The Tire Wear Compounds 6PPD-Quinone and 1,3-Diphenylguanidine in an Urban Watershed

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Abstract
Prompted by a recent report that 6PPD-quinone (6PPD-q), a by-product of a common tire manufacturing additive that is present in road runoff, is toxic to coho salmon (Oncorhynchus kisutch), extracts of water samples collected from an urban river were re-analyzed to determine if this compound was present in stormwater-influenced flows. In addition, extracts were analyzed for 1,3-diphenylguanidine (DPG), which is also used in tire manufacturing. Samples were originally collected in the fall of 2019 and winter of 2020 in the Greater Toronto Area of Canada from the Don River, a highly urbanized watershed in close proximity to several major multi-lane highways. These target compounds were analyzed using ultra-high pressure liquid chromatography with high resolution mass spectrometric detection with parallel reaction monitoring. Both 6PPD-q and DPG were detected above limits of quantification (i.e., 0.0098 µg/L) in all extracts. Maximum concentrations for 6PPD-quinone of 2.30 ± 0.05 µg/L observed in the river during storm events exceeded the LC50 for this compound for coho salmon (i.e., > 0.8 µg/L). In composite samples collected at intervals throughout one rain event, both compounds reached peak concentrations a few hours after initiation of the event (i.e., 0.52 µg/L for DPG and 2.85 µg/L for 6PPD-q), but the concentrations of 6PPD-q remained elevated above 2 µg/L for over 10-h in the middle of the event. Estimates of cumulative loads of these compounds in composite samples indicated that kg amounts of these compounds entered the Don River during each hydrological event, and the loads were proportional to the amounts of precipitation. This study contributes to the growing literature indicating that potentially toxic tire-wear compounds are present at elevated levels and are transported via road runoff into urban surface waters during rain events.

The recent literature indicates that contamination of surface waters by chemicals derived from tire wear is an emerging area of research (Jan Kole et al. 2017; Wagner et al. 2018; Baensch-Baltruschat et al. 2020). In the 1970s, several products of tire wear were first characterized, but the study of road runoff and the toxicity of leachate from tires began to accelerate in the 1990s (Halle et al. 2020). In the 2000s, investigations began on specific chemical classes, such as polynuclear aromatic hydrocarbons, benzothiazoles, and various trace metals (e.g., zinc) in relation to contamination from tires (Halle et al. 2020). It has been demonstrated that some organisms may indiscriminately consume tire material and that this route of exposure may cause short-term and long-term toxicity to aquatic organisms (Wik and Dave 2009; Redondo-Hasselerharm et al. 2018; Khan et al. 2019; Halle et al. 2020). Furthermore, Kolomijeca et al. (2020) observed that leachates from tires caused poor hatching success and various deformities in the embryos of fathead minnow (Pimephales promelas). Capolupo et al. (2020) reported that tire leachate caused both acute and sub-lethal responses in microalgae and in mussels. A recent study showed that the common classes of chemicals in tire leachates, benzothiazoles and aryl-amines, were linked to toxic effects in fathead minnow embryos, although uncharacterized chemicals in the leachates likely also contributed to the observed toxicity (Chibwe et al. 2021).

Several chemicals that are used in the manufacturing of tires and/or the transformation products of these compounds are transported in road runoff into surface waters (Unice et al. 2015; Seiwert et al. 2020). Untreated road runoff is a significant source of these contaminants in the...
aquatic environment (Hou et al. 2019). We recently showed that a tire cross-linking agent, hexamethoxymethylmelamine (HMMM) is transported during rain events from high traffic highways into urban surface waters in the Greater Toronto Area (GTA) of Canada (Johannessen et al. 2021a). This compound is transformed into numerous more watersoluble transformation products (TPs) that are expected to readily partition into the aquatic environment (Alhelou et al. 2019, Johannessen and Parnis 2021), and we recently detected several of these TPs of HMMM in urban rivers in the GTA (Johannessen et al. 2021b).

Another tire wear compound, the aryl-amine compound, 1,3-diphenylguanidine (DPG) has been detected in urban surface waters (Peter et al. 2018; Wagner et al. 2018; Zahn et al. 2019; Seiwert et al. 2020). DPG is used as a catalyst in tire production to accelerate the cross-linking of rubber material with sulfur (Fisherbein 1991). This agent has been shown to also leach from tire wear materials, indicating that this additive is neither completely consumed in the catalytic process nor removed during tire processing (Unice et al. 2015). Recent work has also demonstrated that DPG is a component in tire wear leachate that is toxic to the embryos of fathead minnow (Chibwe et al. 2021). It was recently reported by Tian et al. (2021) that another tire additive, N-phenyl-N’-(1,3-dimethylbutyl)-p-phenylenediamine (6PPD) undergoes oxidation in the environment to form the by-product, 6PPD-quinone (6PPD-q). The 6PPD compound is used as an anti-oxidant in the production of synthetic rubber and it is present in tires in mass fractions ranging from 0.4 to 2% (Krüger et al. 2005; Tian et al. 2021). The oxidation by-product of 6PPD was identified as a likely candidate compound causing mass mortalities of coho salmon (Oncorhynchus kisutch) as they return to spawn in urban streams in the Pacific Northwest region of the USA (Tian et al. 2021). Recently, we reported the presence of 6PPD-q at µg/L concentrations in urban rivers in the GTA (Johannessen et al. 2021b).

The report of 6PPD-q as a highly toxic tire-derived chemical in urban streams prompted us to reinvestigate archived extracts from composite surface water samples collected from an urban watershed (i.e., Don River) in the GTA that is impacted by runoff from several multi-lane transportation corridors and other impermeable urban landscapes. Our previous analyses of these extracts from composite samples collected from the Don River showed that the levels of HM MMM in the river followed the hydrological profile during major rain events, except there was a delay of several hours between the initiation of high flows in the river and the peak in the concentration of this tire-wear compound (Johannessen et al. 2021a). In the present study, we analyzed these archived samples for two additional tire-derived compounds, DPG and 6PPD-q. The aim of this study was to determine the temporal patterns during rain events of these potentially toxic compounds and to determine if the concentrations rise to levels that exceed toxic thresholds (Tian et al. 2021; Chibwe et al. 2021). Composite samples collected over entire hydrological events were also analyzed to estimate the cumulative loads of the compounds (grams per event) entering the watershed. The potential for toxicity of these tire wear compounds to aquatic organisms is of particular concern because several urban rivers that discharge into Lake Ontario are habitat for native fish species, including brook trout (Salvelinus fontinalis). Introduced salmonids (e.g., coho salmon) also enter these rivers during their annual spawning runs.

Methods and Materials

Chemicals

A solid sample of diphenyl guanidine (DPG) was purchased from Sigma-Aldrich (Oakville, ON, Canada) at a reported purity of 97%. A commercial standard of 6PPD-q with a purity of 98.8% was purchased from HPC Standards (Atlanta, GA, USA). HPLC grade solvents (methanol and acetone) and formic acid (88.0%) were purchased from Fisher Scientific (Ottawa, ON, Canada). MilliQ water was produced in-house within the Water Quality Centre at Trent University. For the preparation of a solution of the oxidation biproducts of 6PPD, a solid sample of this compound (N-phenyl-N’-(1,3-dimethylbutyl)-p-phenylenediamine) was also purchased from Sigma-Aldrich, but no purity data were provided by the supplier. However, no previously reported transformation products of 6PPD (Tian et al. 2021) were detected in significant amounts in this standard.

Oxidation of 6PPD

Since there were no commercial standards available for 6PPD-q when this study was initiated, this compound was generated from 6PPD by ozonation using a modified version of the procedure described by Tian et al. (2021). As illustrated in Fig. 1, 500 mg of 6PPD pellets were ground using a ceramic mortar and pestle until a fine, dark gray powder was produced. The 6PPD powder was then spread on a fritted glass disk as a thin layer and placed in an ozone reaction chamber, which consisted of a Pyrex cylindrical fritted tube. Using a Model TOGC2 corona discharge ozone generator manufactured by Triogen O3 (East Kilgide, Scotland, U.K.), ozone was produced from pure oxygen. This ozone was then passed up through the chamber at room temperature (~20 °C) for 80 min at a rate of 0.5 L/min. The dose of ozone used was 20 g/Nm² which is equivalent to 1.5 g O₃ per gram of 6PPD. The ozonated 6PPD was dissolved in 50 mL of ethanol and stored in a refrigerator until analysis.

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Upon analysis of the solution for the concentration of 6PPD by LC-HRMS (as described below) and comparing it to the concentration of 6PPD present before ozonation, the conversion efficiency of 6PPD-q from 6PPD was estimated to be $50 \pm 3.8\%$ or $9.31 \times 10^{-4} \text{ mol}$, assuming that all reacted 6PPD produced 6PPD-q and there were no other ozonated by-products produced. Therefore, the amount of 6PPD-q in the solution was estimated to be $278 \text{ mg} \pm 13.2 \text{ mg}$. Dilutions of this solution were used for analytical calibration.

After the analysis of all samples was completed, a commercial standard of 6PPD-q became available. Using this commercial standard, the retention time and estimated concentration of 6PPD-q in the ozonated solution was confirmed to be $278 \text{ mg}$ by LC-HRMS analysis with a 9-point calibration curve of the commercial standard ($R^2 = 0.993$).

**Archived Samples**

Archived extracts from surface water samples were analyzed for 6PPD-q and DPG. These extracts were prepared from surface water samples originally collected in the fall and winter of 2019 and 2020 at a provincial water quality and water level monitoring station from the Don River at Tömmorden (43° 41′ 09.0″ N 79° 21′ 41.0″ W). This location in the GTA in Southern Ontario, Canada, is adjacent to a major urban highway, the Don Valley Parkway. Downstream of the monitoring station, the Don River discharges into the Toronto Harbour area of Lake Ontario.

Samples were collected as part of a water quality monitoring program by the Ontario Ministry of Environment Conservation and Parks (MECP) using composite samplers, as previously described by Johannesen et al. (2021a). In brief, composite samples were collected in the fall of 2019, as well as in the winter and early spring of 2020. The surface water samples were collected in response to significant hydrological events using an ISCO Avalanche refrigerated automated sampler (Avensys Systems, Toronto, ON, Canada) which was triggered when water levels in the Don River began to rise. On October 16–17th, 2019, “temporal” composite samples were collected at intervals throughout the rain event. These samples were collected hourly over 42 h in 300 mL aliquots and pooled in flow-weighted 3-h increments for a total of 14 temporal samples. On this date, as well as all other dates that sampling was performed (i.e., October 22–23rd, 2019, January 11–12th, 2020, March 29–30th, 2020, and March 2–4th, 2020), “event” composite samples were prepared by pooling together the samples collected in 42 h in flow-weighted proportions. In contrast to the other samples that were all collected in response to rain events, the March 2–4th, 2020 sample collection was triggered by elevated flows in the Don River caused by rapid snow melt.

Extraction was performed as described by Johannesen et al. (2021a) using Oasis hydrophilic-lipophilic balance (HLB) solid phase extraction (SPE) cartridges (6 cc, 500 mg) purchased from Waters (Milford, MA, USA). The cartridges were preconditioned with acetone, methanol, and pH neutral MilliQ water adjusted for pH with 3.0 M H$_2$SO$_4$. Triplicate aliquots (50 mL) of each sample were extracted using a vacuum manifold. The cartridges were eluted with $3 \times 3 \text{ mL}$ of methanol and immediately concentrated to a volume of 1 mL.

**Analysis**

Extracts and standards were analyzed using a Q-Exactive Orbitrap high resolution mass spectrometer (HRMS) coupled to an Ultimate 3000 ultra-high pressure liquid chromatography (UPLC) system, both supplied by Thermo Fisher (Waltham, MA, USA). Samples and standards injected into the UPLC at a volume of 25 µL were separated chromatographically using a Kinetex 2.6 µm C18 column (50×4.6 mm) purchased from Phenomenex (Torrance, CA, USA). The flow rate was 500 µL/min and the
column was maintained at room temperature. The mobile phases and their gradients were as described by Johannessen et al. (2021a, b). In brief, the binary mobile phase consisted of Solvent A, MilliQ water (pH = 7) with 0.1% of formic acid, and Solvent B, methanol with 0.1% of formic acid. The gradient was started with mobile phase B at 2%.

The spray voltage was 3.5 kV. The capillary rate were 50 AU (arbitrary units) and 15 AU, respectively.

The Orbitrap HRMS was operated in positive ionization mode with a heated electrospray ionization source (HESI-II). Parallel reaction monitoring (PRM) was used for data acquisition. The sheath gas flow rate and auxiliary gas flow rate were 50 AU (arbitrary units) and 15 AU, respectively. The spray gas flow rate was 0 AU and the S-lens RF level was 50.0 AU. The spray voltage was 3.5 kV. The capillary was operated at a temperature of 320 °C and the auxiliary gas heater temperature was 300 °C. The MS² had an AGC target of 2 × 10⁵ and the maximum IT was 100 ms. The isolation window used was 4 m/z and the resolution of the MS² was 70,000 at m/z 200.

The exact masses of the precursor ions monitored were m/z 269.20123 for protonated 6PPD, m/z 299.17540 for protonated 6PPD-q and m/z 212.11822 for protonated DPG. The protonated 6PPD was fragmented at a normalized collision energy of 10%, whereas the protonated 6PPD-q and DPG were fragmented at 40%. PRM allowed for the visualization of all the fragments of the precursor ions in the MS² spectra, which was especially important for identifying 6PPD-q in the ozonated standard, since it lacked purity.

Analytes were detected using a processing method with Genesis automated integration on the Xcalibur™ software (version 3.0.63). Samples were processed in a randomized manner. An 11-point calibration curve (diluted in methanol) that spanned 3 orders of magnitude (i.e., 0.488 to 500 µg/L) and had linear regression coefficients of R² ≥ 0.99 was used to quantify the analytes. The calibrations for 6PPD and 6PPD-q were conducted separately to avoid contamination. The limits of quantification (LOQs) for each analyte were considered to be the concentration of the lowest calibration standard. The lowest calibration standard for 6PPD, 6PPD-q, and DPG was 0.488 µg/L, which corresponds to a concentration in water samples of 0.0098 µg/L.

Quality Assurance/Quality Control (QA/QC)

An archived field blank consisting of HPLC-grade deionized water was re-analyzed for the presence of 6PPD-q and DPG. This field blank was collected at the site in a polyethylene ISCO bottle and was transported, processed, transferred to PET bottles. It was stored in the same manner as the archived surface water samples. Archived laboratory blanks of MilliQ water (pH = 7) were re-analyzed, as well. In addition, methanol reagent blanks were run at the beginning, middle, and end of each sample sequence to ensure QA/QC. Furthermore, a quality control (QC) standard consisting of 6PPD-q and DPG was run at the end of the sample sequence to look for instrumental sensitivity drift.

The efficiency of extraction of each target analyte using SPE with Oasis HLB cartridges was determined by spiking 100 mL of dechlorinated tap water with the analyte at a concentration of 1 µg/L. Extractions were performed in triplicate. These spike and recovery samples then underwent the same extraction and analytical procedure as described above and the recoveries were quantified with a 10-point curve that spanned over three orders of magnitude (0.488 to 500 µg/L) and had R² ≥ 0.97.

Databases

Precipitation data were accessed from “Toronto Historical Total Precipitation” available at toronto.weatherstats.ca/metrics/precipitation.html. Hydrometric discharge data (reported every 5 min) were accessed online from a database maintained by Environment and Climate Change Canada (i.e., wateroffice.ec.gc.ca) for Station 02HC024 on the Don River. These data, land-use information for the surrounding area, and sampling site characteristics (e.g., proximity to the highway, etc.) were described previously by Johannessen et al. (2021a, b).

Cumulative Load and Cumulative Volume Curves

Mass loads (g/event) were calculated by taking the weighted discharge from the storm event (L/hr) and multiplying these discharge data by the concentration values (g/L) and the duration of the sample collection period (hr). The runoff volume was calculated using discharge data for the receiving water site. The cumulative load and cumulative volume curves were generated as described by Peter et al. (2020).

Results and Discussion

No target analytes were detected in any field, laboratory, or reagent blanks, indicating that there was no significant source of contamination in the sample collection, extraction, and analysis processes and instrumentation. In addition, the QC standard run at the end of the sample sequence was within 5% of its expected value for all analytes, indicating that there was no drift in sensitivity for the analysis.

It should be noted that no appropriate stable isotope surrogates were spiked into the samples prior to extraction. However, our recent work (Johannessen et al. 2021b) indicated that SPE extraction by HLB cartridges was an efficient
method for extraction of 6PPD-q and DPG from water samples compared to extraction using Oasis mixed-mode, strong anion-exchange (MAX) and Oasis mixed-mode, strong cation-exchange (MCX) cartridges. Spike recovery tests with the target analytes dissolved in dechlorinated tap water showed that the method using HLB cartridges was efficient at extracting 6PPD-q (98% ± 7% recovery) and DPG (93% ± 8% recovery). Although no matched stable isotope surrogates were available for use as internal standards for the target compounds when the samples were extracted, our previous analyses of these archived samples showed that recoveries were > 80% for atrazine-d₅ added as an internal standard for HMMM quantification (Johannessen et al. 2021a).

Both DPG and 6PPD-q were detected at concentrations > LOQ in all archived extracts. There was no significant retention time shift (< 6 s) between sample analyses for both compounds. The identities of both analytes were confirmed by monitoring multiple confirmation ions. For instance, the MS² was monitored for m/z 187.0874 (C₁₁H₁₁N₂O₄), 215.0827 (C₁₂H₁₃N₂O₂), and 241.0970 (C₁₄H₁₃N₂O₂), which were described by Tian et al. (2021) as confirmation ions for 6PPD-q. The mass error for all instances of detection (n = 29) for the protonated analyte masses was below 5 ppm, as listed in Supplemental Information in Table S1.

Composite Sampling Results

Figure 2 illustrates the mean concentrations of DPG and 6PPD-q measured in replicate aliquots of composite surface water samples collected from the Don River during hydrological events in 2019 and 2020. The data on average rainfall, rainfall length, concentrations plus or minus the analytical standard deviation (± SD), and mass loads are also summarized in Table S2 in Supplemental Information. The highest concentrations of both DPG (i.e., 0.22 ± 0.07 µg/L) and 6PPD-q (i.e., 2.30 ± 0.05 µg/L) were detected in the composite samples collected during a rain event in October 22–23rd, 2019. The variations in concentrations of the two compounds are roughly proportional over the sampling dates. For example, the concentrations of both 6PPD-q and DPG increase by ~ 20% in samples from the October 22 event relative to the October 16 event and the levels of both compounds declined by ~ 30% in samples from the January 11th event relative to the October 22 event. The concentrations of DPG are within the range of 0.018–0.87 µg/L reported by Peter et al. (2020) and close to the concentration of 0.54 µg/L detected by Hou et al. (2019) in urban creeks during storm events. Notably, the concentrations of 6PPD-q in three of the five composite samples collected during the hydrological events exceeded the LC₅₀ value for coho salmon (Tian et al. 2021). These concentrations were in the range of those reported by Tian et al. (2021) in urban Seattle watersheds during storm events of < 0.3–3.2 µg/L.

In addition, both compounds were detected in extracts from samples collected during the March 2nd event when there was rapid snow melt, followed by a low amount (i.e., 9 mm) of precipitation (Fig. 2). Therefore, this tire-derived compound may also be sequestered in snow and released during snow melt; a possibility also suggested for HMMM surrogates (Johannessen et al. 2021a). However, sampling of the snowpack should be performed to confirm this hypothesis, as it may just be that the snow melt is mobilizing contaminants from the soil which are then transported to receiving waters.

The lowest concentration of 6PPD-q detected in any of the composite samples was 0.30 (± 0.01) µg/L, observed in composite samples collected during the March 29th event (Fig. 2). The fact that this rain event occurred during a lockdown prompted by the COVID-19 pandemic when there was greatly reduced traffic in the GTA may have contributed to the lower concentration of this chemical. However, this assessment is speculative and additional sampling must be performed to definitively link traffic volumes with contaminant concentrations.

Mass Loadings and Flush Dynamics

Mass loadings, rather than data on concentrations, are important information that indicate trends between pollution and the size of rain events (Peter et al. 2020). For both DPG and 6PPD-q, there was a positive correlation between the size of the storm event (i.e., the total amount of precipitation in mm) and the resulting mass loads (grams) of these contaminants in the Don River, as shown in Fig. 3. This apparent relationship was observed for both chemicals, but especially for DPG where the R² is 0.964. These correlations are largely driven by the data generated from a large rainfall event that occurred in the region on January 11–12th, 2020.

Fig. 2 Mean (±analytical SD) concentrations (µg/L) of diphenyl guanidine (DPG, dark gray bars) and 6PPD-quinone (6PPD-q, light gray bars) in replicate aliquots of composite samples collected from the Don River during rain events in the fall of 2019 and the winter of 2020, as well as during a period of rapid snow melt in March 2–4, 2020.
However, statistical analysis using the Pearson Correlation test indicated that the relationship was significant ($p < 0.05$). Future work should focus on determining whether these relationships are observed over a wider range of precipitation events. These data from the event-composite sampling suggest that increasing precipitation results in larger mass transfer of these contaminants into the urban surface waters. These quantitative results for DPG and 6PPD-q support the conclusions by Peter et al. (2020) that even small hydrological events can prompt the degradation of water quality by initiating the transport of tire-derived chemicals.

The concentrations of both contaminants were tracked throughout the duration of a storm event on October 16–17, 2019, and the results are presented in Table S3 in the Supplementary Information. The concentrations detected during this event ranged from 0.09 to 0.52 µg/L for DPG and 0.93 to 2.85 µg/L for 6PPD-q. The normalized cumulative load vs. normalized cumulative volume curves ($M-V$ curves) for this event is shown in Fig. 4 for both compounds. As seen in this figure, DPG undergoes pronounced first flush effects, as 60% of the contaminant load occurs within the first ~10% of runoff volume. However, DPG also exhibits a middle flush dynamic, as its $M-V$ curve remained consistently above the uniform (1:1) contaminant wash-off rate during the rainstorm event. 6PPD-q exhibits a middle flush dynamic, wherein the contaminant loading is sustained with the increasing volumes of cumulative runoff.

These results support the suggestion by Peter et al. (2020) that there may be reservoirs of tire-wear compounds in urban watersheds that can be considered semi-infinite in relation to the volume of runoff that is available to mobilize them. This “semi-infinite reservoir” hypothesis suggests that increased precipitation results in increased contaminant mobilization from these reservoirs or more distant sources, rather than depleting the reservoirs themselves (Stieglitz et al. 2003; Qin et al. 2016). This explains why a larger mass transfer of contaminants was observed with an increase in precipitation (Fig. 3), due to their transport-limited behavior. Further work is needed to confirm whether similar concentration changes of 6PPD-q are observed in other watersheds.

**Temporal Profiles of Contaminants**

The temporal profile of the concentrations of both DPG and 6PPD-q formed from the samples collected at 3-h intervals during the storm event indicated that there was a delay between the start of the rain event and when the contaminants reached their maximum concentrations (Fig. 5). This delay was between 8 and 11 h for DPG and between 17 and 20 h for 6PPD-q. Our previous work with these same samples determined that there was a 14 to 17-h delay for the concentrations of HMMP to reach a maximum in these samples (Johannessen et al. 2021a). In addition, there is a notable
difference between the time lag between peak discharge and the time needed to reach the peak concentration for DPG and 6PPD-q. The peak concentration of DPG was observed to occur ~3 h after peak discharge, whereas for 6PPD-q, the delay was ~12 h. These results indicate that, although these contaminants are likely coming from the same source, each compound may have different mobilities in soil and water as they are transported by road runoff into the receiving waters. In addition, the rate of oxidation of 6PPD to form 6PPD-q may contribute to the temporal differences.

It is notable that the concentration of DPG immediately declined in the archived samples after reaching a peak but still remained above the assumed baseflow levels represented by hours 2–5 and hours 41–44, whereas the concentration of 6PPD-q remained at a plateau around a maximum of 2.8 µg/L for a period between 12 and 18 h. This means that, over a prolonged period, 6PPD-q concentrations remained at maximum concentrations that were approximately three times greater than the median lethal concentration for coho salmon of 0.80 (± 0.16) µg/L reported by Tian et al. (2021). These data indicate that there is a need to assess the potential for toxic impacts to aquatic organisms in urban streams impacted by runoff of these tire wear compounds.

**Conclusions**

These data generated by the analysis of archived extracts of composite surface water samples indicate that hydrological events, even relatively small ones, can mobilize the tire-wear compounds DPG and 6PPD-q for transport in road runoff into urban receiving waters. Temporal samples collected at 3-h intervals from the Don River after the start of a rain event indicated that peak concentrations of 6PPD-q occurred late in the hydrograph and remained relatively constant for many hours. Similarly, DPG concentrations remained elevated above baseline levels well after the start of the rain event. It was observed that 6PPD-q exhibited a middle flush dynamic, where there was a prolonged influx of this contaminant into the Don River without dilution by a corresponding increase in total precipitation and runoff volumes. In addition, a positive relationship was observed between the size of the rain event and the related mass loading of both DPG and 6PPD-q into the Don River.

The maximum concentrations of 6PPD-q detected during rain events exceeded by 3–4 times the median lethal concentration of this compound for coho salmon reported recently by Tian et al. (2021). Both DPG and 6PPD-q were found in amounts comparable to those detected in surface waters in the Pacific Northwest region of the USA (Tian et al. 2021), supporting the assessment that tire-derived organic chemicals are ubiquitous in urban streams and rivers. We recently detected these compounds at similar concentrations in two urban rivers in the GTA (Johannessen et al. 2021b). Together, these findings highlight the need for effective and practical storm water treatment systems in the urban environment, especially near transportation corridors that are sources of tire wear compounds and other pollutants in road runoff. Notably, the City of Toronto has implemented various storm water treatment projects, including end-of-pipe facilities that treat the runoff both above and below ground prior to its discharge into receiving waters. In order to ensure the protection of urban watersheds, the efficacy of various storm water mitigation strategies must be tested for these emerging pollutants.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00244-021-00878-4.

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Declarations

Conflict of interest The authors declare no conflicts of interest associated with this study.

Data Availability Raw data are available upon request to Chris Metcalfe (cmetcalfe@trentu.ca).

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