



Micron-size tire tread particles leach organic compounds at higher rates than centimeter-size particles: Compound identification and profile comparison[☆]

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ABSTRACT

Tire tread particles (TTP) are environmentally prevalent microplastics and generate toxic aqueous leachate. We determined the total carbon and nitrogen leachate concentrations and chemical profiles from micron (~32 μm) and centimeter (~1 cm) TTP leachate over 12 days. Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were used to measure the concentration of leached compounds. Nontargeted chemical analysis by comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC/TOF-MS) was used to compare the chemical profiles of leachates. After leaching for 12 days, DOC was 4.0 times higher in the micron TTP leachate than in the centimeter TTP leachate, and TDN was 2.6 times higher. The total GC×GC/TOF-MS chromatographic feature peak area was 2.9 times greater in the micron TTP leachate than the centimeter TTP leachate, and similarly, the total relative abundance of 54 tentatively identified compounds was 3.3 times greater. We identified frequently measured tire-related chemicals, such as 6PPD, N-cyclohexyl-N'-phenylurea (CPU), and hexa(methoxymethyl)melamine (HMMM), but nearly 50% of detected chemicals were not previously reported in tire literature or lacked toxicity information. Overall, the results demonstrate that smaller TTP have a greater potential to leach chemicals into aquatic systems, but a significant portion of these chemicals are not well-studied and require further risk assessment.

1. Introduction

Tire tread particles (TTP) are an emerging concern due to their environmental ubiquity and toxicity. Global tire tread emissions are estimated to range from 8045 to 551,155 tons/year, making TTP one of the most abundant microplastics in stormwater and surface waters (Sadiktsis et al., 2012; San Francisco Estuary Institute, 2019). TTP results from the abrasion of tires on road surfaces, creating particles that can enter waterways via stormwater and atmospheric deposition (Wik and Dave, 2009). In environmental samples, TTP varies in size from <10 μm to several hundred microns (Gustafsson et al., 2008; Kreider et al., 2010; Kwak et al., 2013). Generally, smaller particles leach more

compounds due to their larger surface-area-to-volume ratio, so that smaller microplastics are more chemically harmful than larger macroplastics (Beita-Sandí et al., 2019; Halsband et al., 2020; Jeong et al., 2022). However, several studies have found that the size of tire particles does not positively correlate with leaching ability for metals and nitrogenous compounds, and the impact of tire particle size on other nonpolar organic compounds has not been studied (Beita-Sandí et al., 2019; Halsband et al., 2020).

Tires are manufactured with a complex chemical mixture of rubber polymers, fillers, protective agents, vulcanization agents, and other additives including polycyclic aromatic hydrocarbons (PAHs), benzothiazoles, *p*-phenylenediamines (PPDs), amines, and heavy metals

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(Llompart et al., 2013; Perkins et al., 2019; Halsband et al., 2020; Cel-eiro et al., 2021; Peter et al., 2018; Rauert et al., 2022). Currently, TTP and TTP-related chemicals are known to cause toxicity in a variety of aquatic species (Tian et al., 2021; Brinkmann et al., 2022; Chibwe et al., 2022; Siddiqui et al., 2022). 6PPD-quinone, a degradant of the antioxidant 6PPD, was associated with coho salmon mortality in the Pacific Northwest (Tian et al., 2021), as well as in multiple species of trout (Brinkmann et al., 2022). Benzothiazoles and aryl amines have been correlated with fathead minnow embryo deformities (Chibwe et al., 2022).

Apart from these known tire contaminants, multiple unknown and unidentified chemicals have also been detected in tire leachate (Peter et al., 2018; Halsband et al., 2020; Chibwe et al., 2022; Müller et al., 2022). This highlights the need for chemical analysis of tire particles and leachates to determine environmentally relevant contaminants. Nontargeted analysis can provide comprehensive characterization and identification of known and unknown TTP-associated chemicals. Within recent years, several studies have characterized TTP via nontargeted analysis, but many of these studies utilize liquid chromatography (LC) based systems that are more compatible for polar compound identification. Our study utilizes comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC/TOF-MS) to detect relatively nonpolar compounds that may have been missed by LC methods. Together, the use of both techniques allows for complementary characterization of TTP-related chemicals.

Our research aims to 1) determine the leaching potential of chemicals from two size fractions (centimeter vs. micron) of laboratory-generated TTP over 12 days as measured by dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) and 2) establish and compare chemical profiles for the two size fractions of TTP using nontargeted chemical analysis with GC×GC/TOF-MS (Chang et al., 2021; Ishida et al., 2022; Mladenov et al., 2022a). Note, this study focuses on providing foundational knowledge regarding the chemical constituents in pristine tire particles only. Abiotic factors, such as UV exposure, may transform leached chemicals in the environment, but those factors are outside the scope of this study. The results here provide further characterization of tire-related chemicals and additives to better understand TTP's ability and magnitude to leach relatively nonpolar organic compounds.

2. Methods

2.1. Tire particle generation

Tire particles were produced at Oregon State University using tire tread material from a new standard all-season passenger car replacement tire (Caldera Confidence C3 with DOT quality grades of Treadwear 480, Traction A and Temperature A) following the methods described in Cunningham et al. (2022). Briefly, tire tread was removed as shavings using a stainless steel blade and further sliced into 2–4 mm pieces. Two size fractions of TTP particles were generated from the shavings: centimeter and micron sizes. For the centimeter TTP, the tire shavings were hand cut into 1 cm × 1 cm pieces using scissors rinsed three times with ultrapure water. For the micron TTP, approximately 12 mL of tire tread pieces were loaded into a 50 mL chamber with a 25 mm stainless steel ball and milled in a liquid nitrogen cryomill (Retsch CryoMill, Haan, Germany). Cryomilling was achieved with 4 min of chamber precooling at 5 Hz, followed by three 5 min milling cycles at 30 Hz with 1 min of intermediate cooling at 5 Hz. This milling produced particles with an average size of 32.2 µm (±25.5, SD). No liquid nitrogen had direct contact with the tire tread particles, only the outside of the cryomilling chamber.

2.2. Leaching experiments

We tested the 8 treatment conditions (micron or centimeter TTP

leaching for 4 different timepoints) using a series of replicate aqueous-phase leaching experiments (Table 1). The replicate experiments varied in sample size, but each replicate experiment had at least two unique samples per treatment. DOC and TDN were analyzed in all samples (i.e., at all timepoints across the replicate experiments) in duplicate to maximize the statistical power. Due to the relatively low data processing throughput of NTA, we analyzed a single sample from each timepoint (n = 4) within each sample type (micron TTP, centimeter TTP, and control) for a total of 12 NTA samples. We used a larger control sample size to confirm the lack of background contamination.

To prepare the leachates, micron or centimeter TTP was leached for 1, 3, 6, or 12 days in individual 125 mL amber glass vials at aqueous concentrations of 10 g/L (0.25 g TTP/25 mL water). The concentration was chosen based on previous work to ensure the detection of chemicals, rather than at environmental concentrations (Chibwe et al., 2022). All solutions were prepared in laboratory-created freshwater (60 mg/L Instant Ocean Sea Salt in ultrapure water). Particles were gently agitated at 150 rpm at room temperature on a Fisherbrand™ Multi-Platform shaker (ThermoFisher, Waltham, MA). After leaching, the particles were filtered from the water using 0.22 µm PTFE filters (ThermoScientific, Rockwood, TN). Aqueous control samples were prepared and agitated under the same conditions without the presence of the TTP. Aliquots of 20 mL water were used for DOC and TDN analysis and the remaining 5 mL were used for NTA.

2.3. Dissolved organic carbon and total dissolved nitrogen

Filtered aliquots were analyzed in duplicate (10 mL per duplicate sample) for DOC and TDN concentrations using a Shimadzu TOC-L Total Organic Carbon and Total Nitrogen Analyzer (Shimadzu, Kyoto, Japan) with high temperature combustion (EPA 415.3) (Mladenov et al., 2022b). The instrument detection limits were 0.1 mg/L for DOC and 0.03 mg/L for TDN. DOC and TDN standards, ranging from 0 to 15 mg/L and 0–5 mg/L respectively, were used to generate standard curves (each standard curve had an R² value = 0.99) and also analyzed as unknown samples for quality control. Average standard deviations of duplicate samples were within 5% of the means of the samples.

2.4. Nontargeted chemical analysis

Because of the relatively high TTP concentration (10 g/L), we extracted 5 mL of filtered leachate using solid phase extraction (SPE) with Oasis HLB (Hydrophilic-Lipophilic Balance) cartridges (Waters, Milford, MA). Briefly, Oasis HLB cartridges were conditioned with 5 mL methanol, followed by 15 mL LCMS-grade water. The 5 mL of filtered leachate was loaded into the cartridge and eluted at 1 drop/sec. After the sample passed through, an additional 5 mL of LCMS water was loaded into the cartridge to rinse it. The sample was extracted from the cartridges using 5 mL acetone followed by 5 mL dichloromethane. Extracts were dried with 4 g sodium sulfate and loaded onto 6 mL UCT Enviro-Clean cartridge with 2500 mg muffled sodium sulfate (United

Table 1

Number of unique TTP leachate samples analyzed for dissolved organic carbon (DOC), total dissolved nitrogen (TDN), and nontargeted analysis (NTA). All DOC/TDN samples were run in duplicate, whereas NTA used a single sample.

Sample Type		Days Leached			
		1 day	3 day	6 day	12 day
Micron TTP	DOC/TDN	8	4	4	2
	NTA ^a	1	1	1	1
Centimeter TTP	DOC/TDN	4	4	4	4
	NTA ^a	1	1	1	1
Control	DOC/TDN	11	6	6	4
	NTA ^a	1	1	1	1

^a All NTA samples were subsetted from samples analyzed for DOC/TDN.

Chemical Technologies, Bristol, PA, USA) for further drying. Extracts were evaporated to 350 μL under nitrogen and spiked with 50 μL of an isotope labeled PAH standard (4 $\mu\text{g}/\text{mL}$ acenaphthene- d_{10} ; AccuStandard, New Haven, CT, USA). Extracts were analyzed using a Pegasus 4D GC $\times$ GC/TOF-MS (LECO, St. Joseph, MI) following previously described instrumental parameters (Chang et al., 2021; Ishida et al., 2022; Mladenov et al., 2022a). Briefly, RTX-5Ms column (35 m $\times$ 0.25 mm $\times$ 0.25 μm) was used as a primary column, and an Rxi-17 (1.00 m $\times$ 0.10 mm $\times$ 0.10 μm) was used as a secondary column. The modulation time was 3.50 s, injection was splitless, and research grade helium was used as the carrier gas. We used the mass range 50–1000 m/z with a data acquisition rate at 151.51 spectra/sec.

2.5. QA/QC

All glassware and tools were baked at 450 $^{\circ}\text{C}$ for 6 h prior to use. Prior to filtering the samples, the plastic pipettes and PTFE filters were rinsed 3 times with ultrapure water. Three blank samples consisting of only laboratory-generated freshwater were prepared and analyzed concurrently with samples for each timepoint. Controls from 5 leaching experiments ($n = 27$) were analyzed for DOC/TDN, and one control from each timepoint ($n = 4$) was analyzed for the nontargeted analysis. The analytical runs were random selections of micron samples, centimeter samples, and controls, in accordance with nontargeted analysis protocols (Peter et al., 2018).

All samples were spiked with 50 μL of a recovery standard, acenaphthene- d_{10} at 4 ng/ μL (AccuStandard, New Haven, CT, USA), immediately prior to injection on the GC $\times$ GC/TOF-MS. The peak area of the standard was used to normalize peak areas in the samples. Relative abundance was calculated as normalized peak area for all 60 tentatively identified compounds by dividing a compound's peak area by the peak area of the sample's recovery standard.

Recovery rates were measured in 5 mL equivalent samples ($n = 11$) that used an internal standard (acenaphthene- d_{10}) spiked prior to SPE extraction and a recovery standard (2-fluorobiphenyl; AccuStandard, New Haven, CT, USA) that was spiked immediately prior to injection on the GC $\times$ GC/TOF-MS. The equivalent samples used the same source of TTP and water, and they were leached for 1 or 6 days at 10 g/L. The only difference with the current samples was the equivalent samples used a final volume of 300 mL instead of 25 mL because leachate was being generated for future toxicity exposure assessments. The percent recovery in the equivalent samples was $127\% \pm 67\%$, which we consider representative of the current samples.

2.6. Chemical data alignment, selection criteria, and confirmation with authentic standards

Chromatographic and mass spectral data was analyzed using LECO ChromaTOF (v4.72.0.0) and its add-in feature, Statistical Compare (Chang et al., 2021; Ishida et al., 2022; Mladenov et al., 2022a). Briefly, Statistical Compare aligns peaks across samples using retention time and mass spectral similarity. For this alignment, samples were grouped by size instead of leaching time due to small sample size. Therefore, each alignment group consisted of one sample from each timepoint (e.g., 4 samples per group). The final groups were micron TTP ($n = 4$), centimeter TTP ($n = 4$), and controls ($n = 4$).

Once peaks were aligned with Statistical Compare, features were selected for further investigation if: 1) the feature was not detected in the control samples; 2) the feature was present in at least 3 out of 4 samples in a group; and 3) the signal to noise (S/N) ratio was ≥ 20 . All remaining features and their mass spectra were manually reviewed and classified as tentatively identified or not (Chang et al., 2021; Ishida et al., 2022; Mladenov et al., 2022a). The criteria for inclusion as a tentatively identified compounds are: 1) high similarity score match (≥ 600) with the NIST library match, 2) the same molecular ion and 3) at least three of the same prominent quantitative ions.

We searched for authentic standards of all 60 tentatively identified compounds using PubChem, SciFinder, and the compound's Chemical Abstract Services registry number (CASrn). We selected 27 compounds for identity confirmation with commercially available and affordable standards. Standards were run following the same instrumental parameters as the samples, and internal standards were used to account for retention time shifts. Identification of compounds was made with one of the following categories, based on Shaul et al. (2015): [1] Authentic MS RT: experimental mass spectrum and retention time matched that of the authentic standard. [2] Authentic MS (isomer): experimental mass spectrum matched that of the authentic standard, but retention times varied indicating an isomer, if an isomer is available. [3] Reference database MS: experimental mass spectrum was matched to one within the NIST Electron Ionization Mass Spectra.

2.7. Source, persistence, bioaccumulation, and toxicity data

The 60 tentatively identified compounds underwent a literature search to determine their source and use data, as well as their persistence, bioaccumulation, and toxicity (PBT), as quantified by their biodegradation half-life, log-bioconcentration factor, and predicted 96-h fathead minnow LC₅₀. The thresholds for each parameter were: persistence = 60 half-life days, bioconcentration factor = 1000, and toxicity < 10 mg/L, based upon limits set by the European Commission REACH regulations. PubChem and SciFinder were the primary tools for source and use data, and the EPA CompTox Dashboard (v2.1) and the ECOSAR Class Program (v2.2) were used for PBT data. Detailed methods are described in SI-1.

2.8. Data analysis

Data were not normally distributed, therefore non-parametric tests were used. We used Mann-Whitney U tests in SPSS (v28.0.1.14) to test significance ($p < 0.05$) for DOC and TDN concentrations, as well compound abundance between samples. Hierarchical clustering for only tentatively identified compounds was performed in R (v3.6.2) using the function *hclust* (method = *ward.D*) and the heatmap was generated using R package *latticeExtra* (version 0.6–29).

3. Results and discussion

3.1. DOC/TDN

DOC and TDN concentrations were significantly higher in the micron TTP leachate than the centimeter TTP leachate at all time points (Mann-Whitney U test, $p < 0.05$) (Fig. 1). By 12 days, the average DOC and TDN concentrations in the micron TTP were 4.0 times and 2.6 times greater, respectively, than the centimeter TTP.

DOC increased over time in both size fractions, but more quickly in the micron leachate. TDN increased over time in the centimeter leachate, but there was no clear trend in the micron leachate. In the micron leachate, TDN decreased at 6 days, but then increased again at 12 days. This anomaly could be resolved with a larger sample size, but a similar trend has been previously noted in tire particle leachates (Selbes et al., 2015). Additionally, we saw an increase in the DOC and TDN in the controls at 12 days, but the concentrations were still significantly lower than in both the micron and centimeter leachates (SI-1).

TDN is important to consider in addition to DOC because nitrogenous compounds may form nitrosamines, a group of potential carcinogens that could adversely affect human and environmental health. In particular, environmental concentrations of TTP (0.3–197 mg TTP/L) can contribute to nitrosamine formation in drinking water treatment (Beita-Sandí et al., 2019). Together, these results suggest that greater concentrations of compounds are released from micron-sized TTP, likely due to the larger surface-area-to-volume ratio of the micron particles (Beita-Sandí et al., 2019; Halsband et al., 2020; Jeong et al., 2022).

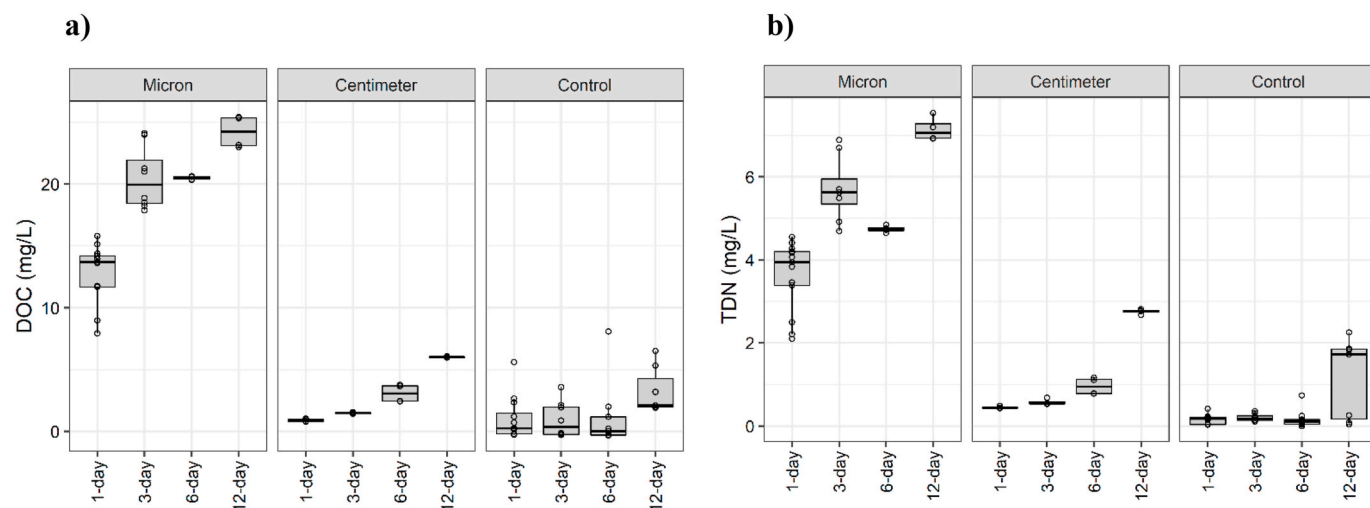


Fig. 1. Boxplots of the 25th quantile, median, and 75th quantile for (a) DOC and (b) TDN concentrations in the TTP leachates. Sample sizes are listed in Table 1.

3.1.1. GC×GC/TOF-MS based nontargeted analysis results

Leaching of micron TTP resulted in both a greater diversity of chromatographic features and a greater abundance of those features than the centimeter TTP. In total, 513 chromatographic features were identified by the analysis, as described in the **Methods**, with 355 and 304 chromatographic features identified in micron and centimeter leachates, respectively. Of these features, 90 were unique to the micron leachate, and 47 were unique to the centimeter leachate. The total unadjusted chromatographic peak area for the features detected in both leachate types was 2.9 times greater in the micron leachate (2.12×10^9 , unitless) compared to the centimeter leachate (7.42×10^7 , unitless).

Of the 513 chromatographic features, 60 compounds were classified as tentatively identified (described above) (Table 2). Their additional analytical information, such as experimental mass spectra and GC retention times, are available in SI-2 and SI-1 Table 1. There were 27 authentic standards tested to verify compound identities. Of those, 89% ($n = 24$) had mass spectral and retention time matches, while 11% ($n = 3$) had mass spectral matches but not retention time matches (e.g., possible isomeric compounds) (Table 2). These verifications rates are similar to those in previous work from our group (87.5%–96%) (Tran et al., 2020; Chang et al., 2021; Ishida et al., 2022; Mladenov et al., 2022a).

Of the 60 compounds, 54 compounds were detected in both the micron and centimeter leachates, four compounds were unique to the micron leachate, and two compounds were unique to the centimeter leachate. The average total relative abundance (described previously in the Methods) of the 54 tentatively identified chemicals was 3.3 times greater in the micron leachate than the centimeter leachate. The average abundance and standard deviation for each of the 60 compounds in the micron and centimeter leachate are available in SI-1 Table 1.

3.1.2. Relative abundance and occurrence data for tentatively identified compounds

We determined that three tentatively identified compounds in the highest abundance category (top five most abundant compounds) were found in both size fractions (Fig. 2). Those three common compounds were: N-cyclohexyl- cyclohexanamine (CASrn: 101-83-7; abbr: DCHA); N-cyclohexyl-N'-phenylurea (CASrn: 886-59-9; abbr: CPU); and N-(1,1-dimethylethyl)-N'-phenyl-urea (no CASrn). Authentic standards confirmed the identity of the first two compounds, but no standard was available for the last compound. The average relative abundance of each of these compounds was nearly two times greater in the micron leachate than the centimeter leachate (SI-1 Table 1).

The other most abundant compounds differed in the micron and centimeter leachates. In the micron leachate, 6PPD (the previously

reported toxic compound) (Tian et al., 2021) and 1-phenyl-1H-tetrazole (CASrn: 5378-52-9) were among the abundant compounds. Although 6PPD also occurred in the centimeter leachate, it was not among the most abundant compounds. Two other compounds, 1,2-dihydro-2,2,4-trimethyl-quinoline (CASrn: 147-47-7; abbr: TMQ) and N-methyl-1,1'-bicyclohexyl]-2-amine (no CASrn) were among the most abundant in the centimeter leachates. Again, of these 4 additional compounds, each was confirmed with authentic standards except N-methyl-1,1'-bicyclohexyl]-2-amine, for which there was no authentic standard.

Overall, the top 5 most abundant tentatively identified compounds comprised ~40% of total normalized abundance in the micron leachate (e.g. for all chromatographic features, not only the tentatively identified compounds), indicating that a relatively small number of compounds were responsible for a large proportion of the total abundance. In the centimeter leachate, the top 5 most abundant tentatively identified compounds comprised ~31% of the total normalized abundance.

Of the tentatively identified compounds, 38.3% ($n = 23$) were significantly more abundant in the micron leachate (Mann-Whitney *U* test, $p < 0.05$). This, along with the higher DOC concentrations in the micron leachates, indicates that while the larger TTP is leaching similar compounds, they are leaching at a slower rate or to a lesser extent. This pattern has been observed previously in several targeted analyses of tire particles, where leached constituents decrease as particle size increases (Beita-Sandí et al., 2019; Halsband et al., 2020). Only 9 compounds (15%) were significantly more abundant in the centimeter leachate, including quinoline-related compounds, benzothiazoles, and amines (each class is discussed in greater detail below). While most studies indicate that smaller particles leach chemicals more readily than larger particles, one prior study did find that larger tire particles leached similar or greater concentrations than smaller tires particles (Halsband et al., 2020). Additionally, the centimeter particles in the present study were not subjected to cryomilling whereas the micron particles were. It is possible that the freezing associated with cryomilling influenced temperature-dependent compounds, such that chemical changes occurred in the TTP while chemicals were preserved in the centimeter particles. Overall, organic compound leaching is likely driven by particle size, but additional factors, like temperature or matrix, may still play a role (Wagner et al., 2018; Halsband et al., 2020).

3.2. Comparison of micron and centimeter TTP leachate chemical profiles

Hierarchical clustering was used to compare the overall chemical profiles of the tentatively identified compounds in the leachates (Fig. 3). Micron leachate samples clustered separately from centimeter leachate samples, indicating significant differences in their chemical profiles. In

Table 2

List of the 60 tentatively identified compounds and their associated CASrn in the micron and centimeter leachates, based on siabundance in the micron TTP leachate. The degree of identification is included as described in the Methods: 1 = authentic MS RT; 2 = authentic MS RT (isomer); 3 = reference database MS. The abundance shows which TTP leachate size had significantly greater abundance (Mann-Whitney *U* test, $p < 0.05$) (<Micron = greater abundance in micron TTP leachate; <Centimeter = greater abundance in millimeter TTP leachate). The persistence, bioaccumulation, and toxicity (PBT) data is provided. Bold text indicates which PBT values are in exceedance of thresholds, as described in the Methods.

Compound Name	CAS #	Degree of identification	Source/ Use ^a	Abundance (sig.)	P (half-lifedays)	B (factor)	T (mg/L)
Cyclohexanamine, N-cyclohexyl-	101-83-7	1	<i>j</i>		34.70	4.75	0.97
1H-Tetrazole, 1-phenyl-	5378-52-9	1	<i>f</i>	<Micron	4.09	2.55	5150.00
6PPD	793-24-8	1	<i>a</i>	<Micron	3.16	403.00	0.86
Urea, N-cyclohexyl-N'-phenyl-	886-59-9	1	<i>k</i>		3.11	19.40	9.09
urea, N-(1,1-dimethylethyl)-N'-phenyl-	n/a	3	<i>k</i>		n/a	n/a	n/a
1H-Tetrazole, 1-phenyl- (isomer)	5378-52-9	2	<i>f</i>	<Micron	4.09	2.55	5150.00
Urea, N,N'-diphenyl-	102-07-8	1	<i>k</i>		7.08	26.05	23.50
Proximpnam	2828-42-4	2	<i>c</i>		4.51	8.89	476.00
Acetamide, N-phenyl-	103-84-4	1	<i>k</i>	<Micron	4.82	3.57	715.00
[1,1'-Bicyclohexyl]-2-amine, N-methyl-	n/a	3	<i>i</i>		n/a	n/a	n/a
Hexa(methoxymethyl)melamine	68,002-20-0	3	<i>k</i>	<Micron	n/a	n/a	n/a
Quinoline, 1,2-dihydro-2,2,4-trimethyl-	147-47-7	1	<i>a, k</i>	<Centimeter	19.87	8.62	9.62
4-Anilinoquinazoline	34,923-95-0	3	<i>d</i>		12.00	44.07	n/a
phenol, 2-(1,1,3,3-tetramethylbutyl)-	3884-95-5	1	<i>i</i>		n/a	n/a	0.19
n-Decanoic acid	334-48-5	1	<i>e</i>	<Micron	4.99	7.37	21.90
Carbamic acid, phenyl-, ethyl ester	101-99-5	1	<i>j</i>	<Centimeter	4.66	8.29	82.20
2,8-Dimethylquinoline	1463-17-8	2	<i>a</i>	<Centimeter	4.32	12.53	10.00
4-Cyclopentyl-N-phenylbutanamide	56,051-98-0	3	<i>f</i>		3.51	21.57	6.77
Ethanone, 1-phenyl-2-(4-pyridinyl)-	1620-55-9	3	<i>d</i>	<Micron	3.54	7.95	n/a
4,4'-(p-Phenylene)diisopropylidene)diphenol	2167-51-3	3	<i>e</i>	<Micron	44.63	2715.27	n/a
1,4-Benzenediamine, N-phenyl-	101-54-2	1	<i>f</i>		22.39	50.38	25.90
Aniline	62-53-3	3	<i>j</i>	<Micron	5.60	1.63	40.30
2-Benzothiazolamine, N-phenyl-	1843-21-6	1	<i>j</i>	<Micron	40.96	48.60	n/a
2-Benzothiazolesulfenamide, N-(1,1-dimethylethyl)-	95-31-8	1	<i>j</i>		12.40	17.03	61.20
3-Methyl-3H-benzothiazol-2-one	2786-62-1	3	<i>c</i>		3.36	3.36	93.40
4-Isopropylbenzenethiol, TMS derivative	n/a	3	<i>i</i>		3.16		n/a
Quinolin-6-ol, 1,2-dihydro-2,2,4-trimethyl-	72,107-05-2	1	<i>a</i>		6.14	3.89	n/a
Cyanamide, methylphenyl-	18,773-77-8	3	<i>e</i>	<Micron	3.67	4.39	n/a
Hexa(methoxymethyl)melamine related 1	n/a	3	<i>c</i>	<Micron	n/a	n/a	n/a
2-(2',4',6',8',8'-Heptamethyltetrasiloxan-2'-yloxy)-2,4,4,6,6,8,8,10,10-nonamethylcyclopentasiloxane	145,344-72-5	3	<i>e</i>	<Micron	n/a	n/a	n/a
3-(4-Methylbenzoyl)acrylic acid	20,972-36-5	3	<i>d</i>		n/a	n/a	n/a
1,2-Benzisothiazole	n/a	3	<i>i</i>		n/a	n/a	n/a
5-(1-Ethyl-2(1H)-quinolyldiene)-3-phenylrhodanine	32,136-89-3	3	<i>c</i>		4.84	27.89	n/a
Benzenamine, N-[2-(1-phenyl)-5-tetrazolyl]ethenyl]-	274,690-45-8	3	<i>i</i>		n/a	n/a	n/a
N-Phenylsuccinimide	83-25-0	1	<i>h</i>		5.27	3.31	31.00
1(2H)-Naphthalenone, 3,4-dihydro-3,3,6,8-tetramethyl-	5409-55-2	3	<i>c</i>		4.81	306.92	0.83
Caprolactam	105-60-2	3	<i>k</i>	<Micron	4.57	2.15	1470.00
1,2-Dimethoxy-4-n-propylbenzene	5888-52-8	1	<i>a</i>	<Micron	4.31	46.22	n/a
2-Benzothiazolamine	136-95-8	3	<i>j</i>		7.50	2.92	16.20
Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	5654-86-4	3	<i>d</i>	<Micron	3.35	2.43	n/a
Diphenylamine	122-39-4	3	<i>a</i>		6.65	150.97	9.62
1-Phenanthrenecarboxaldehyde, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-, [1 S-(1a,4aa,10aa)]-	24,035-50-5	3	<i>b</i>	<Micron	44.23	486.17	0.16
1H-Isindole-1,3(2H)-dione, 2-phenyl-	520-03-6	1	<i>f</i>		4.76	15.99	7.61
Oxazolidine, 2-ethyl-2,3-dimethyl-	109-01-3	3	<i>i</i>	<Micron	6.69	2.42	965.00
3-Aza-6-oxabicyclo[3.3.0]octane-2,4-dione, 3-phenyl-	136,309-86-9	3	<i>k</i>		n/a	n/a	n/a
Caffeine	58-08-2	1	<i>g</i>	<Micron	3.82	1.88	7220.00
2-Furanmethanol	98-00-0	3	<i>f</i>		4.45	5.26	2010.00

(continued on next page)

Table 2 (continued)

Compound Name	CAS #	Degree of identification	Source/Use ^a	Abundance (sig.)	P (half-lifedays)	B (factor)	T (mg/L)
Anisole, o-octyl-	20,056-59-1	3	e		4.54	513.29	0.04
Hexanoic acid, phenyl ester	7780-16-7	3	c		4.47	161.67	2.67
Benzoic acid, 2-(1,3-benzothiazol-2-yl)-	883-93-2	1	j		34.30	178.84	3.19
Benzoic acid, 2-benzoyl-, methyl ester	606-28-0	1	e		4.39	14.16	7.40
Acetamide, N-(3-oxo-5-phenylpyrazolidin-4-yl)-	16,242-08-3	3	k		n/a	n/a	n/a
2,6,6-Trimethyl-2-cyclohexene-1,4-dione	1125-21-9	1	b	<Centimeter	214.67	27.84	n/a
2H-Indol-2-one, 1,3-dihydro-3-(phenylimino)-	33,828-98-7	3	d		3.34	11.74	84.60
Benzothiazole, 2-(ethylthio)-	2757-92-8	1	j	<Centimeter	4.40	31.01	4.62
Benzenamine, N-2-propyn-1-yl-	14,465-74-8	3	d		4.06	9.10	n/a
Silane, chlorodisopropylethyl-	98-54-4	3	i	<Centimeter	5.37	67.60	6.52
Benzothiazole, 2-methyl-	120-75-2	1	j	<Centimeter	8.93	13.31	27.90
2(3H)-Furanone, 5-ethenyl(dihydro-5-methyl-	1073-11-6	3	b		4.90	2.85	68.30
N-Phenyl-N-(1,3,2-dioxaphospholane-2-yl)methylcarbamate	2603-10-3	1	f		4.47	3.78	208.00

^a Source/use categories: a = antioxidant; b = flavoring/fragrance; c = general manufacturing; d = pharmaceutical/general manufacturing; e = plasticizer/polymer manufacturing; f = resin/rubber-related; g = softener/conditioner; h = tire-related manufacturing; i = unknown; j = vulcanization accelerator; k = vulcanization agent.

particular, the fewest number of compounds were detected in the 1-day centimeter leachate, including the absence of chemicals that were abundant in the micron leachate, such as N-cyclohexyl-cyclohexanamine (DCHA), 1-phenyl-1H-tetrazole, and N,N'-diphenylurea (CASrn: 102-07-8; abbr: DPU). Common classes of chemicals and their occurrence in the micron and centimeter leachates are described below. Source and use information for each chemical is described in Table 2.

3.2.1. Benzothiazoles

Benzothiazoles are often added to tires as vulcanization agents (Dröge and Hulschotte 2018; EPA, 2019). We detected multiple benzothiazole-related compounds, including: 2-methyl-benzothiazole (CASrn: 120-75-2; abbr: MeBT), 2-benzothiazolamine (CASrn: 136-95-8; abbr: 2-ABT) (Zhang et al., 2018; Rauert et al., 2020), 2-(1,3-benzothiazol-2-yl)-benzoic acid (CASrn: 883-93-2; abbr: PBTZ) (Skoczyńska et al., 2021), and N-(1,1-dimethylethyl)-2-benzothiazole-sulfenamide (CASrn: 95-31-8; abbr: TBBS) (Perkins et al., 2019). Each of these compounds, except 2-benzothiazolamine, was confirmed with an authentic standard. The compound 2-methyl-benzothiazole was significantly more abundant in the centimeter leachate, while 2-benzothiazolamine was significantly more abundant in the micron leachate. Previous studies with road dust have also shown that 2-benzothiazolamine has been found in higher concentrations in the sub-PM_{2.5} fraction of road dust compared to the PM_{2.5-10} fraction (Zhang et al., 2018).

In addition to these well-known benzothiazoles, we confirmed the identities of two additional benzothiazole-related compounds: 2-(ethylthio)-benzothiazole (CASrn: 2757-92-8) and N-phenyl-2-benzothiazolamine (CASrn: 1843-21-6). No toxicity data was available for either of these two compounds. Given the known toxicity of 2-methyl-benzothiazole (Chibwe et al., 2022), these additional benzothiazole-related compounds should be assessed for environmental risk.

3.2.2. Amines

Amines are most often used in tires as vulcanization accelerants, and several types have been found consistently across studies. N,N'-diphenylguanidine (DPG), the parent compound of aniline and N,N'-diphenylurea, is often identified as a highly abundant compound in tire leachates (Peter et al., 2018; Capolupo et al., 2020; Müller et al., 2022). While DPG may be too polar for us to detect with the GC methods, we detected aniline, N,N'-diphenylurea, and N-cyclohexyl-N'-phenylurea as abundant compounds in the leachates. These amines are all associated with aquatic or human toxicity. Aniline is listed as an EPA probable human carcinogen, and N,N'-diphenylurea and N-cyclohexyl-N'-phenylurea are associated with deformities in fathead minnows (Capolupo et al., 2020; Chibwe et al., 2022).

Additional amine-related compounds were detected, including diphenylamine (CASrn: 122-39-4) (Halsband et al., 2020; Skoczyńska et al., 2021), carbamic acid (CASrn: 101-99-5); caprolactam (CASrn: 105-60-2) (Seiwert et al., 2020); and acetanilide (CASrn: 103-84-4), which has been detected in stormwater runoff and in fish livers (Du et al., 2017). All amine-related compounds were significantly more abundant in the micron leachate than the centimeter leachate, except for carbamic acid, which is a reaction product of aniline. Given the low relative abundance of aniline in the centimeter leachate, it is possible that aniline transformed into carbamic acid in these leachates more readily than in the micron leachates. Each of these chemicals, except carbamic acid, have been previously associated with tire particles and leachates.

3.3. Hexa(methoxymethyl)melamine

We detected HMMM in all micron and centimeter samples, and it was significantly more abundant in the micron leachate. Additionally, we detected a possible HMMM isomer. Both HMMM and its possible isomer were detected at all timepoints in the micron leachate, but only appeared after 3 days of leaching in the centimeter leachate. HMMM is

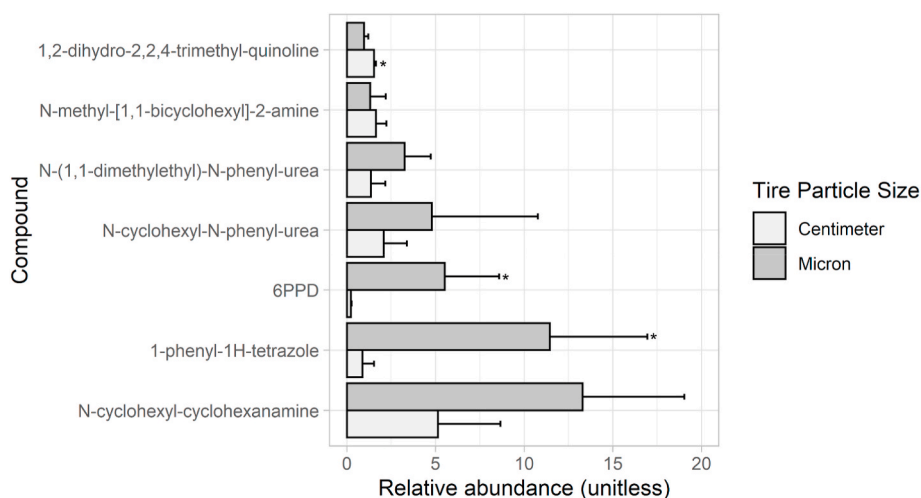


Fig. 2. The average relative abundance (response) of the top five most abundant compounds found in each tire particle size group. Asterisks indicate where a compound was significantly more abundant in one group, based on Mann-Whitney U tests.

widely used in industrial and plastic products and can degrade into greater than 20 transformation products, some of which can be persistent (Alhelou et al., 2019). Isomers of the parent HMMM compound are not well-known, and additional characterization is needed to understand their potential toxicity and environmental impact (Wiener and Lefevre 2022).

3.4. Quinoline-related compounds

Quinolines are often used in tires as antioxidants (Moretto, 2007). We detected the common quinoline-related compound 1,2-dihydro-2,2,4-trimethyl-quinoline (TMQ) (Halsband et al., 2020; Thomas et al., 2023). It was present in significantly higher abundance in the centimeter leachate. TMQ is an antioxidant used in tires that may be a persistent leachate compound (Thomas et al., 2023).

We confirmed the identity of two additional quinoline-related compounds. The first was 2,8-dimethylquinoline (CASrn: 1463-17-8), which has been previously detected in tire particles (Moretto, 2007). The second was 1,2-dihydro-2,2,4-trimethyl-quinolin-6-ol (CASrn: 72, 107-05-2), which has limited environmental and toxicological data.

3.5. Newly detected tire-related compounds

Of the 60 tentatively identified compounds, nearly 50% were not previously reported in the tire literature reviewed. This is similar to other TTP nontargeted analysis studies that also report new and unknown chemicals not detected in other tire research (Peter et al., 2018; Halsband et al., 2020; Seiwert et al., 2020; Chibwe et al., 2022; Müller et al., 2022; Sakai et al., 2022). The lack of overlap with previous studies may be because much of the prior work on TTP leachate used liquid chromatography/electrospray ionization (LC/ESI), rather than the gas chromatography/electron ionization (GC/EI) mass spectrometry method used in the present study. The use of both methods is important for determining the full chemical composition of TTP leachate (Ulrich et al., 2019).

New or underreported tire-associated compounds from this study included amines, benzothiazoles, and quinoline-related compounds. We also detected a possible new isomer of HMMM (Rauert et al., 2020; Seiwert et al., 2020; Thomas et al., 2023). Of these new or underreported compounds, several were within the most abundant leached compounds. The compound 1-phenyl-1H-tetrazole has been detected in crumb tire rubber but additional environmental impact information is lacking (Nilsson et al., 2008). Two of the most abundant compounds did not have authentic standards available, which will make future

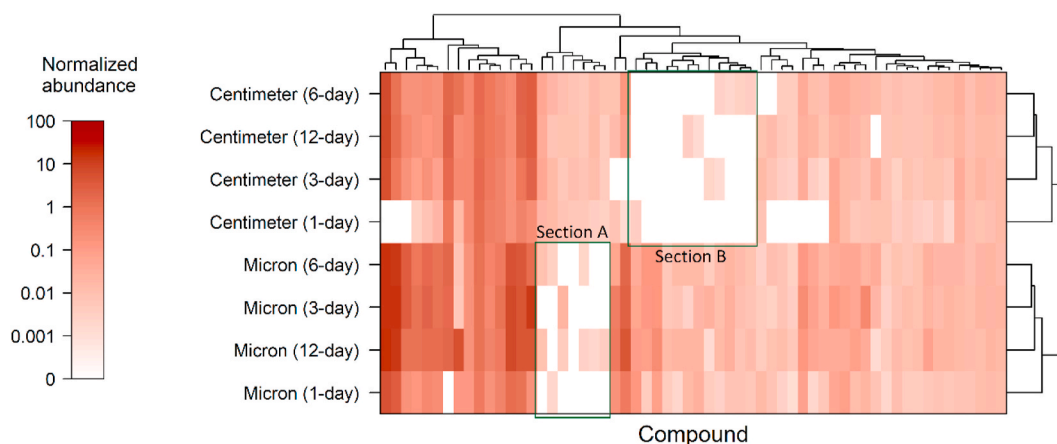


Fig. 3. Heatmap produced via hierarchical clustering comparing each leaching time point and particle size (micron vs centimeter). Clustering on the x-axis is determined by the 60 tentatively identified compounds. Section A highlights compounds missing from the micron leachate: 2(3H)-furanone and 5-ethenyldihydro-5-methyl- (CASrn: 1073-11-6). Section B highlights compounds missing from the centimeter leachate: 1,4-benzenediamine, N-phenyl- (CASrn: 101-54-2); 4-cyclopentyl-N-phenylbutanamide (CASrn: 56,051-98-0); 2-furanmethanol (CASrn: 98-00-0); and 2-benzothiazolamine.

toxicology evaluations difficult. It is possible that some of the detected compounds, including several of the most abundant compounds, are degradation or transformation products of the original compound, and these products must be better understood because these are likely the compounds that will be found in the environment.

Known toxic chemicals were also identified, including *o*-octyl-anisole and 1,2-dihydro-2,2,4-trimethyl-quinoline. Both are predicted to be genotoxic by EPA CompTox, but few studies, if any, have assessed their possible environmental impacts. While 6PPD-quinone has been recently highlighted in literature for its toxicity to coho salmon and other aquatic species, there are several other *p*-phenylenediamines that can leach from tires. We detected one such compound (1,4-benzenediamine, *N*-phenyl; CASrn: 101-54-2) in only the micron leachate that could belong to this larger chemical class (Stephensen et al., 2003).

3.6. Persistence, biodegradation, and toxicity (PBT) data

Of the 60 tentatively identified compounds, 49 had available persistence and bioaccumulation data and 38 had toxicity data. One compound (2,6,6-trimethyl-2-cyclohexene-1,4-dione; CASrn: 1125-21-9) exceeded the persistence threshold with a predicted half-life of 215 days (>60 days), indicating long-term presence in the environment. Another compound, bisphenol P (CASrn: 2167-51-3), exceeded the expected bioconcentration threshold (>1000). Sixteen compounds exceeded the predicted toxicity threshold (<10 mg/L) (Table 2), including the highly abundant compounds 6PPD, *N*-cyclohexyl-cyclohexanamine (DCHA), *N*-cyclohexyl-*N'*-phenylurea (CPU), *N*-cyclohexyl-*N'*-phenylurea (CPU), and 1,2-dihydro-2,2,4-trimethyl-quinoline (TMQ). While these values are only predictive of toxicity, it highlights that tire leachates contain complex mixtures of toxic chemicals that could pose deleterious environmental effects.

4. Conclusion

Based on the increased DOC, TDN, and relative abundance in the micron TTP leachate, we determined that smaller TTP leach a greater abundance of chemicals than larger particles. The micron TTP used in this study were ~32 µm in diameter, which is within the range of environmental TTP (Mathissen et al., 2011; Kim and Lee, 2018; Wagner et al., 2018). Therefore, the results suggest that TTP in the environment is likely to release significant amounts of organic compounds. Smaller tire particles thus pose a greater environmental risk because, for the same mass, smaller particles release greater abundances of potentially toxic chemicals. For example, 6PPD, a parent compound of 6PPD-quinone, was one of the most abundant compounds in the micron TTP leachate, but it was not one of the most abundant compounds in the centimeter TTP leachate. Further, nearly 50% of the identified compounds were not discussed in reviewed tire literature and are identified in the present study for the first time as leached tire constituents. Additionally, several of the most abundant compounds have limited toxicity data and no authentic standards. While some of these compounds may be transformation or degradation products of the original additive or compound, chemical and toxicological evaluations of these constituents are needed to determine their environmental impacts.

Our study examined a single tire, and alternate additives or chemicals may be used in other tire types. Further, different mixtures of tire-related particles may yield different chemical profiles (e.g., tire and road wear particles vs. tire tread). There is an urgent need for composite samples of TTP from multiple manufacturers and tire types (e.g., winter vs. performance, pristine vs. used) so that common or ubiquitous chemicals may be identified and evaluated for their environmental impact. Additionally, the concentrations chosen in this study were higher than environmental concentrations in order to determine the types of chemicals in tires, but future studies may focus on environmentally relevant concentrations for risk assessments. Continued detection and identification of tire-related chemicals, and their

degradation or transformation products, in laboratory and environmental samples, as well as toxicological testing, will be important to inform policy regarding the use and inclusion of individual chemicals in tire manufacturing.

CRedit authorship contribution statement

M.E. Stack: Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Project administration. **K. Hollman:** Methodology, Formal analysis, Investigation, Writing – review & editing. **N. Mladenov:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition. **B. Harper:** Resources, Writing – review & editing. **F. Pinongcos:** Methodology, Investigation. **K.E. Sant:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition. **C.M. Rochman:** Conceptualization, Writing – review & editing, Funding acquisition. **W. Richardot:** Investigation, Writing – review & editing. **N.G. Dodder:** Conceptualization, Writing – review & editing, Funding acquisition. **E. Hoh:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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