires relative to other aquatic taxa from earlier studies.[27,36,37] This is especially relevant because most previous studies generated TWP leachates at higher temperatures and for longer durations than in the current study, and/or used extreme conditions such as low pH or solvent extraction. Leachates produced under these aggressive conditions would likely contain higher concentrations of TWP-derived chemicals. Previous studies also exposed test organisms for longer durations (48−96 h) than in the current study (24 h). These methodological differences would be expected to enhance toxicity in test organisms. The unusual vulnerability of coho to TWP leachate further reinforces the importance of this species as a sentinel for runoff-driven ecological decline in rapidly urbanizing watersheds of the Pacific Northwest.[12] To this end, green stormwater infrastructure that demonstrably and consistently protects coho from stormwater toxicity[7,8] are likely to also protect many other aquatic species from roadway-derived contaminants.

The physiochemical and behavioral characteristics of the acute coho distress syndrome elicited by TWP leachate were nearly identical to those previously documented in coho exposed to urban roadway runoff.[14,15] In both cases, the syndrome corresponded with significantly increased hematocrit and decreased plasma ions and pH. Furthermore, principal component analysis of blood parameters showed comparable changes in direction and magnitude in response to TWP leachate (this study) and roadway runoff.[14] The weight of evidence supports that TWP-derived chemicals are driving the coho urban runoff mortality syndrome.[8,12,14] This is further reinforced by the recent discovery of 6PPD-quinone as the driver of the acute mortality in juvenile coho exposed to TWP leachate.[25]

In the contiguous United States, coho salmon are native to watersheds from the central California coast north to Canada. Their long-term conservation risk is generally greater in lowland areas where human development is most dense. Coho salmon populations are listed under the U.S. Endangered Species Act as "endangered" in the central California coast (including greater San Francisco), "threatened" in the lower Columbia River basin (including greater Portland-Vancouver metropolitan area), and a "species of concern" in the Puget Sound basin (including greater Seattle). Many factors contribute to declines in populations of coho and other Pacific salmonids, including loss of physical spawning and rearing habitat. To date, there have been only limited efforts to evaluate the relative contributions of physical vs chemical habitat degradation as limiting factors for salmon recovery.[38] The role of water quality therefore remains poorly understood but could be a dominant factor for coho in watersheds with densely trafficked roadways. For example, extinction of local coho populations was predicted on a time course of decades by life history modeling[39] incorporating adult coho mortality rates observed in the field.[12]

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In contrast to coho, chum salmon showed no evidence of toxicity in response to TWP leachate. While the leachate (320 mg/L TWP) was consistently lethal to coho, all of the coexposed chum survived without significant changes in blood chemistry or behavior relative to unexposed controls. This finding agrees with the relative insensitivity of chum exposed to roadway runoff[14] and the notable survival of chum in mixed-species spawning habitats impacted by urban runoff where coho spawner die-offs recur each autumn.[12] The underlying mechanistic basis for these striking, species-specific differences in toxicity will require additional studies: a line of research considerably more tractable with the discovery of 6PPD-quinone as the primary causal agent. We have recently associated premortem behavioral changes in coho exposed to roadway runoff with apparent loss of vascular integrity in the brain and other organs.[16] Identifying phenotypically anchored receptors in target tissues should lead to understanding mode of action, and ultimately the molecular initiating event for this highly consequential adverse outcome pathway[40] in coho but not chum.

In the past decade, there have been notable advances in analytical techniques for identifying currently unregulated chemicals that may pose significant risks to biota. Nontargeted analysis and suspect screening approaches based on HRMS have detected thousands of organic chemicals in source waters,[41] including stormwater[19,20] and receiving waters.[24,42] From a public policy perspective, these techniques demonstrate the current natural resource management challenge, i.e., the true chemical complexity of urban-impacted surface waters. For example, Du et al.[19] reported >4000 unique chemicals in roadway runoff from a multilane highway, hundreds of which were also detected in tissues of experimentally exposed adult coho salmon. This refined exposure profiling has demonstrated a large number of bioavailable chemicals in urban-impacted runoff with no corresponding guidelines or criteria for the protection of aquatic life. Effects-directed analysis, bioassays in combination with nontargeted analysis to screen chemical fractions, was used to identify the primary toxicant in TWP leachate for coho salmon as 6PPD-quinone: a transformation product of the antioxidant 6PPD ubiquitously added to tire formulations to protect rubber polymers against degradation from ozone.[25] 6PPD-quinone was detected in TWP leachate, roadway runoff, and some surface waters at concentrations near or above the LC_{50} in juvenile coho (∼0.8 μg/L). Detection of acutely lethal concentrations of the previously unrecognized, yet likely widespread, 6PPD-quinone suggests the need for more effective management of nonpoint source pollution threats to water quality.

We have shown that environmentally realistic concentrations of TWPs leach chemicals that trigger an acute stormwater mortality syndrome in coho salmon. Beyond coho, numerous other species, freshwater and saltwater, vertebrate and invertebrate, are sensitive to tire rubber leachates,[23,27] although relatively few species (<20) have been evaluated (Supporting Information). Thus, our current understanding of species sensitivity distribution for this complex chemical mixture is far from complete. Roads, motor vehicle traffic, tire wear, rain events, and untreated stormwater discharges are global phenomena. Although estimates of TWP and TWP-derived chemical concentrations in aquatic environments remain uncertain,[27,43,44] it is clear that large numbers of TWP are transported into surface waters,[45] and chemicals leaching from these particles are ubiquitous in receiving waters and sediments.[24,27,46,47] Importantly, the current study suggests that it is primarily the chemicals leaching from TWP rather than the TWP themselves that pose a threat in receiving waters. An estimated 50% of TWP deposited on roads are not washed off by precipitation.[48] Instead, they are an *in situ* source of water-soluble contaminants, including those with toxic properties like 6PPD-quinone, for at least several years.[48] As such, there is a need to evaluate the relative contribution of vehicle tires on-road vs worn tire particles, particles embedded in the road vs free, and particles leaching *in situ* vs mobilized with runoff to the load of tire-derived chemicals in receiving waters. In concert, future research will invariably address the occurrence of 6PPD-quinone and other TWP-derived chemicals in aquatic environments and begin assessing additional species for adverse outcomes. Finally, scrap tire yards, crumb rubber in playgrounds and artificial turf fields, and other reuses of tires must be evaluated for their contribution of tire-derived chemicals to receiving waters.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.1c03569.

Methods for TWP generation; methods for chemical analysis by LC-HRMS; methods for TWP leachate concentration assessment; methods for summarizing species toxicity data for tire leachates; description of tires used to generate TWP; symptom severity scale; biometrics for adult coho and chum; principal component analysis for coho blood parameters; TWP leachate concentration assessment; image of a replicate exposure tank with PVC tubes containing salmon; overview of experimental design for TWP leachate exposures; hematocrit comparison of iSTAT and microcapillary results; dose−response and timing of acute mortality of coho spawners; calibration curve for TWP-derived chemicals in leachate (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Jenifer K. McIntyre − *Washington State University, School of the Environment, Puyallup Research and Extension Center, Puyallup, Washington 98371, United States;* orcid.org/0000-0003-3480-7083; Email: jen.mcintyre@wsu.edu

Authors

Jasmine Prat − *Washington State University, School of the Environment, Puyallup Research and Extension Center, Puyallup, Washington 98371, United States*

James Cameron − *Ocean Associates, under contract to Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Seattle, Washington 98112, United States*

Jillian Wetzel − *Washington State University, School of the Environment, Puyallup Research and Extension Center, Puyallup, Washington 98371, United States*

Emma Mudrock − *Washington State University, School of the Environment, Puyallup Research and Extension Center, Puyallup, Washington 98371, United States*

Katherine T. Peter − *Center for Urban Waters, Tacoma, Washington 98421, United States; Interdisciplinary Arts and Sciences, University of Washington Tacoma, Tacoma,*

Washington 98421, United States; orcid.org/0000-0001-7379-265X
Zhenyu Tian – *Center for Urban Waters, Tacoma, Washington 98421, United States; Interdisciplinary Arts and Sciences, University of Washington Tacoma, Tacoma, Washington 98421, United States;* orcid.org/0000-0002-7491-7028
Cailin Mackenzie – *Washington State University, School of the Environment, Puyallup Research and Extension Center, Puyallup, Washington 98371, United States*
Jessica Lundin – *National Research Council Research Associateship Program, Under contract to Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Seattle, Washington 98112, United States*
John D. Stark – *Washington State University, Washington Stormwater Center, Puyallup Research and Extension Center, Puyallup, Washington 98371, United States*
Kennith King – *United States Fish and Wildlife Service, Environmental Contaminants Program, Lacey, Washington 98503, United States*
Jay W. Davis – *United States Fish and Wildlife Service, Environmental Contaminants Program, Lacey, Washington 98503, United States*
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;* orcid.org/0000-0002-7968-4198
Nathaniel L. Scholz – *Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Seattle, Washington 98112, United States*

Complete contact information is available at:
https://pubs.acs.org/10.1021/acs.est.1c03569

Notes

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