Solvent Extraction & Aqueous Leaching of Tire Tread Particles: Understanding Tire Compositions by Quantifying PPD antioxidants and Transformation Products Using Non-Targeted Analysis

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Introduction

For over 20 years in the Puget Sound region, researchers have documented urban runoff mortality syndrome (URMS), a phenomenon in which urban stormwater exposure causes widespread acute mortality in 100s-1000s of adult coho salmon (*Oncorhynchus kisutch*) annually (Scholz et al., 2011). Urban stormwater has also been shown to be lethally toxic to juvenile coho salmon (Chow et al., 2019). Coho salmon have tremendous cultural, ecological, and economic value in Washington and they also are a widely accepted sentinel/indicator species for habitat quality in the western US. In addition to substantial value in recreational fisheries, which may exceed commercial fisheries value by over 10-fold, Washington State commercial fisheries averaged $4.3M/year for coho from 2000-2014 ($30.2M/year across all the US) (Fisheries, 2021).

URMS is caused by polluted urban stormwater derived from roadway runoff (Feist et al., 2017; McIntyre et al., 2018), and after initial studies documenting correlations with traffic and roads, it was more specifically been linked to chemicals derived from tire rubber (McIntyre et al., 2021; Peter et al., 2018). Recently, the primary causal toxicant for URMS was identified as 6PPD-quinone (6PPDQ), a previously unknown ozonation transformation product of the commonly used (likely 100% of global tires use 6PPD as the primary tire anti-degradant) tire rubber anti-ozonant 6PPD (Tian et al., 2021b). 6PPDQ is acutely toxic to juvenile coho salmon at concentrations near or below 100 ng/L, with sensitive individuals perishing at concentrations as low as 20 ng/L. Absent current regulations, such concentrations provide quantitative goals for treatment and water quality management efforts. Driven by its widespread use in tire rubbers and ubiquitous presence in roadway runoff (at concentrations up to 1000s ng/L for busy roads), we expect this toxic chemical to occur in all receiving waters downstream of busy roadways (Klöckner et al., 2021; Tian et al., 2021b, 2021a), likely at lethal concentrations during some storm events.

Notably, 6PPDQ is a transformation product; it is generated from the reaction of an antioxidant ‘parent’ chemical 6PPD that is added to all vehicle tires. Both 6PPD and 6PPDQ are high risk aquatic toxicants (OSPAR Commission, 2006c; Tian et al., 2021b). While this new chemical and its suspected provenance have been identified and verified in the scientific literature, considerable research is needed to characterize its environmental transport and fate, its stability and leaching from tire wear particles under varied physical, chemical, and biological solution conditions and its fate in complex rubber-sediment-water systems such as in roadside environments, receiving waters and storm drains.

Currently, given that 6PPDQ was recently discovered, there exist many data gaps and uncertainties regarding its presence and management in the environment and in roadway systems. Notably for this study, a key data gap exists around understanding the compositions of rubber tires that act as the primary source of 6PPD, 6PPDQ, and many other tire rubber chemicals to aquatic systems. Chemical compositions of tire rubbers are protected as confidential and proprietary business information; such information is not routinely shared to researchers and the general public beyond a few basic chemical ingredients. Therefore, because the absolute and relative amounts of tire chemicals discharged by vehicular activity is uncertain, it is especially difficult to relate vehicle traffic compositions and activities to water pollution potential. Additionally, the relative impact of source control activities (e.g. altering tire chemical compositions by removing 6PPD from passenger car, light truck, or heavy truck tires) remains unknown because the specific compositions, of these tire classes, or individual tires from different manufacturers, remain unknown. This research effort was intended to further our understanding of tire wear chemical composition and leaching rates to better inform exposure risk to vulnerable species and pollutant management, as well as to inform ongoing green chemistry and source control efforts directed at replacing 6PPD in global tires.

Project Objectives

The aim of this project is to improve our understanding of tire wear particle composition and leaching through laboratory experiments and mass spectrometry-based analytical methods, with the specific objective to assess the effects of tire composition on relative mass loads and leaching dynamics of 6PPD-quinone and other tire-derived chemicals from tire wear particles that impair water quality and adversely affect salmonid health. This study will primarily utilize non-target high resolution mass spectrometry (LC-HRMS) for qualitative detection and compositional analysis of 6PPD-quinone and a broader suite of tire-derived organic contaminants. These methods will be applied to assess contaminant occurrence in tire wear particles generated from tires of different types, and to evaluate relative tire-derived contaminant leaching dynamics under different environmentally relevant solution conditions. For select samples, HRMS analysis will be used to complement and extend results from LC/MS/MS analysis.

Specific goals of the project were to:

- Characterize the occurrence and relative abundance of 6PPD and other emerging tire-derived organic contaminants across TWPs derived from different type types and classes,

- Survey different tire types (passenger car, light truck, heavy truck; 3-5 brands per type; including examples of both new and used tires) to evaluate the relative concentrations and relative abundance of 6PPD-quinone and other tire-derived contaminants,

- Characterize the leaching dynamics into water of 6PPD-quinone and other tire-derived organic contaminants as a function of time and environmental variables (e.g., pH, ionic strength).

Materials and Methods

<u>Chemicals and Reagents.</u> Analytical standards of 6PPDQ (10 mg, 98.8% purity, solid), 6PPDQ-*d5* (solution in acetonitrile, 100 mg/L) and 6PPDQ-C^{13} (solution in acetonitrile, 100 mg/L), were purchased from HPC (Atlanta, GA). Other analytical standards for HRMS suspect

screening were listed in **Table 1**. Water (LCMS grade), Methanol (MeOH, LCMS grade), ethanol (absolute, 200 proof), formic acid (HPLC grade) and monopotassium phosphate, monohydrate and dipotassium phosphate, and sodium phosphate dibasic heptahydrate were obtained from Fisher Scientific (Fair Lawn, NJ, USA). A Milli-Q EQ 7000 ultrapure water purification system (Burlington, MA, USA) was used to provide 18 MΩ deionized water when needed.

Table 1. Summary of analytical standards used for HRMS targeted analysis and suspect screening.

Analyte	**Abbreviation**	**CAS Number**	**Formula**	**Source**
***p*-phenylenediamine (PPD) antioxidants**				
N-(1,3-dimethylbutyl)-*N'*-phenyl-*p*-phenylenediamine	6PPD	793-24-8	$C_{18}H_{24}N_2$	Usolf Chemicals
N-isopropyl-*N'*-phenyl-1,4-phenylenediamine	IPPD	101-72-4	$C_{15}H_{18}N_2$	Richest Group Ltd
N-(1,4-dimethylpentyl)-*N'*-phenyl-*p*-phenylendiamine	7PPD	3801-01-4	$C_{19}H_{26}N_2$	Richest Group Ltd
N,*N'*-diphenyl-*p*-phenylenediamine	DPPD	74-31-7	$C_{18}H_{16}N_2$	Richest Group Ltd
N,*N'*-ditolyl-*p*-phenylenediamine	DTPD	68953-84-4	$C_{20}H_{20}N_2$	Richest Group Ltd
N,*N'*-di-2-naphthyl-*p*-phenylenediamine	DNP	93-46-9	$C_{26}H_{20}N_2$	Richest Group Ltd
PPD transformation products				
4-hydroxydiphenylamine	4-HDPA	-	$C_{12}H_{11}NO$	Fisher Scientific
4-aminodiphenylamine	4-ADPA	-	$C_{12}H_{12}N_2$	Sigma-Aldrich
4-nitrodiphenylamine	4-NDPA	836-30-6	$C_{12}H_{10}N_2O_2$	Sigma-Aldrich
4-nitrosodiphenylamine	4s DPA	156-10-5	$C_{12}H_{10}N_2O$	ChemService Inc
1,3-dimethylbutylamine	1,3-DMBA	108-09-8	$C_6H_{15}N$	Sigma-Aldrich
Vehicle-related chemicals				
1,3-diphenylguanidine	DPG	102-06-7	$C_{13}H_{13}N_3$	Sigma-Aldrich
hexa-(methoxymethyl)melamine	HMMM	3089-11-0	$C_{15}H_{30}N_6O_6$	Combi-Blocks ltd
N-cyclohexyl-1,3-benzothiazole-2-amine	NCBA	28291-75-0	$C_{13}H_{16}N_2S$	Enamine
1,3-dicyclohexylurea	DCU	2387-23-7	$C_{13}H_{24}N_2O$	Sigma-Aldrich
Corrosion inhibitors / UV stabilizer				
benzotriazole	1-H-BTR	95-14-7	$C_6H_5N_3$	Sigma-Aldrich
5-methyl-1-H-benzotriazole	5-Me-1-H-BTR	136-85-6	$C_7H_7N_3$	Sigma-Aldrich
BTZ & UV-234	UV-234	70321-86-7	$C_{30}H_{29}N_3O$	Aquatic Contaminants Research Division (Canada)
BTZ & UV-326	UV-326	3896-11-5	$C_{17}H_{18}ClN_3O$	Aquatic Contaminants Research Division (Canada)

2-amino-benzothiazole	2-NH2-BTH	136- 95-8	$C_7H_6N_2S$	Sigma-Aldrich
2-hydroxy-benzothiazole	2-OH-BTH	934-34-9	C_7H_5NOS	Sigma-Aldrich
2-(4-morpholinyl)benzothiazole	2-Mo-BTH	4225-26-7	$C_{11}H_{12}N_2OS$	Sigma-Aldrich

Tire Grinding. The preparation of TWP (focused on new and used passenger car and light truck tires) for leaching studies followed protocols described elsewhere [1]. Tire tread wear particles (TWP) were made by physical abrasion of the treads of selected tires (brand, vehicle type, and use history in **Table 2**) using an angle grinder with a steel carbide disk at 11,000 rpm with a 4.6” Parkes tire grinding disc (Medium MCM 90, 7/8” arbor hole) and collected in a high-efficiency filter bag with a ShopVac. The TWP were stored capped in pre-weighed, pre-cleaned, food-grade buckets under dark at room temperature (~20 ℃) until use.

Table 2. Make, model, and other identifiers of passenger car, light truck, and heavy truck tires evaluated in the study. *Indicates the tire was included in the 9-tire mixture.[1-6] [a]Indicates the tire was used in aqueous leaching experiments with varied conditions. Abbreviations: P = passenger car, LT = light truck, S = heavy-truck, or “semi” tires.

ID	Make	Model	Season/Type	New/ Used/ Retread	Tire Code (P, LT) or DOT # (S)
P1*	Firestone	Winterforce	Winter	New	175/65R14 82S
P2*	Goodyear	Assurance Fuelmax	A/S	New	185/65R15
P3*	Cooper	Zeon RS3G1	A/S	New	215/45R17
P4*[a]	Toyo	Eclipse	A/S	Used	P205/60R15 90T
P5*	Mastercraft	MC-440	A/S	New	P205/60R16
P6*	Michelin	Premier AS	A/S	Used	205/55R16 91H
P7*	Epic	Tour AS	A/S	Used	195/70R14 91H
P8*	BF Goodrich	Premier Touring	A/S	Used	P185/65R15 86T
LT1*	Goodyear	Wrangler HT	A/S	Used	LT235/85R16
LT2[a]	Hankook	Dynapro HT	A/S	New	LT245/75R16
LT3	Hankook	Dynapro HT	A/S	Used	LT245/75R16
LT4	Goodyear	Wrangler HT	A/S	Used	LT265/70R16
S1	Westlake	CR918	Trailer	Used, Retread	8D2J 4314
S2	Toyo	M154	All-Position	Used, Retread	N33t MVV1113
S3	Roadmaster	RM852	Long Haul Drive	Used, Retread	CR37 LWF
S4	Bridgestone	R250 ED	All-Position	Used	Y7 AB 3K6 5020
S5	Michelin	XZE	All-Position	Used	66H6 AHN 458
S6	Doublecoin	FT115	Trailer	New (likely)	02J 3K GJY1 1922
S7	Continental	HDL2	Long-Haul Drive	New	1A3 054GUH 3422
S8	Michelin	XZE2	All-Position	New	HA EJ 453X 1022
S9[a]	Doublecoin	RR150	All-Position	New	02J 3T GR1 0722

TWP Aqueous Leaching. To generate the TWP leachate stock, TWP was immersed in 500 mL amber glass jars (capped) and contacted with LC-MS grade water (24 h) from on the shaker table under room temperature. Tire leachates were generated in triplicate for each TWP. The optimal TWP/water ratio was determined from preliminary trail studies to maximize the extractable chemical masses while ensuring the leachates were not over-saturated. TWP leachate stock was generated at a 300 mg TWP/L water concentration (150 mg TWP/ 500 mL pH7 buffer solution in the jar), roughly matching TWP chemical concentrations in runoff from busy, multilane highway settings. The TWP leachate was filtered to remove TWP particles after the leaching process with glass fiber filters (pore size: 0.6 micron; 47 mm, Advantec, Japan). After being collected, tire leachate was stored in 1 L amber glass jars at 4 °C until extraction, typically within 12 hrs. A 200 mL leachate aliquot was spiked with 5 ng 6PPD-*d5* (50 µL of 100 ng/mL methanolic stock; yielding 25 ng 6PPDQ-d_5 / L water) and extracted using preconditioned (10 mL methanol, 25 mL water) SPE cartridges (Oasis HLB) at 5-10 mL/min. SPE cartridges were then rinsed with 10 mL DI water, air-dried (30 min), then eluted with methanol (2 × 5 mL). The methanolic eluents were evaporated to 1 mL under nitrogen gas and stored at -20 °C until QTOF analysis.

Complementary leaching experiments for quantitative LC/MS/MS analysis (largely described elsewhere) were conducted with representative TWPs (P4, LT2, S9) from different tire types. The selected TWPs were leached under varied pH (4, 7, 9; 10 mM phosphate buffer), temperature (4, 20, 40 ºC; achieved with temperature-controlled walk-in chambers, pH-adjusted buffers stored in chamber overnight prior to leaching), ionic strength (0.01, 0.1, 1% NaCl), and dissolved organic matter (DOC; 0.1, 1, 10 mg C/L) to evaluate the effects of conditions that impact leaching of 6PPDQ and other TWP-derived chemicals under environmental conditions. Temperature was held at 20 ºC for studies that varied pH, ionic strength, and DOC compositions; pH was adjusted to 7 with 10 mM phosphate buffer for studies with varied temperature, ionic strength, and DOC compositions.

<u>TWP Solvent Extraction.</u> TWP were also extracted with organic solvent (MeOH) in triplicate. The optimal TWP/MeOH ratio was determined from preliminary trail studies to maximize the extractable chemical masses while ensuring the extracts were not over-saturated. For each TWP, 50 mg sample was added into a 10 mL glass centrifuge tube and spike with 5 ng 6PPDQ-*d5* (50 µL of 100 ng/mL methanolic stock solution; yielding 0.1 µg 6PPDQ-*d5* / g TWP). The MeOH was allowed to evaporate off overnight. TWP was then extracted on the following day by adding 5 mL MeOH (equiv. to 10 mg TWP/mL MeOH), the centrifuge tube was then vortexed for 1 min, shaken for 10 min and sonicated for 20 min. After 20 min centrifugation, the supernatant was transferred to a new glass centrifuge tube with glass pipette. The TWP within the original tube was extracted again with the same procedure. Two supernatants were combined then (10 mL total) and concentrated under dry N_2 gas to 1 mL (with post volume adjustments accomplished by adding MeOH, if needed). The samples were stored overnight under -20 ℃. The samples were then filtered (0.22 µm PTFE syringe filter) and diluted (10-fold) in the following day and stored under -20 ℃ until instrumental analysis.

<u>LC-QTOF analysis.</u> All samples were analyzed using an Agilent 1290 Infinity II ultrahigh performance liquid chromatograph (UHPLC) coupled to an Agilent 6546 quadrupole time-of-flight high-resolution mass spectrometer (QTOF-HRMS; Santa Clara, CA, USA). A reverse-phase C18 column (Agilent ZORBAX Eclipse Plus 2.1×100 mm, 1.8 µm) with a C18 guard column

(2.1×5 mm, 1.8 µm) was used for the UHPLC separation at 45 ºC with 5 µL injection volume (infused with 1 µL QTOF ISTD during injection for QA/QC purpose). Separation employed a gradient elution with mobile phases of 0.1% formic acid in each of deionized water (A) and methanol (B) as follows: 5% B at 0-1 min, 50% B at 4 min, 100% B at 17-20 min, 5% B at 20.1 min; stop time 22.5 min; post-time 2 min. The flow rate was 0.4 mL/min. Full scan data were acquired under 10 GHz Extended Dynamic Range mode at a range of 100-1700 m/z.

HRMS data reduction. After data acquisition, raw data files (Agilent .d format) were converted to .abf format (Reifycs Abf Converter); MS-DIAL (version 3.46) [11] was used for primary data processing (i.e., non-target feature extraction and alignment). Parameter settings were as follows: *m/z* tolerance for feature extraction 0.005; minimum peak height 5000; *m/z* slice 0.01 Da; alignment *m/z* tolerance 0.015 Da; alignment retention time tolerance 0.1 minutes. Initial data reduction used Python programming language (version 3.9.12) with Pandas (version 1.5.1) to isolate features with maximum peak area (across all samples) > 100000, *m/z* between 100 and 900 Da, and retention time between 2-18 min; and present at peak area 10-fold greater than peak area in any of the solvent blanks or the ISTD samples. Features satisfying all these criteria (n = 24957) were retained.

Complementary LC-MS/MS Analysis for 6PPDQ quantification. LC-MS/MS analysis to quantify 6PPDQ followed established methods.[1] Chromatography used a reverse phase C18 column (Agilent Poroshell HPH-C18, 2.1 × 100 mm, 2.7 µm particle size) with a C18 Security Guard Cartridge (4 × 2.0 mm ID) at 20 °C, injection volume 10 µL, and binary gradient of DI water (A) and methanol (B) both with 0.1% formic acid: 10% B 0-1 min, 60% B at 1 min, 100% B at 10-12 min (0.2 mL/min flow rate), 100% B at 13-16 min (0.5 mL/min flow rate), 10% B at 17-24 min (0.2 mL/min). Detection used electrospray ionization (ESI+) and multi reaction monitoring (MRM) mode, with a capillary voltage of 3500 V. Qualitative and quantitative ion transitions are provided in Tian et al.[1] The calibration curve range (1/x weighting) was 0.025 – 100 ng/mL, with at least five points, calculated accuracy 70-130%, and determination coefficient (R^2) >0.99 (calibration curve details are provided in **Table 3**).

Table 3. Experiment summary, including extraction date, QA/QC sample details, and (re)analysis date. Method blanks (MB) and matrix spike/recovery (SR) experiments followed aqueous leaching (AL) and/or solid phase extraction (SPE) protocols. N/A indicates a given QA/QC sample was not prepared with the experimental batch.

Preparation Batch #	**Experimental Conditions (TWP used)**	**Extraction date**	**QA/QC batch* #**	**Method blanks concentration [µg 6PPDQ /g TWP]**	**LCS [% recovery]****	**Matrix Spike/Recovery [% recovery]*****	**Analysis (& re-analysis) date(s)**
1	Aqueous leaching, 20C (S1, S2, S3, S4. S5. S6. S7. S8. S9, 9-tire mix, CMTT)	2/21/23	7	AL/SPE-MB, n=3 < MQL	n=3 [103 ± 2]	SPE-SR (9-tire mix), n=3, [225 ± 143]	2/27/23
2	Solvent extraction (S1, S2, S3, S4. S5. S6. S7. S8. S9, 9-tire mix, CMTT)	2/21/23	16	Solvent Ext-MB, n=3 <MQL	N/A	Solvent Ext SR (9-tire mix),n=3, [98 ± 36]	2/27/23
3	Solvent extraction (LT2, LT3)	5/4/23	16	Solvent Ext-MB, n=3 <MQL	N/A	Solvent Ext SR (9-tire mix), n=3, [98 ± 36]	5/5/23
4	Solvent extraction (P1, P2, P3, P4, P5. P6, P7, P8, LT1)	6/27/23	16	Solvent Ext-MB, n=3 <MQL	N/A	Solvent Ext SR (9-tire mix), n=3, [98 ± 36]	8/9/23
5	Aqueous leaching, pH5 (P4, LT2, S9)	7/14/23	5	AL/SPE-MB, n=3 [0.02 ± 0.02]	n=3 [105 ± 9]	SPE-SR (9-tire mix), n=3, [66 ± 61]	9/28/23 (& 10/4/23)
6	Aqueous leaching, pH9 (P4, LT2, S9)	8/11/23	6	AL/SPE-MB, n=3 < MQL	n=3 [96 ± 4]	SPE-SR (9-tire mix), n=3, [119 ± 23]	9/28/23 (& 10/4/23)
7	Aqueous leaching, pH7 (P4, LT2, S9)	9/8/23	7	AL/SPE-MB, n=3 < MQL	n=3 [103 ± 2]	SPE-SR (9-tire mix), n=3, [225 ± 143]	9/28/23 (& 10/4/23)

8	Aqueous leaching, 0.01% NaCl (P4, LT2, S9)	9/18/23	8	AL/SPE-MB, n=3 < MQL	n=3 [95 ± 2]	SPE-SR (9-tire mix), n=3, [66 ± 61]	10/5/23
9	Aqueous leaching, 0.1% NaCl (P4, LT2, S9)	9/20/23	9	AL/SPE-MB, n=3 < MQL	n=3 [79 ± 6]	SPE-SR (9-tire mix), n=3, [79 ± 46]	10/5/23
10	Aqueous leaching, 1.0% NaCl (P4, LT2, S9)	9/27/23	10	AL/SPE-MB, n=3 < MQL	n=3 [84 ± 4]	SPE-SR (9-tire mix), n=3, [26 ± 69]	10/5/23
11	Aqueous leaching, 4C (P4, LT2, S9)	10/13/23	11	AL/SPE-MB, n=3 < MQL	n=3 [300 ± 7]	SPE-SR (9-tire mix), n=3, [40 ± 120]	10/24/23
12	Aqueous leaching, 0.1 mg C/L (P4, LT2, S9)	11/17/23	12	AL/SPE-MB, n=3 [0.02 ± 0.005]	n=3 [65 ± 5]	SPE-SR (9-tire mix), n=3, [230 ± 240]	11/27/23
13	Aqueous leaching, 10 mg C/L (P4, LT2, S9)	11/21/23	13	AL/SPE-MB, n=3 < MQL	n=3 [72 ± 7]	SPE-SR (9-tire mix), n=3, [32 ± 80]	12/7/23
14	Aqueous leaching, 40C (P4, LT2, S9)	12/7/23	14	AL/SPE-MB, n=3 < MQL	n=3 [75 ± 3]	SPE-SR (9-tire mix), n=3, [16 ± 73]	12/12/23
16	Aqueous leaching, 1 mg C/L (P4, LT2, S9)	12/18/23	15	AL/SPE-MB, n=3 < MQL	n=3 [86 ± 4.8]	SPE-SR (9-tire mix), n=3, [79 ± 14]	12/20/23

* For samples prepared prior to the QAPP draft, QA/QCs were not conducted due to the time limitations. Therefore, samples from later batches prepared following identical protocols were used as representative QA/QCs.

** Laboratory Control Samples (LCS) were DI water spiked with 6PPDQ (25 ng, 0.05 ng/mL), following SPE protocols.

*** For AL/SPE-SR experiments, 6PPDQ was spiked onto TWP prior to aqueous leaching (25 ng spike; 0.17 µg/g 6PPDQ/TWP); for SPE-SR experiments, 6PPDQ was spiked into aqueous TWP leachate prior to SPE (25 ng/200 mL; equiv. to 0.42 µg/g 6PPDQ/TWP).

Quality Assurance / Quality Control (QA/QC). For quality assurance and quality control (QA/QC), we monitored detector performance by checking mass accuracy of the QA/QC samples that injected repeatedly during the analysis process. For HRMS instrument analysis, all samples were analyzed within the same injection batch, the sample preparation information is summarized in **Table 3**. The detector performance was validated by checking mass accuracy and retuning if mass error exceeded 2 ppm. Mass calibration is also conducted before each analytical batch with the real-time mass accuracy correction by continuous infusion of purine and HP-921 calibrants. No column carryover was detected in solvent (MeOH) blanks (analyzed every 8−12 samples). A mixture of external reference standards (**Table 4**) was also analyzed every 8−12 samples. The instrument was retuned, and samples reanalyzed if mass accuracy was >5 ppm or area counts were >30% of initial within-batch sensitivity for QA/QC samples. Failed injection samples (n=2; evaluated by no detection of ISTD chemicals) were excluded from the sample analysis. Relative standard deviation of area counts in the reference standard were <30% across all analytical batches (>9 month). 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 (Du et al., 2017).

Table 4. ISTD Chemicals for instrument performance evaluation.

Compound Name	Retention Time [min]	ESI	CAS #	Chemical Formula	Vendor	Injection Concentration [ng/mL]
2,4-Di-tert-butylphenol-d19	12.7	-	96-76-4	$C_{14}H_3D_{19}O$	SCBT	100
5-methyl-1H-benzotriazole-d6	5.05	+/-	1246820-65-4	$C_7HD_6N_3$	Toronto Research Chemicals	100
Atrazine-d5	7.14	+/-	163165-75-1	$C_8H_9D_5ClN_5$	Sigma Aldrich	100
Bis(2-ethylhexyl)phthalate-d4	16.9	+	93951-87-2	$C_{24}H_{34}D_4O_4$	CDN isotopes	100
Caffeine-13C3	4.5	+	78072-66-9	$C_5[^{13}C]_3H_{10}N_4O_2$	Sigma Aldrich	50
Carbamazapine-d10	6.53	+	132183-78-9	$C_{15}H_2D_{10}N_2O$	Sigma Aldrich	25
Cotinine-d3	3.46	+	110952-70-0	$C_{10}H_9D_3N_2O$	Sigma Aldrich	100
DEET-d7	7.27	+/-	1219799-37-7	$C_{12}H_{10}D_7NO$	CDN isotopes	100
Docosahexaenoic acid-d5	15	-	25167-62-8	$C_{22}H_{27}D_5O_2$	Cayman	100

Ethylparaben-d4	5.82	+/-	1219795-53-5	$C_9H_6D_4O_3$	Toronto Research Chemicals	100
Ibuprofen-d3	9.08	-	121662-14-4	$C_{13}H_{15}D_3O_2$	Sigma Aldrich	100
Linolenic Acid-d4	13.3	+	463-40-1	$C_{18}H_{26}D_4O_2$	Cayman	100
Lithocholic Acid-d4	15	+	434-13-9	$C_{24}H_{36}D_4O_3$	Cayman	100
Metolachlor-d6	10	+	1219803-97-0	$C_{15}H_{16}D_6ClNO_2$	Toronto Research Chemicals	100
Nicotine-d3	1.99	+	69980-24-1	$C_{10}H_{11}D_3N_2$	Sigma-Aldrich	500
Prometon-d3	8.05	+	1219803-43-6	$C_{10}H_{16}D_3N_5O$	CDN isotopes	100
Propylparaben-d4	6.99	+/-	1219802-67-1	$C_{10}H_8D_4O_3$	Toronto Research Chemicals	500
Sulfadimethoxine-d6	4.99	+/-	73068-02-7	$C_{12}H_8D_6N_4O_4S$	Sigma Aldrich	100
Sulfamethoxazole-d4	4.27	+/-	1020719-86-1	$C_{10}H_7D_4N_3O_3S$	Toronto Research Chemicals	100
Theobromine-d6	3.1	-	117490-40-1	$C_7H_2D_6N_4O_2$	Sigma Aldrich	200
Vanillin-d3	4.23	+/-	74495-74-2	$C_8H_5D_3O_3$	Sigma Aldrich	100

For LC-MS/MS data, experimental conditions, extraction and analysis dates, and QA/QC samples are summarized in **Table 3**. In addition to the many replicate analyses, three types of QA/QC samples were prepared for these sample batches: method blanks (MB), laboratory control samples (LCS; only prepared with aqueous leaching experiments), and matrix spike/recovery (SR) samples. Method blanks and matrix spike/recovery experiments followed solvent extraction (SE), aqueous leaching (AL), and/or solid phase extraction (SPE) protocols. All experimental samples, QA/QC samples, and calibrants were spiked with $^{13}C_6$-6PPDQ immediately prior to analysis as an injection internal standard to support quality control evaluation.

For aqueous leaching experiments, method blanks that corresponded to SPE (SPE-MB) used 200 mL DI water spiked with 5 ng 6PPDQ-d_5 (50 µL of 100 ng/mL 6PPDQ-d_5; 25 ng 6PPDQ-d_5/L) immediately prior to SPE. Method blanks that corresponded to the entire experimental procedure (24 h aqueous leaching and subsequent SPE; AL/SPE-MB) used 500 mL DI processed identically to TWP leaching samples, without inclusion of TWP.

Matrix spike/recovery samples that corresponded to SPE (SPE-SR) were prepared by spiking 200 mL aliquots of aqueous leachate from P2 or the 9-Tire Mix with 25 ng 6PPDQ (25 µL of 1000 ng/mL of 6PPDQ; 125 ng 6PPDQ/L) and 5 ng 6PPDQ-d_5(50 µL of 100 ng/mL 6PPDQ-d_5; 25 ng 6PPDQ-d_5/L), immediately prior to SPE. Matrix spike/recovery samples that corresponded to the entire experimental procedure (spikes prior to 24 h aqueous leaching and subsequent SPE; AL/SPE-SR) were prepared by spiking 150 mg dry TWP (P4 or the 9-Tire Mix) with 25 ng 6PPDQ (25 µL of 1000 ng/mL of 6PPDQ; 0.17 µg 6PPDQ/ g TWP) and 5 ng 6PPDQ-d_5(50 µL of 100 ng/mL 6PPDQ-d_5; 0.03 µg 6PPDQ-d_5/ g TWP), then allowing methanol to evaporate off overnight prior to aqueous leaching.

Additionally, for most aqueous leaching studies, laboratory control samples (LCS) were prepared by spiking 200 mL of DI water with 25 ng 6PPDQ (25 µL of 1000 ng/mL 6PPDQ; 125 ng 6PPDQ/L) and 5 ng 6PPDQ-d_5 (50 µL of 100 ng/mL 6PPDQ-d_5; 25 ng 6PPDQ-d_5/L water), then processed through SPE.

For LC-MS/MS 6PPDQ quantification, the minimum detection limit (MDL, 0.5 ng/mL in vial) and minimum quantitation limit (MQL, 1.02 ng/mL in vial) were determined as the lowest concentrations (in the final extract) giving signal to noise (S/N) ratios of 3 and 10, respectively. Relating instrument response to quantification of 6PPDQ in TWP (as µg 6PPDQ/g TWP), the MDL and MQL correspond to 0.01 ug/g and 0.02 ug/g for solvent extraction, and 0.0083 ug/g and 0.017 ug/g for aqueous extraction. If 6PPDQ was detected at concentrations >MQL in solvent or method blanks, the highest detected concentration in a blank from a given batch was subtracted from the observed concentration in experimental samples. Detections in method blanks and method blanks for which concentration of 6PPDQ was <MQL are noted in **Table 3**.

If observed peak area responses were above the calibration curve range, a common issue for abundant analytes in tire rubbers that also have high peak area responses (as many aromatic amines do), the original sample extract was diluted further with clean methanol (10-fold for water leaching extracts, 50-fold for solvent extraction extracts) and re-analyzed. Data was used from the least dilute extract with a peak area response within the calibration curve range.

Results

Chemical leaching potential from different TWPs. The total chemical features that were detected, a metric of chemical complexity, using HRMS varied based on the type of extraction solvent (**Figure 1**). Total features detected ranged from 3988-7315 features for methanolic solvent extractions of TWPs and 663-2259 features for aqueous leachates. While it is clear that solvent extractions (using methanol) are more effective at extracting chemicals from TWP, as measured by both number of features and average feature peak area, substantial numbers of TWP-derived chemical features are clearly water-extractable under ambient conditions. On average, aqueous leachates contained much less (28 ± 5%) features compared to the solvent extracts, suggesting that the aqueous leaching process was only partially capable of mobilizing surficial tire additives or their transformation products from rubbers. Therefore, most of the mass of tire additive chemicals and transformation products would remain in the TWP and be subjected to future release upon subsequent leaching events. Note here that the current estimates do not consider further transformation processes of the chemicals, such as the ongoing formation of 6PPDQ from 6PPD as gaseous ozone contact occurs with remaining 6PPD antioxidant mass within the tire rubber.

Alternatively, it is also possible that desorption processes more strongly affect aqueous systems, where even efficient leaching and mass transport of TWP chemicals away from rubber phases was counterbalanced by effective uptake and sorption of TWP back onto rubber surface environments. Given the substantial numbers of leachate chemicals detected here, most remaining unidentified, we expect that a large variety of chemicals will be leached from TWP over its service life, with identities and abundances of most chemicals in these complex mixtures remaining unknown. These differences may be driven by factors such as the different distribution of tire types, or different leaching dynamics due to differences in particle size/surface area.

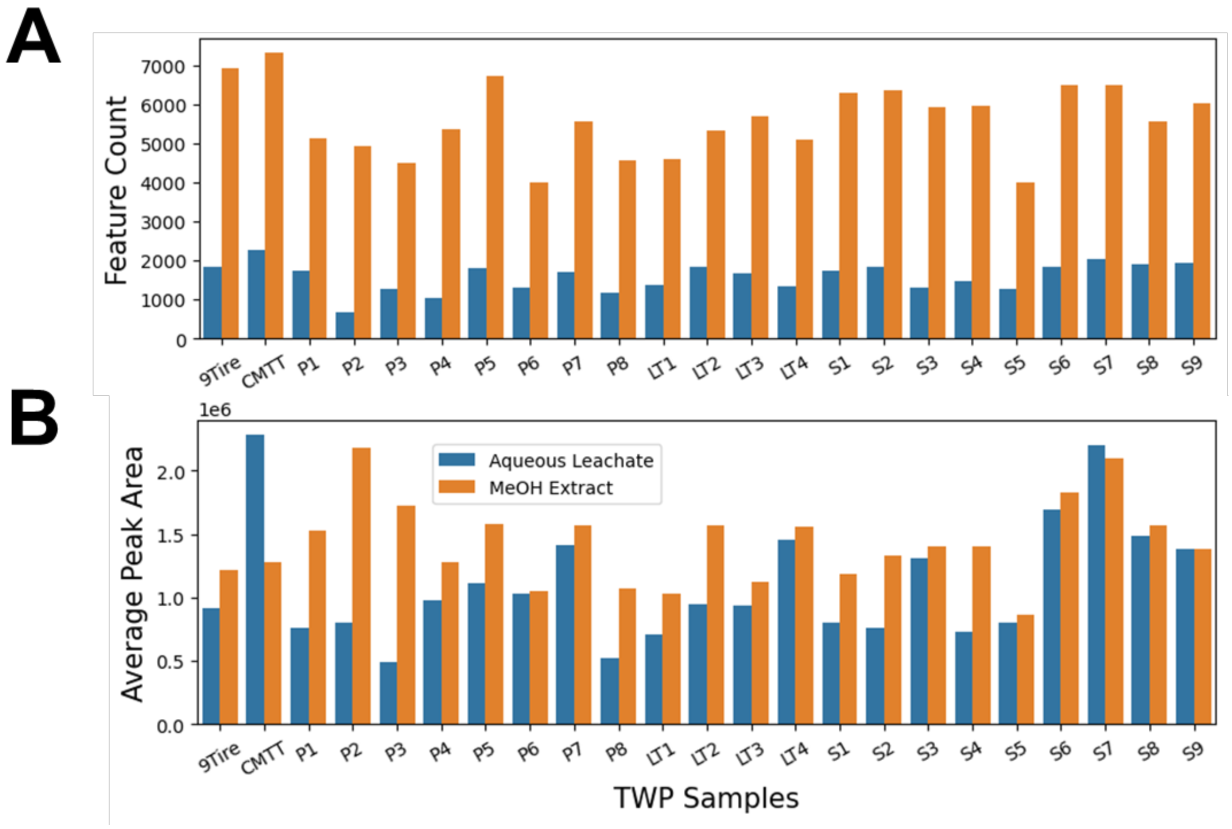

Figure 1. Summary of detected HRMS features from from aqueous leachates (300 mg/L TWP) and MeOH extracts (5g/L TWP) of 21 different TWPs, plus two composite TWP mixtures, using LC-QTOF-HRMNS analysis. (A) total numbers of features detected from different TWP samples and (B) average peak areas detected from different TWP samples. The feature counts were calculated from the average of experimental replicates. Average peak areas were calculated from the average of detected features of experimental replicates.

<u>Tire characteristics and chemical composition.</u> Understanding differences between types of tire and tire use characteristics was a primary goal of this study, so various types of HRMS data were collected and analyzed to try and resolve chemical compositions of various tire types. The total feature counts from different tire used conditions and types are summarized in **Figure 2**. For different tire used conditions, although the detected features in tire TWPs were slightly higher for

new tires than detections from used tires (total features detected: 1665 (new tire) vs 1434 (used tire) in aqueous leachates; 5683 (new tire) vs 5285 (used tire) in MeOH extracts), no significant differences (p-value > 0.05; t-test) were observed between total feature counts of TWPs from new and used tires for these numbers of analyzed samples. The average lower feature counts from the used tire TWPs indicated the possible transformation, consumption or leaching of tire additive chemicals over tire lifetimes, although it also is possible that matrix differences affect these data. During the tire service life, the tire rubber derived chemicals could be transformed into transformation products and released during storm events or directly deposited into the environment via roadway runoff (Councell et al., 2004; Johannessen et al., 2022, 2021, "Road Hazard," n.d.). Additionally, with respect to total feature numbers, as the difference between new and used types was not statistically significant here, the result also suggests that most HRMS-detectable chemicals used different tire types or conditions weren't depleted during the service period (i.e., there were still residual masses in the TWP). Therefore, after being deposited into the field, and because TWPs are designed for stability and are relatively resistant to environmental degradation processes, those residual TWP chemicals may consequently induce acute, subchronic or chronic environmental effects, or be subsequently converted into other transformation products with unknown environmental toxicity characteristics.

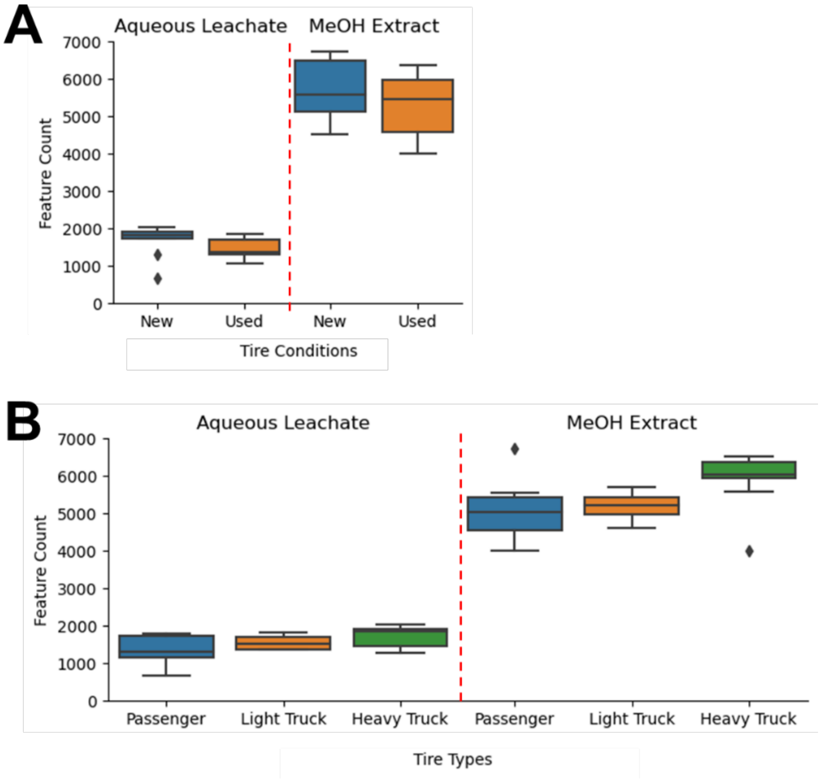

Figure 2. Feature count distributions of HRMS features detected from different TWPs from aqueous leachates (300 mg/L TWP) and MeOH extracts (5g/L TWP). (A) Feature counts comparing new versus used TWPs; and (B) feature counts of TWPs from different types of tires (passenger, light truck or heavy truck). The box and whisker plots indicated the quartiles of all the

datapoints, calculated from the average of the experimental triplicates from individual TWPs. The individual points outside the boxes are outliers.

When comparing different tire types (passenger, light truck and heavy truck tires), while heavy truck tire TWPs had the most detected features as compared to the TWPs from other two tire types (total features detected: 1337 (passenger tire) vs. 1551 (light truck tire) vs 1698 (heavy truck tire) in aqueous leachates; 5092 (passenger tire) vs. 5175 (light truck tire) vs 5904 (heavy truck tire) in aqueous leachates), the differences between different tire types were again not statistically significant (p-value > 0.05; one-way ANOVA test) for these numbers of analyzed samples. The result implies that the different types of tires analyzed here have relatively similar numbers of additives, such as rubber, carbon black, accelerator or antioxidant (Sheridan, 2010), as well as their related transformation products (e.g., 6PPDQ). Nevertheless, the result indicates that more tire additives were detected in heavy truck tire TWPs compared to the other tire types, potentially due to heavy duty weight and wear demands placed upon these tires.

HRMS features detected from new and used TWPs and across tire classes are summarized in **Figure 3**. The data are organized by both features common to all TWP samples and by all features detected. For different tire used conditions (**Figure 3A**), most HRMS features, including tire additives such as PPDs or transformation products such as 6PPDQ, were common across both different condition groups. Both used and new tire TWPs had some features that were exclusively detected within the particular group, likely arising from different tire rubber compositions and different transformation products formed during the tire service periods. On the other hand, for different tire types (**Figure 3B**), strongly overlapped HRMS features were also often observed between different groups, indicated the presence of commonly used tire rubber additives or shared transformation products and underscoring the further research need regarding tire rubber marker chemicals, either reflecting common ingredient compositions or unique chemical formulations for specific tires, manufacturers, or tire types. We note that most of the tire recipes and ingredient lists remain unknown, which poses substantial challenges for researchers to investigate tire rubber additives and their transformation products, due to issues around confidential and proprietary business restrictions. Inaccurate information about relative chemical compositions is a major barrier to progress toward safe tire compositions. The current HRMS data can partially evaluate the chemical composition of different TWPs and prioritize ubiquitous or unique tire rubber additives for future studies, although many of the specific chemicals used remain unidentified. The results here implied that major components of the tire rubber were often chemically similar (Sheridan, 2010) regardless of the used conditions and tire types, although substantial opportunity for uniqueness also was present within the tire rubber compositions from different samples due to the differences in tire recipes. Also, for both unique features and common features, the data indicated that there existed HRMS features that could be potentially used as tire rubber markers, markers of new versus used, or markers reflecting tire type. While these data remain somewhat poorly understood, and are not always internally consistent here, these detected compositions inherently enable opportunities for source tracking and field quantification of TWP compositions in the environment.

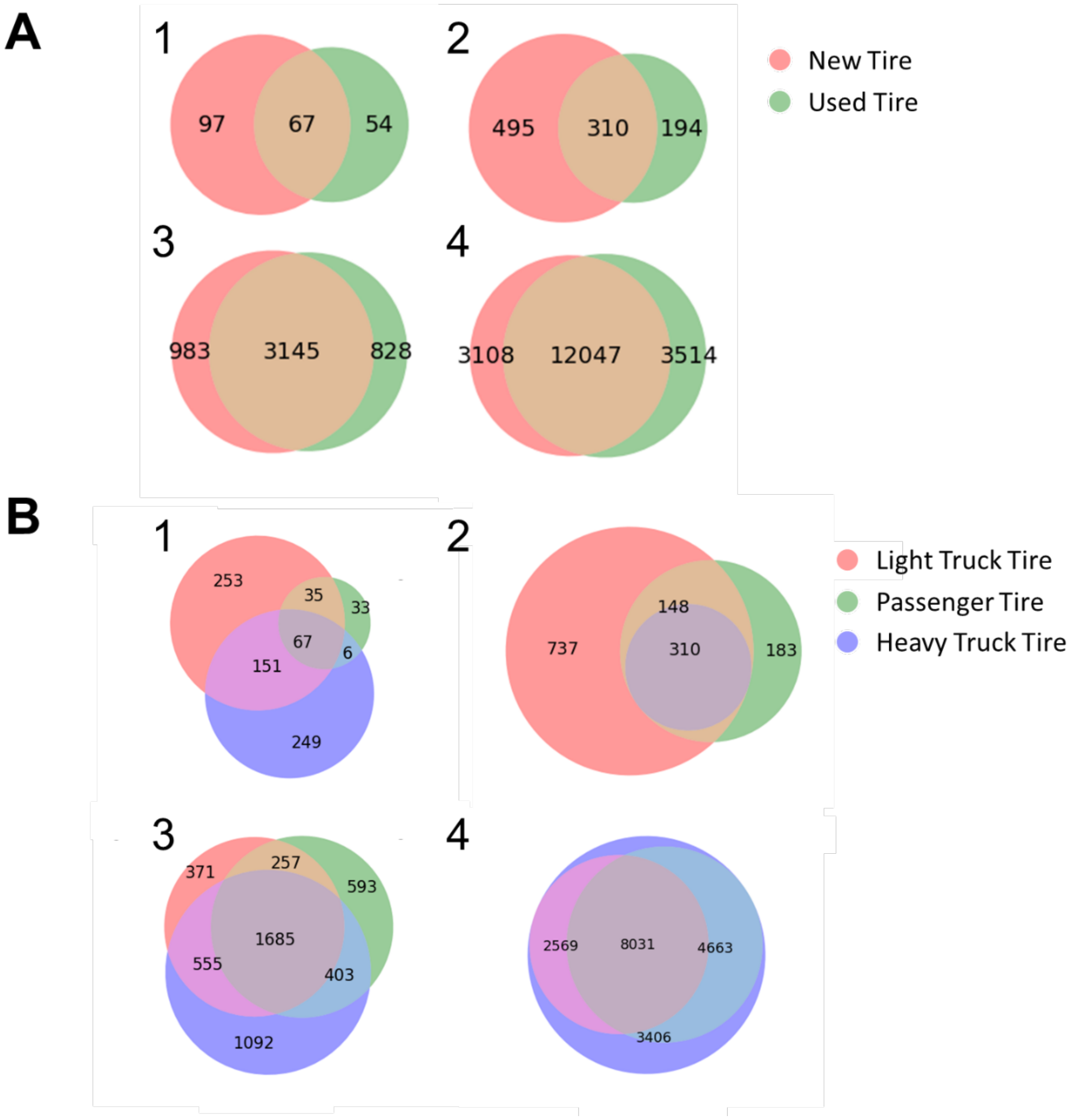

Figure 3. Venn diagram for total HRMS feature detections in (A) TWP samples with different tire used conditions and (B) TWP samples from different tire types. Subplots were representing different feature filtering criteria or TWP extraction method: (1) represents HRMS features detected in all TWPs within the same group from the aqueous leachate (300mg/L TWP) samples, (2) represents HRMS features detected in all TWPs within the same group from the MeOH extract (5g/L TWP; right), (3) represents HRMS features detected in any TWPs within the same group from the aqueous leachate (300mg/L TWP) samples and, (4) represents HRMS features detected in any TWPs within the same group from the MeOH extract (5g/L TWP; right).

Occurrence of PPDs. Global interest in PPD class antioxidants is high because 6PPDQ is far more acutely toxic than 6PPD and researchers globally have now documented many PPDs and PPDQs ubiquitously in environmental systems. Also, another PPD compound may potentially serve as a replacement for 6PPD as an antioxidant in tire rubbers. Given the widespread and deep interest in PPDs as a chemical class, understanding the sources and fate of reactive PPD parents and related transformation products in TWP materials was another key goal of this study. Currently, several PPD class compounds and related TPs have commercial standards available (**Table 5**), including 6PPD, 7PPD, DNP, DPPD, DTPD and IPPD, as well as some 6PPD transformation products, including 6PPDQ, 1,3-DMBA, 4-HDPA, 4-NDPA, 4-sDPA, 4-ADPA. We built targeted and suspect screening lists around these chemicals to conduct HRMS data analysis.

Table 5. Targeted screening list for PPD class compounds and 6PPD related TPs. The HRMS screening were conducted with commercial standards for the below compounds.

Compound	Formula	Exact Mass (Da)	Retention Time (min)	CAS
PPD Antioxidants				
6PPD	$C_{18}H_{24}N_2$	268.19	6.51	793-24-8
7PPD	$C_{19}H_{26}N_2$	282.21	7.31	3801-01-4
IPPD	$C_{15}H_{18}N_2$	226.15	4.89	101-72-4
DPPD	$C_{18}H_{16}N_2$	260.13	10.75	74-31-7
DTPD	$C_{20}H_{20}N_2$	288.16	12.72	68953-84-4
DNP	$C_{26}H_{20}N_2$	360.16	13.77	93-46-9
6PPD Transformation products				
6PPDQ	$C_{18}H_{22}N_2O_2$	298.17	10.90	2754428-18-5
1,3-DMBA	$C_6H_{15}N$	101.12	3.04	108-09-8
4-HDPA	$C_{12}H_{11}NO$	185.08	6.03	122-37-2
4-NDPA	$C_{12}H_{10}N_2O_2$	214.07	8.58	836-30-6
4-sDPA	$C_{12}H_{10}N_2O$	198.08	7.02	156-10-5
4-ADPA	$C_{12}H_{12}N_2$	184.10	4.08	101-54-2

We screened the above compounds within our LC-QTOF HRMS data and semi-quantified the relative concentrations of all those that had commercial standards based upon a 7-point calibration response. We especially note the analytical uncertainty for parent PPD antioxidants, these chemicals are inherently unstable, are designed to react, and are sensitive to the presence of multiple trace constituents in solution. We note that our analytical methods are not optimized for stabilization and quantitative accuracy for parent PPDs, and while we expect relative comparisons are reasonably accurate, the following results are semi-quantitative and potentially biased, especially in terms of absolute concentration. Given these instabilities, it is likely that all quantitative estimates are biased low, and actual extract/leachate concentrations are higher.

For all the PPDs measured (**Figure 4 and 5**), and mirroring results for total chemical features detected, detected water leachable mass of PPDs was much lower than the solvent extractable mass, likely due to the substantial hydrophobicity of the PPD family chemicals. 6PPD was ubiquitously detected (0.036 to 18 µg/g in aqueous leachates; 6.4 to 260 µg/g in MeOH extracts) in all the TWPs analyzed here, while both DPPD and DTPD were detected at high concentrations (0.14 to 7.9 µg/g for DPPD detection in MeOH extracts, 0.36 to 12.9 µg/g for DTPD detection in MeOH extracts; 53 to 160 µg/g for DPPD detection in MeOH extracts, 324 to 652 µg/g for DTPD detection in MeOH extracts) in a few TWPs (LT1, LT4, S3, S7, P2 and P5). Compared to 6PPD, DPPD and DTPD, 7PPD and IPPD have lower detection frequency and detected abundances. P5 was the only TWP where 7PPD was detected (0.15 µg/g in aqueous leachates; 47 µg/g in MeOH extracts). IPPD was detected in S6, S8 and S9 with lower detected abundances (0.11 to 0.5 µg/g for aqueous extracts; 3.0 to 13 µg/g for MeOH extracts). There was no DNP detection in any TWP samples, indicating that it was not a common tire rubber additive for the tires analyzed here, although it is reported to be used as rubber antioxidant ("Westco^{TM} DNPD Western Reserve Chemical Corporation," n.d.). We also note that the 9 tire and USTMA mixture contained all the PPDs (except DNP) analyzed here, likely because those two sample types are aggregated TWP mixtures. 6PPDQ concentrations detected in different TWPs ranged from 6.4 to 260 µg/g in solvent extracts and 0.036 to 18 µg/g in aqueous leachate. Among all the TWPs measured, LT1 and P8 had the lowest detected 6PPD concentrations, potentially because they were both used tires, and potentially even older used tires. Additional analysis to derive tire ages is ongoing. For other TWPs, 6PPD concentrations were more similar, ranging across 110 - 260 µg/g in solvent extract and 1.1-18 µg/g in water leachate, further underscoring the ubiquitous use of 6PPD across different tire rubber products.

Other PPDs were detected in TWPs, but typically at much lower concentrations and detection frequency relative to 6PPD. For example, 7PPD was mostly detected in the P5 solvent extract (47 µg/g) and was not quantifiable in any aqueous leachate samples (< 0.1 µg/g). The leaching of 7PPD into water was potentially limited due to its very low solubility (67 µg/L; https://americasinternational.com/wp-content/uploads/2020/03/SANTOFLEX-7PPD-SDS-EASTMAN.pdf), which is considerably lower than the 1-2 mg/L solubility of 6PPD (DTSC and CalEPA, 2022; OSPAR Commission, 2006c). DPPD was detected in multiple TWPs, including LT1, LT4, S3, S7, P2 and P5. The solvent extractable DPPD concentrations from the TWPs were relatively high, ranging from 53 - 160 µg/g, suggesting that DPPD is added into the TWPs intentionally, or is a major component of PPD technical mixtures, or was a major impurity of some type. However, when comparing water leachable DPPD concentrations to solvent extractable concentrations, the relative extracted mass (e.g., how much mass leached from the TWP compared to other TWPs under the same condition) results were variable between different tires. For example, the S7 water leachate contained the highest concentration (7.9 µg/g) of DPPD. This is potentially due to different TWP shape/surface area characteristics, which may have affected leaching rates. Alternatively, PPD parent compounds may be relatively more reactive in solution, reducing their analytical stability and subsequent detected peak areas. Additional analyses, for example, characterization of TP compositions and decay rates, would be needed to resolve these issues.

Similar to DPPD, DTPD was also detected in multiple TWPs. The solvent extractable DPPD from those TWPs were relatively high, approximately ranging from 8.3 to 650 µg/g, and also suggesting that DTPD broadly used as tire rubber additive. Similar to results for other PPDs, only a relatively small proportion of the solvent extractable DTPD mass could be leached by water, resulting in low detected concentrations (highest detection from S7: 13 μg/g) of DTPD in water leachates. Overall, despite significant mention of its use in older tire formulations, IPPD had the lowest detected concentration in the individual tires or TWP mixtures, with the highest detection in both solvent extract and water leachate from S9 (13 and 0.5 μg/g from the solvent and water extract, respectively).

Compared to 6PPD, which is widely used and detected in different tire samples, other PPDs were used in fewer products or at lower concentrations, or were minor constituents of unpurified mixed PPD technical mixtures. In the TWPs tested, DPPD and DTPD were always detected concurrently (LT1, LT4, S3, S7, P2, P5), indicating that those two PPDs might be applied as a mixture (in which DPPD mass are 20±4% of DTPD mass applied; calculated from the MeOH extract detections) (Hägg et al., 2023). The P5 sample apparently contained all PPDs analyzed here, indicating the potential complexity of the PPD chemical matrix within some individual tire compositions. IPPD was detected at the lowest relative concentration; it therefore might not be commonly used in these tire rubbers.

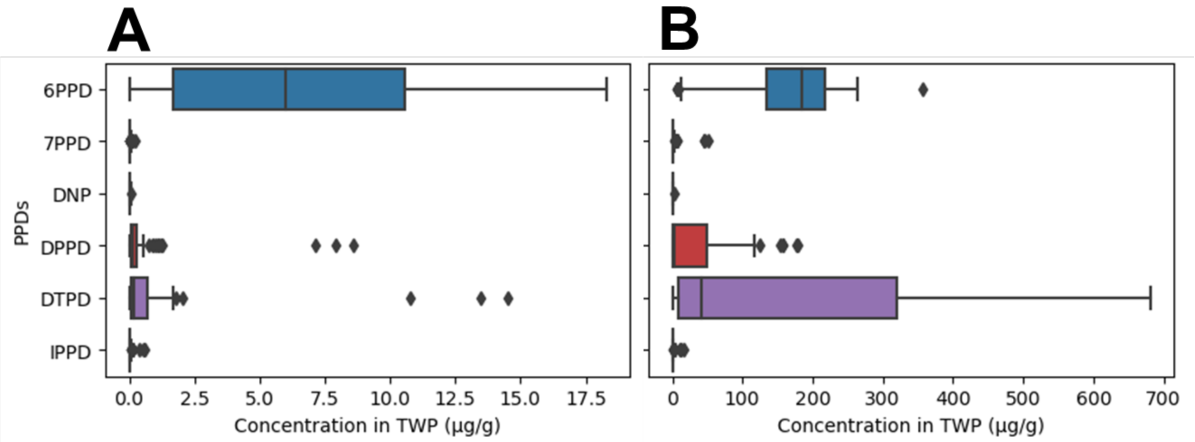

Figure 4. Distribution of semi-quantified PPD concentrations in different TWPs from LC-QTOF-HRMS analysis. (A) Semi-quantified PPD concentrations from TWP aqueous leachates (300mg/L TWP) and (B) Semi-quantified PPD concentrations from TWP MeOH extracts (5g/L TWP). The PPD concentrations were determined from 7-point calibration curve, and back calculated as TWP concentration (μg/g analyte/TWP). Note that DNP was not detected (detections below the baseline peak area with S/N < 3) in any samples. The box and whiskers indicated the quartiles of all the datapoints, with individual points calculated from the average of the experimental triplicates from individual TWPs. The individual points outside the boxes are outliers.

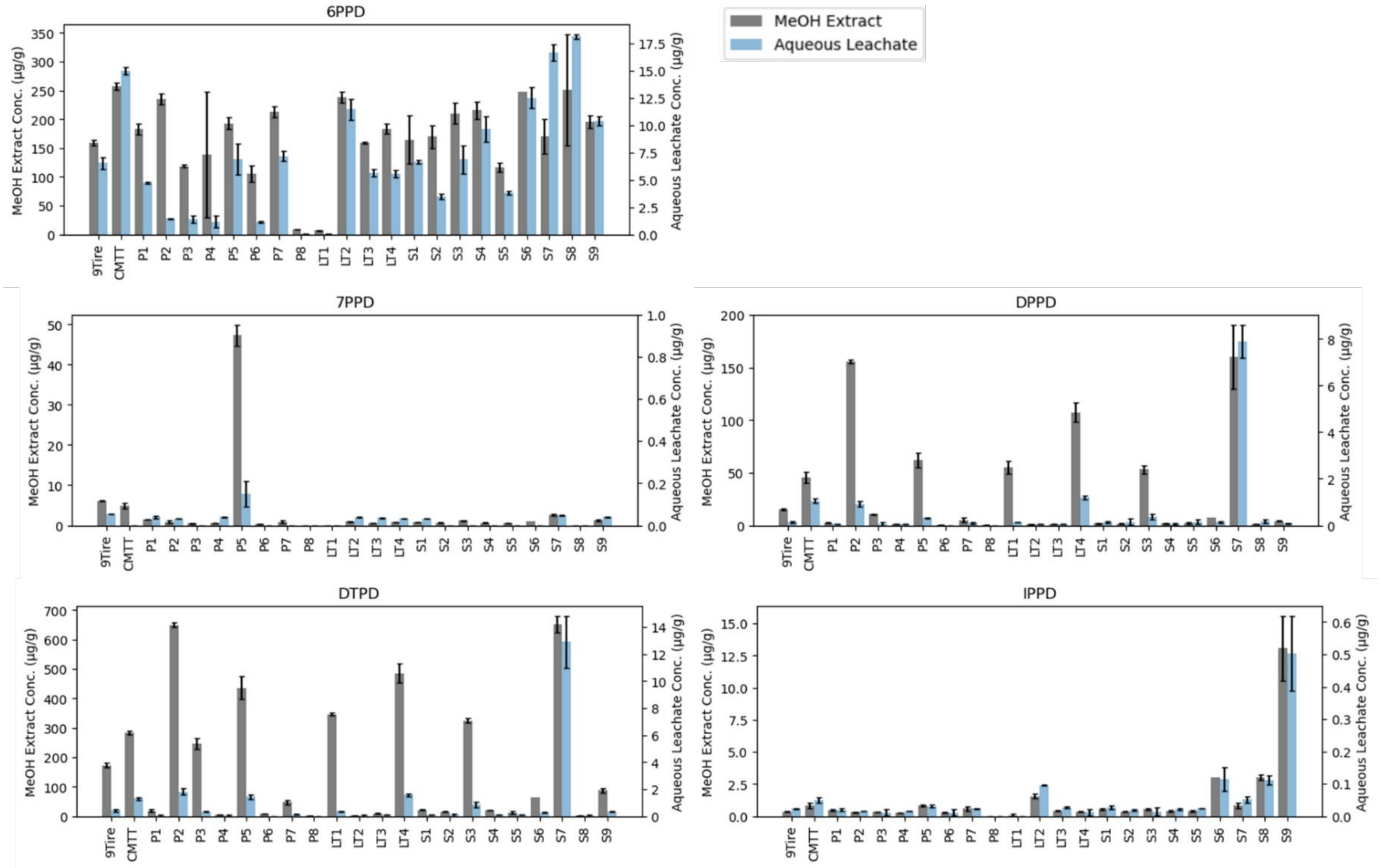

Figure 5. Semi-quantification results of PPDs from different TWP aqueous leachates (5g/L TWP) and MeOH extracts (300mg/L TWP) from LC-QTOF-HRMS analysis for the 21 individual tires and 2 TWP mixtures. The chemical concentrations were determined from 7-point calibration curve, and back calculated as TWP concentration (µg/g analyte/TWP). DNP was not detected (detected below baseline peak area and S/N < 3) in any samples. The error bars were calculated from the experimental triplicates.

Given the ubiquitous presence of 6PPD in all the TWPs, the 6PPD TPs were also present and semi-quantified in the different TWP samples (**Figures 6 and 7**). All the 6PPD TPs were detected in these TWPs, including LT1 and P8, which only had low 6PPD detection abundances. We also note that some of the detected TP compounds might be derived from multiple PPD parent compounds that share common functional groups. These parent-product relationships will merit further investigation in the future to resolve source attribution of specific product compounds and to understand PPD mass balances in complex mixtures. Given that both TWPs reflected used tires, it is reasonable that low detection of 6PPD was due to aging and the consumption of 6PPD over time, potentially indicating that these tires were older models or 6PPD depleted in some fashion. 6PPDQ, the environmental toxicant that induces acute toxicity to coho salmon and other salmonids, also was detected in all TWPs, ranging from 7.3 to 31 μg/g in solvent extracts and 0.035 to 1.3 μg/g in aqueous leachates. The water-leachable 6PPDQ consisted of less than 5% of the solvent extractable mass, indicating the majority of the 6PPDQ mass would be expected to remain in the TWPs after the leaching process and be available for subsequent mobilization. This residual mass could be released repeatedly into the environment during subsequential storm events, inducing some potential for chronic environmental effects. Additionally, given the widespread observations of 6PPD in different TWP surface samples, the residual 6PPD could be converted into 6PPDQ if exposed to the atmosphere, thus replenishing the 6PPDQ mass in TWPs and eventually resulting in additional 6PPDQ mass discharges of tire rubber product lifetimes, including for recycled and reused tire rubber products such as crumb rubbers. In fact, the relatively low 6PPDQ concentrations detected in these tire samples (as compared to 6PPD) may be indicative of the importance of ongoing formation processes for 6PPDQ, relative to the presence of a large mass fraction of previously formed 6PPDQ that is then continuously released over time.

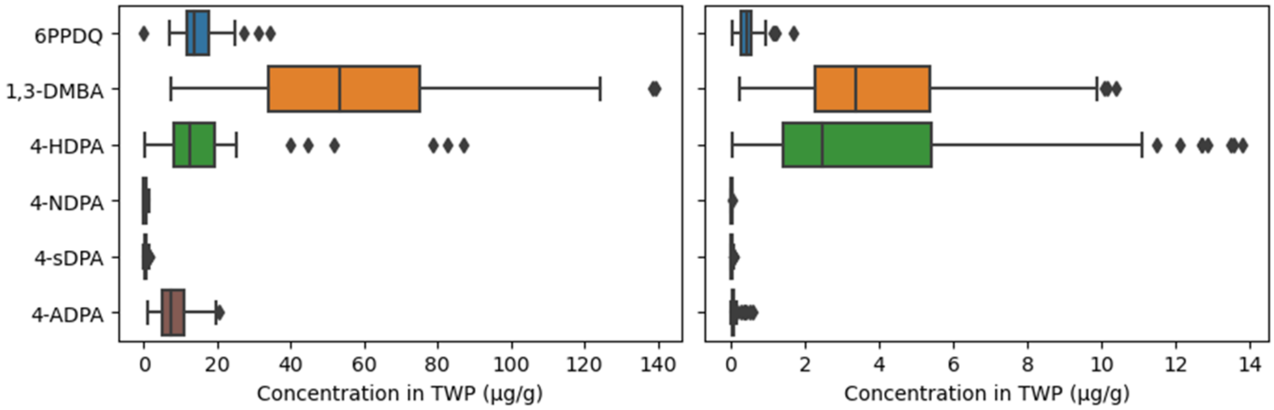

Figure 6. Distribution of semi-quantified 6PPD-derived TP concentrations in different TWPs from LC-QTOF-HRMNS analysis. (A) Semi-quantified 6PPD-derived TP concentrations from TWP aqueous leachates (300 mg/L TWP) and (B) Semi-quantified 6PPD-derived TP concentrations from TWP MeOH extracts (5 g/L TWP). The 6PPD-TP concentrations were determined from 7-point calibration curve, and back calculated as TWP concentration (μg/g analyte/TWP). Note that DNP was not detected (detections below the baseline peak area with S/N < 3) in any samples. The

box and whiskers indicated the quartiles of all the datapoints, with individual points calculated from the average of the experimental triplicates from individual TWPs. Any individual points outside the boxes are outliers.

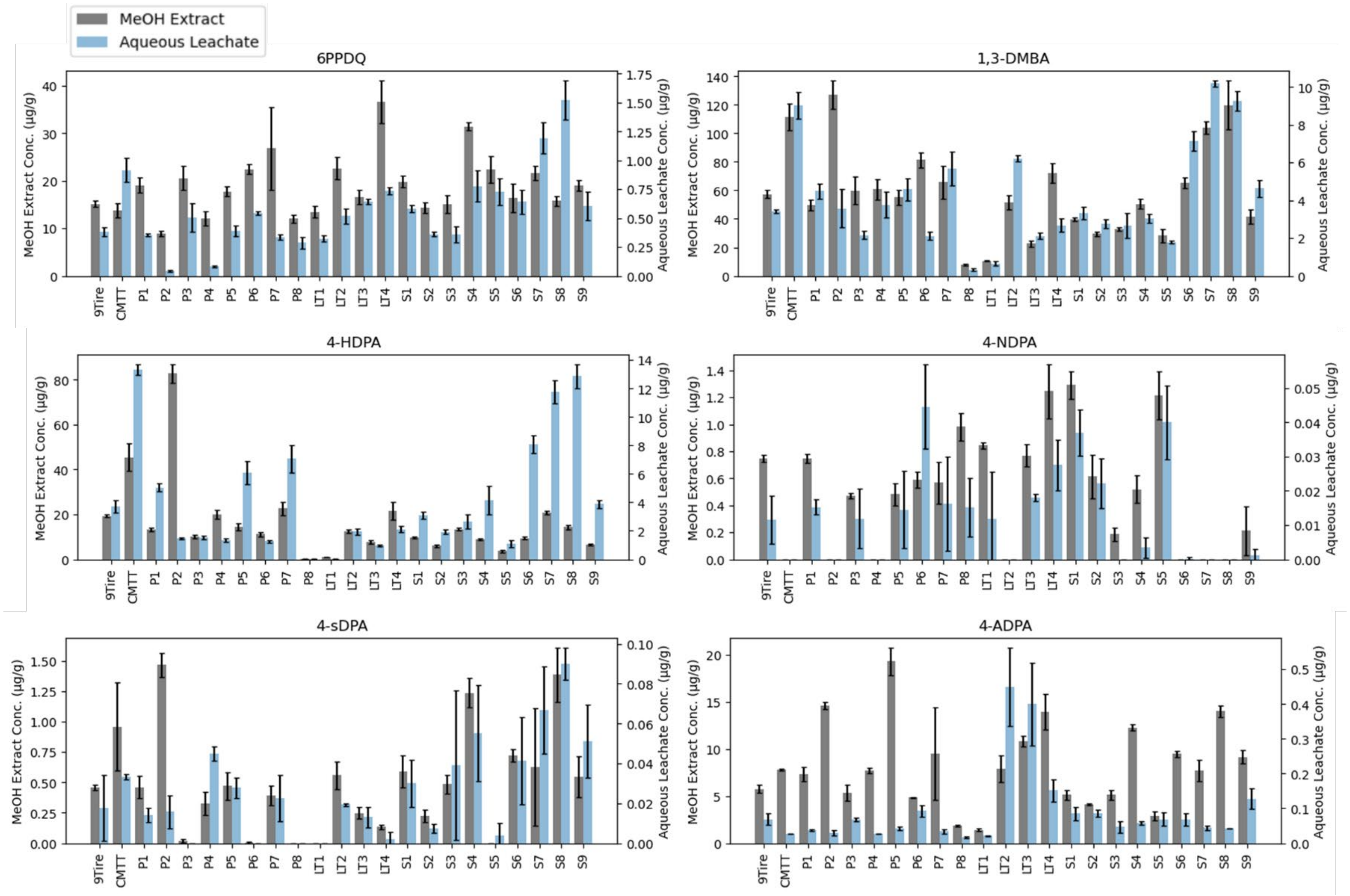

Figure 7. Semi-quantification of 6PPD-derived TPs from different TWP aqueous leachates (300mg/L TWP) and MeOH extracts (5g/L TWP) from LC-QTOF-HRMS analysis. Concentrations were determined by 7-point calibrations and calculated as TWP concentration (µg/g analyte/TWP). The error bars reflect experimental triplicates. Note the differing y-axis scales throughout.

Other TPs besides 6PPDQ were generated and detected across all TWPs. Both 1,3 DMBA and 4-HDPA were previously proposed as aqueous degradation products of 6PPD (Hu et al., 2023; Seiwert et al., 2022) and were also detected in all TWPs tested at a relatively high concentration, ranging from 7.9 to 130 µg/g for 1,3-DMBA (in methanol extract) and 0.32 to 10 µg/g in the aqueous leachate. 4-HDPA concentrations ranged from 0.37 to 83 µg/g in the solvent extract to 0.038 to 13 µg/g in the water leachate. Note that LT1 and P8 again had the lowest detected concentrations of both chemicals, well aligned with their low detection concentrations of 6PPD. 4-NDPA and 4-sDPA were proposed as transformation products from 6PPD ozonation (Hu et al., 2022; Zhao et al., 2023b) and were detected at generally very low levels in these samples (<1.5 µg/g in solvent extract and <0.1 µg/g in water leachate). The chemicals could potentially further react during the storage of the TWPs, prone to strong sorption or other loss processes, or could be lost during the TWP preparation process. Lastly, 4-ADPA, another oxidative transformation product from 6PPD, was detected in all the TWPs. 4-ADPA concentrations ranged from 1.4 to 19 µg/g in solvent extracts and 0.018 to 0.45 µg/g in water leachates, indicating that it is a relatively minor, but still frequently detected, transformation product compared to the more abundant 6PPDQ, 1,3-DMBA and 4-HDPA TPs.

The ratios between 6PPD TPs and 6PPD were estimated and used to interpret potential TWP/tire ages, as reported previously (Zhao et al., 2023a). As shown in **Figure 8,** used tires had a higher average ∑[6PPD-TPs]/[6PPD] ratio compared to the new tires (0.6 vs. 1.2). The results indicate the potential use of 6PPD-derived TPs as indicator chemicals that can reflect tire ages, enabling some insight into the fate and environmental risk assessment of the TWPs and associated chemical mixtures. The observations also indicated that some of these TPs were relatively more stable and environmentally persistent than PPD parent compounds and likely dominate fate and exposure outcomes for PPD-derived chemicals where parent PPDs are expected to be more reactive and shorter lived in most tire and environmental systems.

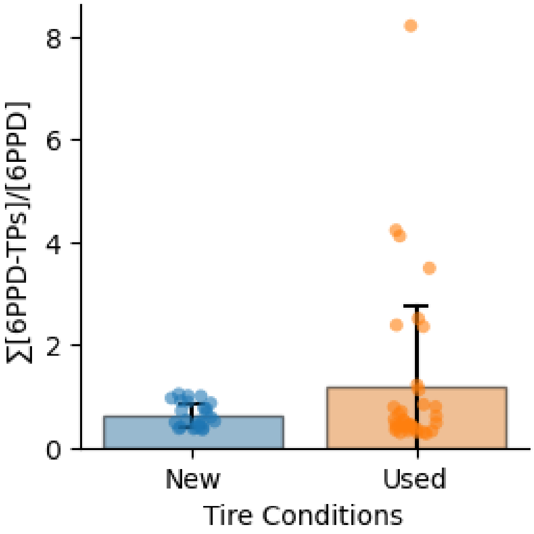

Figure 8. Distributions of average ∑[6PPD-TPs]/[6PPD] ratios calculated from TWP from different tire used condition groups from TWP MeOH extracts (5g/L TWP). The bar plot indicated the average ∑[6PPD-TPs]/[6PPD] ratios in different groups, with the error bar indicated the standard deviation from all samples from the same group. The individual points were calculated from the average of the experimental triplicates from individual TWPs. The individual points outside the boxes are outliers.

Suspect screening of other chemicals that are leachable from different TWPs. In addition to PPD and PPD-derived TPs, other common tire additives (**Table 1**) were lightly screened using the HRMS analysis. Data for seven representative chemicals, reflecting compounds we commonly detected in the aquatic environment, were detected in both TWP MeOH extracts and aqueous leachates, including vehicle-related chemicals (DPG, HMMM, NCBA and DCU) and corrosion inhibitors (2-NH2-BTH, 2-OH-BTH, 2-Mo-BTH) (**Figures 9 and 10**). Many other similar chemical data could also be presented, although the majority of HRMS features remain unidentified in such tire types. Complementary data arising from LC-QQQ analysis also is available for these extracts.

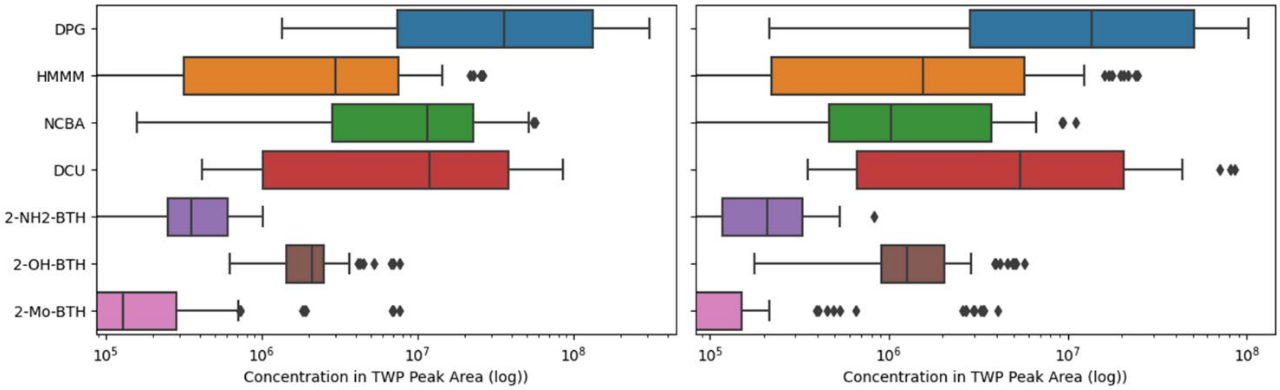

Figure 9. Distribution of analytical standard confirmed tire additive peak areas in different TWPs from LC-QTOF-HRMS analysis. (Left) Peak areas of analytical standard identified tire additives from TWP aqueous leachates (300mg/L TWP); and (right) peak areas of tire additives from TWP MeOH extracts (5g/L TWP). The peak areas were log-transformed (log10) for visualization purposes. The box and whiskers indicated the quartiles of all the datapoints, with individual points calculated from the average of the experimental triplicates from individual TWPs. The individual points outside the boxes are outliers.

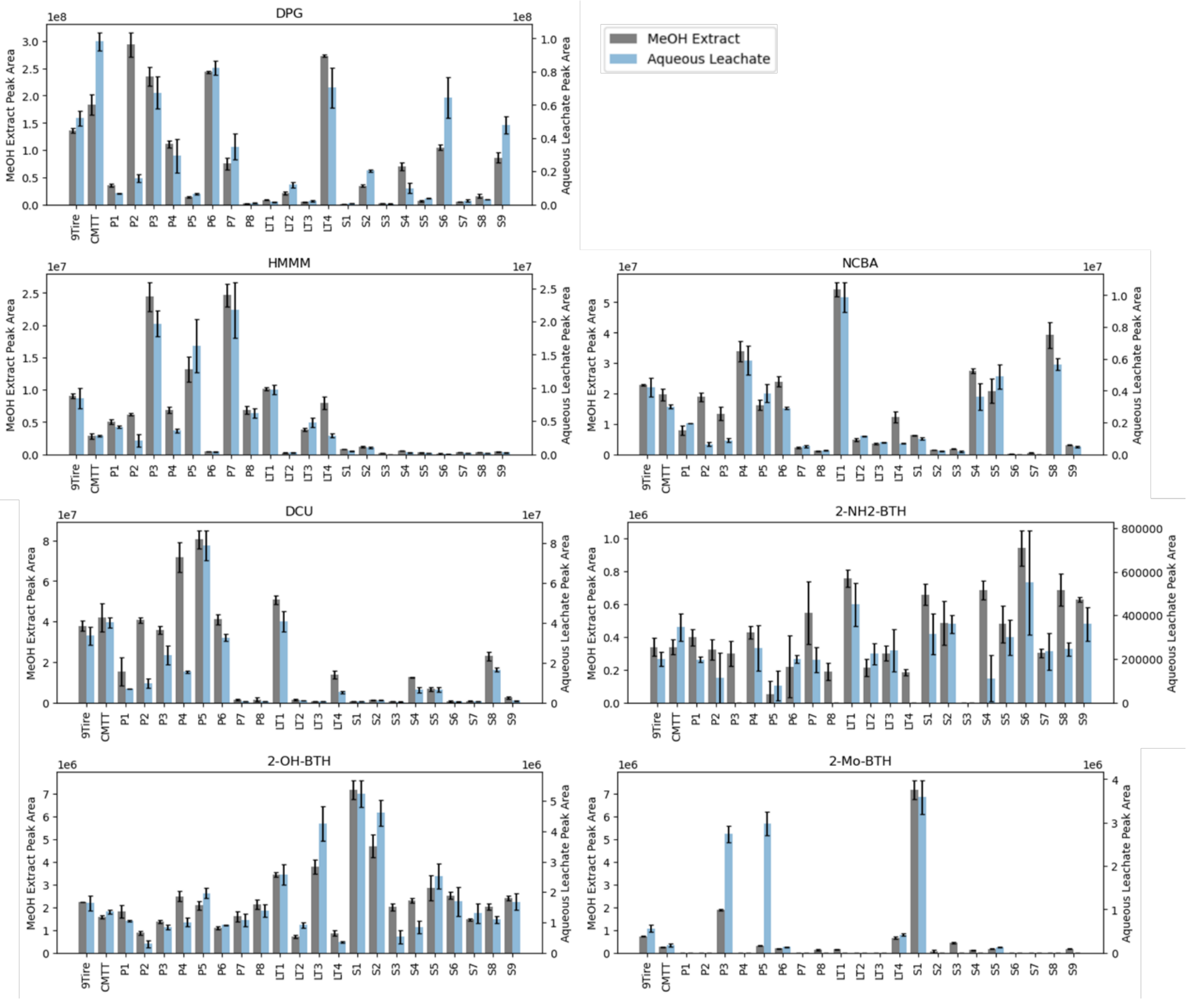

Figure 10. Peak areas of analytical standard identified tire additives different TWP aqueous leachates (300 mg/L TWP) and MeOH extracts (5 g/L TWP) from LC-QTOF-HRMS analysis. The peak areas were log-transformed (log10) for visualization purposes. The error bars were calculated from the experimental triplicates.

Although the quantification of those tire additives was not conducted for current result, the relative use of chemical additives in different TWPs could be compared with the HRMS peak areas. We note that further investigation and data analysis (e.g., expanding the suspect screening list, quantification of the chemicals) of those tire additive chemicals is still ongoing. Nevertheless, the current result represented different chemical compositions from different tires. For instance, passenger tires contained much higher HMMM and DCU compared to the other two tire types. Notably, heavy truck tires apparently contained limited use of HMMM, especially relative to some of the passenger car tires where quite high HMMM concentrations were evident. DPG abundance was also surprisingly variable across the individual tires, especially given its critical role within tire production as a vulcanization accelerator and the environmental observations of detected concentrations within roadway runoff that are sometimes well into the many 10s of µg/L.

Complementary LC-QQQ analysis I: Leaching potentials of 6PPDQ from different TWPs. For preliminary comparison, we included here quantitative 6PPDQ data collected using LC-QQQ methods, including such data for all of the individual tires evaluated throughout this study as well as data for new/used tires and the different tire types. Full description and data results are presented and discussed elsewhere. In the 9-tire mix, the solvent extraction-based 6PPDQ concentration was 18 ± 0.7 µg 6PPDQ/g TWP, while the water leaching-based concentration was 4.2 ± 0.06 µg/g 6PPDQ/g TWP (**Table 6**, **Figure 11**). The solvent extraction-based 6PPDQ concentration observed here was similar to that reported by Zhao et al. for both TWP (12 µg/g; same 9-tire mix used in this study) and crumb rubbers (0.3 – 25 µg/g, median 9.8 µg/g, n=9).[3] As expected, the gentler conditions of aqueous leaching resulted in lower detected surface available concentrations (as was observed across all TWP materials evaluated here) over the short term, although longer term leaching conditions may be better capable of mobilizing additional 6PPDQ mass from tire rubber particles as diffusion, leaching, and extraction processes are optimized.

For the individual passenger and light truck tires in the 9-tire mix, we observed 6PPDQ concentrations of 3.5 – 43 µg/g 6PPDQ/TWP (median 18 µg/g 6PPDQ/TWP) by solvent extraction and 1.0 – 7.8 µg/g 6PPDQ/TWP (median 4.1 µg/g 6PPDQ/TWP) by aqueous leaching (**Table 6**, **Figure 11**). For the heavy-truck tires, we observed 6PPDQ concentrations of 4.1 – 43 µg/g 6PPDQ/TWP (median 20 µg/g 6PPDQ/TWP) by solvent extraction and 1.8 – 10 µg/g 6PPDQ/TWP (median 4.5 µg/g 6PPDQ/TWP) using water (**Table 6**, **Figure 11**). With respect to comparing types of tires, median semi-truck tire concentrations were notably similar to concentrations observed for the 9-tire mixture and were not significantly different from concentrations observed for the individual tires from the 9-tire mix (Wilcoxon rank sum test, p=0.86 for water-leached 6PPDQ, p=0.56 for solvent-extracted 6PPDQ), while noting that substantial variability does exist within tires as a class. This data indicated that the 9-tire mixture (composed of eight passenger car tires and one light truck tire) was pretty representative of not only the passenger car and light truck tire class, but also applied to 6PPDQ compositions for a range of new and used heavy-truck tires. Notably, concentrations of 6PPDQ in used and new heavy-truck tires were also not significantly different from each other (Wilcoxon rank sum test, p=0.29 for water-leached 6PPDQ, p=0.06 for solvent-extracted 6PPDQ), although we note that the age and use conditions of the used tires, which may influence such a comparison, were unknown.

Table 6. Concentrations of 6PPDQ in water after leaching (150 mg TWP / 500 mL water), solvent extraction (50 mg TWP / 10 mL MeOH) and corresponding mass-per-mass concentrations of 6PPDQ for all individual tires tested.

	Concentration in aqueous leachate [ug/L]	**Concentration in MeOH extract [ug/L]**	**Aqueous leachate-based conc in TWP [ug/g]**	**Solvent extraction-based conc in TWP [ug/g]**	**Ratio of aqueous to solvent extractable mass [%]**
9Tire	1.3 ± 0	89 ± 4.4	4.17 ± 0	18 ± 0.87	23 ± 0
CMTT	0.46 ± 0.02	24 ± 1.5	1.5 ± 0.06	4.9 ± 0.31	31 ± 1.2
P1	1.4 ± 0	82 ± 6.2	4.5 ± 0	16 ± 1.3	27 ± 0
P2	0.3 ± 0.05	26 ± 1.7	1.0 ± 0.18	5.2 ± 0.35	19 ± 3.5
P3	2.3 ± 0.35	167 ± 12	7.6 ± 1.2	33 ± 2.3	23 ± 3.6
P4	0.32 ± 0.02	17 ± 0.58	1.1 ± 0.06	3.5 ± 0.12	32 ± 1.8
P5	1.1 ± 0.05	71 ± 3.1	3.7 ± 0.17	14 ± 0.61	26 ± 1.2
P6	2.4 ± 0.05	217 ± 29	7.8 ± 0.17	43 ± 5.8	18 ± 0.38
P7	0.76 ± 0.03	62 ± 2.5	2.6 ± 0.1	12 ± 0.5	21 ± 0.77
P8	1.1 ± 0.08	98 ± 11	3.7 ± 0.25	20 ± 2.2	19 ± 1.3
LT1	1.9 ± 0.13	107 ± 5.8	6.2 ± 0.44	21 ± 1.2	29 ± 2.1
LT2	0.6 ± 0.05	33 ± 2.7	2 ± 0.17	6.6 ± 0.53	30 ± 2.5
LT3	1.9 ± 0.06	123 ± 5.8	6.2 ± 0.19	25 ± 1.2	25 ± 0.78
LT4	1.4 ± 0.12	163 ± 21	4.6 ± 0.38	33 ± 4.2	14 ± 1.2
S1	1.7 ± 0.1	160 ± 17	5.6 ± 0.35	32 ± 3.5	17 ± 1.1
S2	1.4 ± 0.09	101 ± 8.5	4.5 ± 0.29	20 ± 1.7	22 ± 1.4
S3	0.53 ± 0.11	73 ± 3.5	1.8 ± 0.36	15 ± 0.7	12 ± 2.5
S4	2.2 ± 0.34	213 ± 5.8	7.3 ± 1.1	43 ± 1.2	17 ± 2.7
S5	2.9 ± 0.26	180 ± 17	9.8 ± 0.9	36 ± 3.5	27 ± 2.4
S6	0.63 ± 0.08	30 ± 1.2	2.1 ± 0.25	5.9 ± 0.23	36 ± 4.3
S7	0.8 ± 0.2	24 ± 1.5	2.7 ± 0.67	4.7 ± 0.31	56 ± 14
S8	0.78 ± 0.15	20 ± 2.5	2.6 ± 0.51	4.1 ± 0.5	64 ± 13
S9	1.6 ± 0.25	107 ± 5.8	5.4 ± 0.84	21 ± 1.2	25 ± 3.9

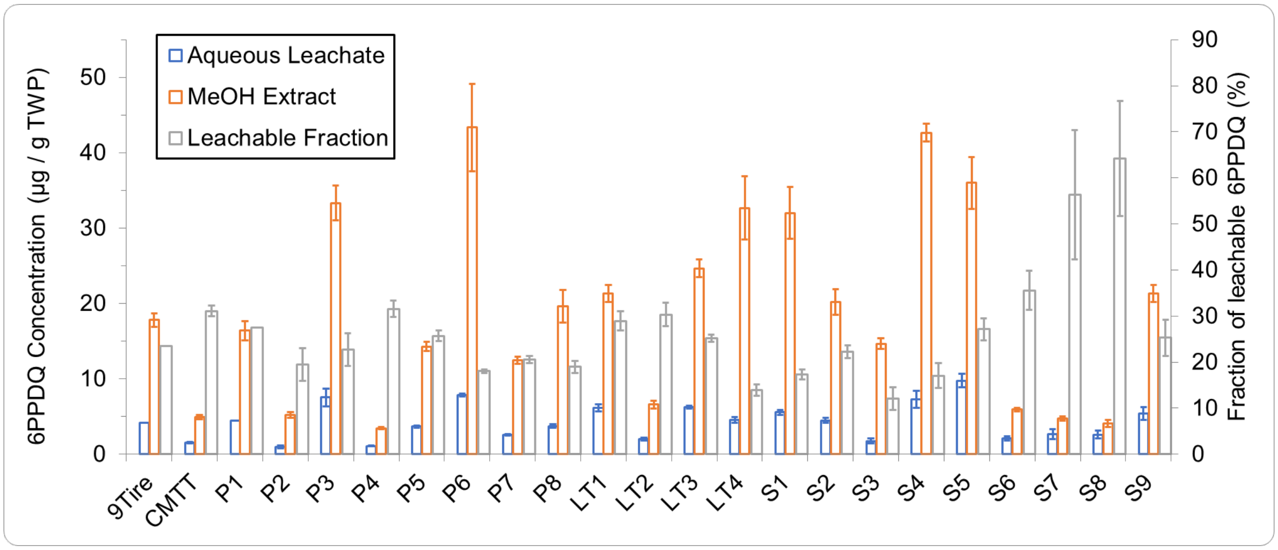

Figure 11. 6PPDQ concentrations detected in 24h aqueous leachates, solvent extracts and fraction of leachable 6PPDQ (%) of different TWP materials. The error bars represent standard deviation derived from the experimental replicates.

Across all the TWPs measured, only 26.7 ± 11.9% of solvent extractable 6PPDQ subsequently was leached into water under the short-term conditions tested. The proportion of solvent-extracted 6PPDQ that leached into water was 14 – 32% (median 24%) for the individual 9 passenger/light truck tires, and 12 – 64% (median 25%) for the heavy truck tires (**Table 6**, **Figure 11**). Concentrations of solvent-extracted and aqueous-leached 6PPDQ in all TWP samples were positively correlated (Spearman r=0.91, p<0.01), indicating that a single extraction method could reasonably be representative of the magnitudes of both solvent- and aqueous- leaching (albeit, with an adjustment factor based on the proportions observed here). We also note that the widest variability, and highest values for leachable mass, came from the heavy truck tires. Additionally, the TWPs that have less 6PPDQ mass tend to yield a higher extractable fraction of 6PPDQ compared to those TWPs with higher 6PPDQ masses (e.g., S6-8 vs. P3, P6, S4 and S5). This observation indicates that TWPs with less 6PPDQ mass would release the 6PPDQ more completely, presumably due to this process is limited by surface areas.

From the TWPs tested, 6PPDQ concentrations weren't significantly different between different types of TWPs if organized by vehicle use class (e.g., passenger car-light truck-heavy truck; **Figure 12**; p = 0.81 for solvent extract, p = 0.57 for aqueous leachate, one-way ANOVA test). The result indicated that 6PPDQ is omnipresent in different types of tire rubber products with relatively similar leaching potentials. This result also indicated the ubiquitous application of 6PPD in different tire rubber products, which could be transformed into 6PPDQ during environmental transformation processes after the TWPs being deposited into the environment. We also note that passenger tires had relatively lower (by median value) 6PPDQ compared to the other two tire types, which is potentially due to the fact that passenger tires are designed for light-duty applications that may require less 6PPD addition (Sheridan, 2010). Additionally, compared to light and heavy trucks, passenger car tires would be expected to have less abrasion and roadway pressure during operation, which should generate lower 6PPDQ mass per vehicle mile traveled if TWP particles are the primary source of these compounds to the aquatic environment.

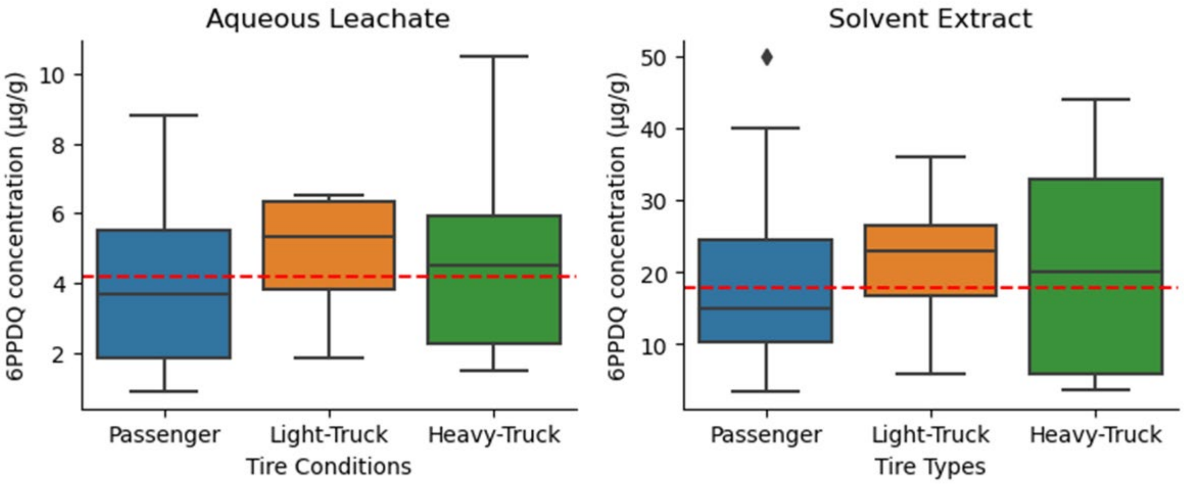

Figure 12. 6PPDQ concentration in 24h aqueous leachates and solvent extracts in different types of TWPs. The box and whiskers indicated the quartiles of all the datapoints. The individual points outside the boxes are outliers. The red dashed line represents an average 6PPDQ concentration measured from the 9-tire mix used as a reference sample. There were no significant differences between different groups ($p > 0.05$, one way ANOVA test).

With respect to new versus used TWP characteristics (**Figure 13**), the 6PPDQ concentrations detected in the new TWPs (on average 3.5 and 12.4 μg/g 6PPDQ/TWP from aqueous leachates and solvent extracts, respectively) are significantly ($p = 2.9\times10^{-5}$ for solvent extract, $p = 0.0091$ for aqueous leachate; t-test) lower than concentrations (on average 5.1 and 25.3 μg/g 6PPDQ/TWP from aqueous leachates and solvent extracts, respectively) detected in the used TWPs. The observation suggests that initial leachable 6PPDQ mass from new tires is lower than the masses that arise during in-use transformation processes of tire rubbers that generate 6PPDQ and other 6PPD transformation products. As they age and are used on roadways, the used tires would be expected to continuously generate substantial 6PPDQ mass during the service time that could then be discharged into the environment via leaching and TWP transport processes. While detection of 6PPDQ in aqueous leachates represents the discharge potential of this chemical during storm events and other water contact events (e.g. car washing, roadway sweeping, and other wet processes), the solvent extractable 6PPDQ measurements indicate that TWP contains substantial quantities of 6PPDQ after initial water contact and may serve as a continuing source of 6PPDQ across multiple rain events or when TWP are deposited and transported into aquatic environments.

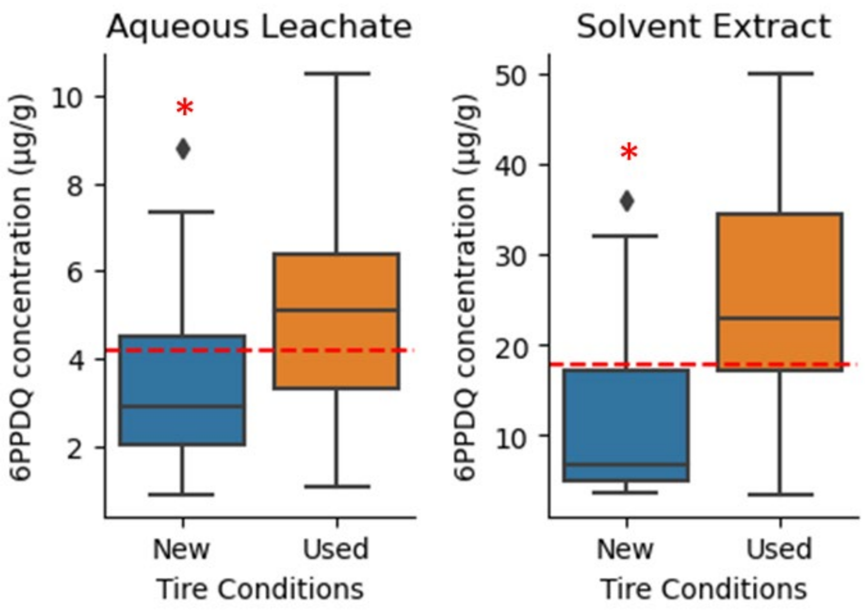

Figure 13. 6PPDQ concentration in solvent extracts and 24h aqueous leachates from used and new TWPs. The box and whiskers indicated the quartiles of all the datapoints. The individual points outside the boxes are outliers. The red dashed line represents the average 6PPDQ concentrations measured from 9-tire mix (reference sample). The red asterisk indicates results that are significantly different ($p < 0.05$, t-test).

Complementary QQQ analysis II: 6PPDQ release under different leaching conditions. The leaching potential of 6PPDQ as a function of solution conditions was further evaluated using the TWP samples from representative different tire types (Passenger tire: P4, Light truck tire: LT2 and Heavy truck tire: S9). Different representative environmental conditions were tested across the range of expected values for common environmental systems, including temperature, ionic strength, DOM, and pH. The measured 6PPDQ concentrations (normalized μg/g 6PPDQ/TWP) are summarized in **Table 7**, while **Figure 14** summarizes 6PPDQ masses leached into solution under the different conditions. During the data processing, one-way ANOVA statistical tests were performed to determine if there are any significant differences between different treatment conditions (**Table 8**).

Table 7. For the P4, LT2 and S9 tire types: detected concentrations of 6PPDQ in water using LC/QTOF-HRMS analysis after leaching (150 mg TWP / 500 mL water) and corresponding mass-per-mass concentrations of 6PPDQ in TWP, grouped by leaching condition variable (temperature, ionic strength, pH, and DOC).

	TEMPERATURE					
	4 °C		25 °C		40 °C	
TWP	**Conc. in Water (µg/L)**	**Conc. in TWP (ug/g)**	**Conc. in Water (µg/L)**	**Conc. in TWP (ug/g)**	**Conc. in Water (µg/L)**	**Conc. in TWP (ug/g)**
P4	0.18±0.01	0.59±0.02	0.24±0.02	0.78±0.06	0.2±0.0	0.8±0.0
LT2	0.34±0.03	1.1±0.10	0.48±0.02	1.6±0.05	0.4±0.0	1.5±0.2
S9	0.38±0.02	1.3±0.05	0.50±0.00	1.7±0.00	0.50 ± 0.04	1.67±0.12
	IONIC STRENGTH					
	0.01% NaCl		0.10% NaCl		1.0% NaCl	
TWP	**Conc. in Water (µg/L)**	**Conc. in TWP (ug/g)**	**Conc. in Water (µg/L)**	**Conc. in TWP (ug/g)**	**Conc. in Water (µg/L)**	**Conc. in TWP (ug/g)**
P4	0.25±0.03	0.84±0.11	0.24±0.01	0.79±0.03	0.22±0.02	0.72±0.07
LT2	0.52±0.03	1.7±0.10	0.47±0.03	1.6±0.10	0.49±0.07	1.6±0.23
S9	0.42±0.18	1.4±0.59	0.49±0.01	1.6±0.03	0.44±0.03	1.5±0.11
	PH					
	pH 5		pH 7		pH 9	
TWP	**Conc. in Water (µg/L)**	**Conc. in TWP (ug/g)**	**Conc. in Water (µg/L)**	**Conc. in TWP (ug/g)**	**Conc. in Water (µg/L)**	**Conc. in TWP (ug/g)**
P4	0.46±0.01	1.5±0.04	0.24±0.02	0.78±0.06	0.28±0.00	0.93±0.01
LT2	0.72±0.06	2.4±0.19	0.48±0.02	1.6±0.05	0.53±0.03	1.8±0.10
S9	1.4±0.16	4.7±0.54	0.50±0.00	1.7±0.00	0.45±0.17	1.5±0.58
	DISSOLVED ORGANIC CARBON					
	0.1 mg C/L		1.0 mg C/L		10 mg C/L	
TWP	**Conc. in Water (µg/L)**	**Conc. in TWP (ug/g)**	**Conc. in Water (µg/L)**	**Conc. in TWP (ug/g)**	**Conc. in Water (µg/L)**	**Conc. in TWP (ug/g)**
P4	0.20±0.02	0.68±0.08	0.2±0.0	0.6±0.1	0.25±0.02	0.83±0.05
LT2	0.44±0.02	1.5±0.07	0.4±0.0	1.4±0.1	0.41±0.03	1.4±0.09
S9	0.39±0.02	1.3±0.07	0.50±0.05	1.65±0.15	0.41±0.01	1.4±0.0

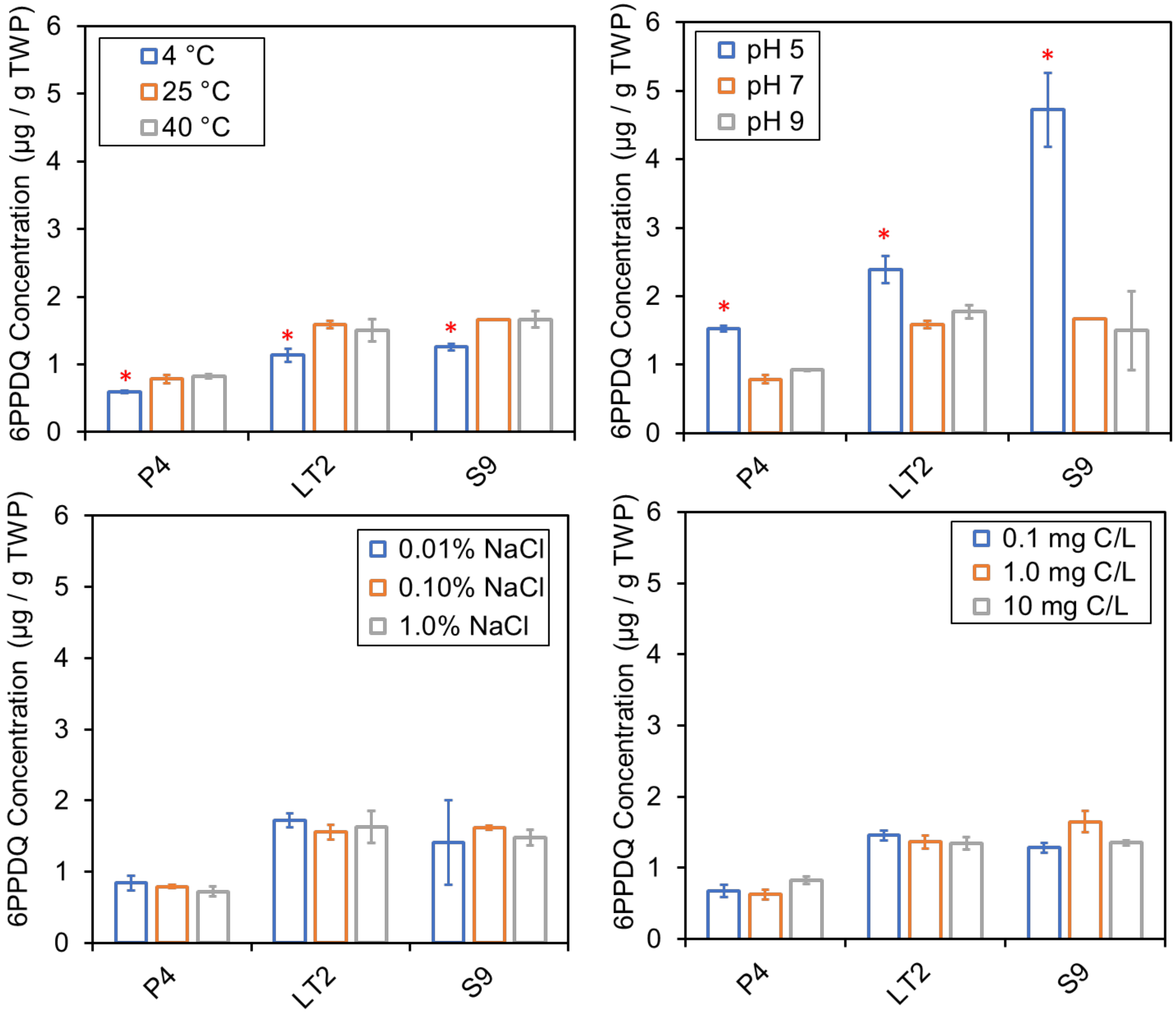

Figure 14. Summary of detected 6PPDQ concentration (as ug/g TWP) in aqueous leachates from different TWPs (P4, LT2 and S9) under varied solution conditions. The error bars represent standard deviation derived from the experimental replicates. Red asterisks indicated the condition result is significantly different ($p<0.05$) than all other conditions for the same TWPs.

Under the control conditions (25°C, pH 7), 0.78 ± 0.06 µg/g 6PPDQ/TWP were leached from P4, 1.6 ± 0.05 µg/g 6PPDQ/TWP were leached from LT2 and 1.7± 0.00 µg/g 6PPDQ/TWP were leached from S9. Additionally, P4 was constantly yielding the lowest 6PPDQ detections compared to the other two tires. This difference is essentially derived from different tire types. Compared to the truck tires (LT2 and S9) that are designed and used for heavy-duty applications, passenger tires (P4) tend to have less workload. Therefore, the latter samples might contain less 6PPD mass (which would potentially be transformed into 6PPDQ) (Sheridan, 2010).

Table 8. One-way ANOVA test result, p-values ($p<0.05$) indicated there are significant differences between the samples from different conditions.

	p-value (one-way ANOVA test)			
TWP	Temperature	Ionic Strength	pH	DOM
P4	1.30×10^{-3}	0.217	1.57×10^{-5}	0.0390
LT2	6.01×10^{-3}	0.771	2.07×10^{-4}	0.304
S9	7.28×10^{-3}	0.462	6.20×10^{-4}	0.0460

Temperature effects on leaching were evaluated across the range of 4 - 40 °C. Somewhat surprisingly, 6PPDQ release was most favored under room temperature (25 °C), compared to either the lower (4 °C) or higher (40 °C) temperatures. The impact of temperature on 6PPDQ leaching process was significant (p-value < 0.05; one-way ANOVA test; **Table 8**). At lower temperatures, leaching process could sometimes be limited by lower mass transfer rates governed by lower diffusion rates (i.e., 6PPDQ diffusion into water as a temperature sensitive process) or slower kinetic rates for partitioning processes. Also, given the low solubility of 6PPDQ (~40 µg/L), lower temperature would further reduce the solubility of 6PPDQ, therefore reducing the mass that was leachable into the water. The 6PPDQ leaching process significantly increased (p-value<0.05 for all TWPs tested) over the temperature range of 4-25 °C, indicating that leaching was clearly temperature dependent under such conditions. Elevated temperature tends to facilitate leaching processes due to enhanced solubility of chemicals as well as higher diffusion and mass transfer coefficients (Lu et al., 2021; Wang et al., 2022), which tend to yield higher kinetic rates. However, the 6PPDQ leaching potentials were plateaued at the higher temperature (40 °C; compared with 25 °C). This phenomenon could potentially be due to increased resorption of 6PPDQ back to TWPs or an accelerated aqueous transformation rate (i.e. reduced stability) of 6PPDQ at the higher temperature. The annual average temperature is 3 °C (10th percentile) to 26 °C (90th percentile) in the Seattle area (Weather Spark, 2024), which implies that in aquatic environments, the 6PPDQ leaching processes would be correlated with seasonal temperature shift under the ambient conditions (i.e., higher temperatures facilitate contaminant discharge).

The measurements suggest that 6PPDQ leaching processes also are significantly (p-value < 0.05; one-way ANOVA test; **Table 8**) affected by pH conditions. Compared to neutral and basic conditions (pH 7 and 9), the release of 6PPDQ from TWPs was favored under the acidic conditions (pH 5). As discovered in the other study (Hu et al., 2023), The aqueous stability of 6PPDQ indicated slightly higher stability of the chemical under acidic conditions. This measured stability indicates less degradation of 6PPDQ under pH 5 condition during the 24h leaching processes, and eventually lead to higher observed leachable 6PPDQ mass. While 6PPDQ (predicted $pK_a = -4.02$ from Marvin) should be almost exclusively in its neutral form across all pH 5-9 conditions, the more acidic pH was likely to affect the TWP surface matrix and associated, and eventually facilitate the release of 6PPDQ. Additionally, the pH of rainwater and creek water is usually around 5-6 (U.S. EPA, n.d.), suggesting that 6PPDQ discharge in the environment is promoted by typical slight acidity conditions in roadway runoff and surface water.

On the other hand, different ionic strength conditions, which are representative to the environmental conditions (the typical sea water ionic strength is approximately around 0.7 M), didn't have significant effect on TWP leaching process (p-value > 0.05; one-way ANOVA test; **Table 8**). The measurements from different samples are not significantly different from the control condition samples as well (p-value > 0.05; t-test). As pointed out above, while 6PPDQ should be in its neutral form under the tested conditions, the ionic interactions between the added salt and 6PPDQ are not likely affecting its solubility or leaching processes.

Lastly, DOC posed mixed effect on 6PPDQ leaching behaviors (p-value < 0.05 for P4 and S9; **Table 8**). This inconsistent observations of 6PPDQ leaching dynamics in different TWPs could be potentially due to the interferences of experimental or analytical errors onto the low mass load of 6PPDQ in P4 (< 1 µg/g 6PPDQ/TWP) and S9 (< 2 µg/g 6PPDQ/TWP). Alternatively, clues can be found from previous studies for the mixed effect of DOC on 6PPDQ leaching behaviors. As reported previously (Hu et al., 2023), 6PPDQ is hydrophobic ($\text{Log}K_{OW}=4.3$), implying that 6PPDQ has higher leaching potential with higher DOC concentrations. On the other hand, higher DOC concentrations would presumably also facilitate 6PPDQ-DOC reactions during the leaching process, resulting in faster 6PPDQ degradation kinetics. Overall, further investigation would be needed to test the 6PPDQ partitioning behavior and stability under different DOC conditions for validation.

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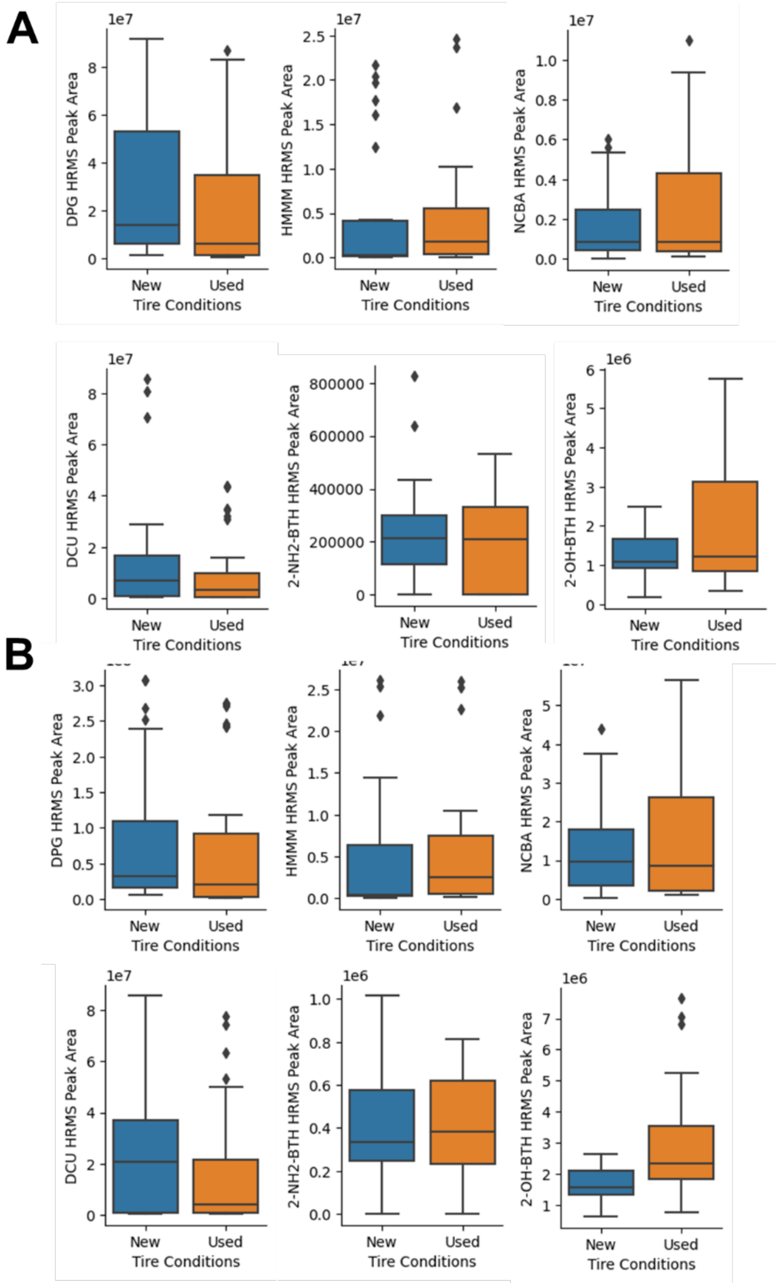
APPENDIX



Appendix Figure A1. HRMS peak area distributions of HRMS features detected from different TWPs from aqueous leachates (A; 300 mg/L TWP) and MeOH extracts (B; 5g/L TWP) from TWPs with different tire used conditions. The box and whisker plots indicated the quartiles of all the datapoints, calculated from the average of the experimental triplicates from individual TWPs. The individual points outside the boxes are outliers.