# Contaminants of Emerging Concern in the Urban Aquatic Environment:

# Targeted and Non-targeted Analysis

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A Thesis
In the Department
of
Chemistry & Biochemistry

Presented in Partial Fulfillment of the Requirements
For the Degree of Master of Science (Chemistry) at
Concordia University
Montreal, Quebec, Canada

December 2023

## CONCORDIA UNIVERSITY

## SCHOOL OF GRADUATE STUDIES

This is to certify that the thesis prepared

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and submitted in partial fulfillment of the requirements for the degree of

**Master of Science (Chemistry)**

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# Abstract

## Contaminants of Emerging Concern in the Urban Aquatic Environment: Targeted and Non-targeted Analysis

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The urban aquatic environment is a repository of anthropogenic organic chemicals. Some of these are classified as contaminants of emerging concerns (CEC) due to their newly discovered environmental and health risks. These chemicals enter the aquatic environment through various routes, including urban runoff, wastewater discharge and snow melting. While some efforts have been invested in monitoring these substances along these routes, the coverage is limited to a small set of chemicals. This thesis aims to address environmental exposures of CEC and identifying lesser-known chemicals of environmental concerns, e.g., transformation products (TPs). Targeted and non-targeted analyses were conducted on 42 surface water and 30 snow samples in Montreal. The analyses focused on automobile-derived compounds used as industrial antioxidants and vulcanization accelerators. Samples were processed using solid-phase extraction (SPE). Liquid chromatography-mass spectrometry (Orbitrap LC-MS) was used for the sample analyses. Fifteen target compounds were quantified using internal standard calibration. Non-targeted screening of lesser-known contaminants and their TPs generated over 30,000 features in snow and 17,000 in surface water. Feature prioritization was based on peak intensity (>10000) and detection frequency (≥ 50%) while identification relied on retention time and substructural information. To further facilitate the identification of TPs, an additional oxidation experiment was conducted by exposing the parent compounds to ultra-violet (UV) radiation in a multi-lamp photochemical reactor. From the non-targeted analysis and transformation experiments, 37 analytes were identified. Our findings highlight the presence of lesser-known CEC including chemicals used as tire additives and their transformation products in the urban aquatic environment. This study also underscores the importance of using targeted and non-targeted screening approaches to identify and assess these compounds and their TPs.

Keywords: urban pollution, surface water, snow, non-targeted analysis, contaminants of environmental concern, chemical additives, rubber, transformation products.

# Acknowledgments

I would like to express my unreserved gratitude to all those who have contributed to the successful completion of this academic journey. This phase of study has been both exciting and challenging but the reward stretches into years to come.

May I first extend my heartfelt appreciation to my supervisor, Dr. Xianming Zhang whose guidance and expertise have been invaluable throughout the study process. His prompt response to academic queries, insightful feedback, and commitment to academic excellence remains critical in shaping this work into its best possible form.

I am also thankful to the members of my advisory committee, Prof. John Oh and Prof. Dajana Vuckovic for their scholarly input, and valuable suggestions that have significantly enriched the content and quality of this research.

To Dr. Heng Jiang, bringing answer to my endless question on HRMS played a crucial role in the successful execution of this research.

To the academic community of Chemistry and Biochemistry and especially the graduate school led by Dr. Cuccia, I am grateful for your support.

How about my TA corner, Mr Khal, Aunty Neema, Marika, Rita, and TA mates. It was fun working with you all.

To my lab group, Cassandra, James, Kevin, Xue, Meng; your incredible support is greatly appreciated.

Special thanks are due to all my dear friends, and you know yourselves. Your calls, food, advice, encouragement, and prayers were the biggest incentive. Your sacrifices are eternally etched on my mind.

Lastly, I would like to acknowledge the support and encouragement received from my family. Your unwavering belief in my abilities have been a constant source of motivation. It has not only facilitated this journey to its completion but has also inspired me to strive for excellence in general academic pursuits.

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## List of Abbreviations

| | |
|---|---|
| CMP | Chemical Management Plan |
| CEC | Contaminants of emerging concerns |
| DF | Detection frequency |
| DNA | Deoxyribonucleic acid |
| DS | Dumping site |
| HR-MS | High-Resolution mass spectrometry |
| HLB | Hydrophilic-Lipophilic Balanced |
| IAs | Industrial antioxidants |
| NPRI | National Pollutant Release Inventory |
| NEP | Non exhaust pollutants |
| NTA | Non-targeted analysis |
| PPDs | Para-phenylenediamines |
| PLS-DA | Partial least square-discriminant analysis |
| PAHs | Polycyclic aromatic hydrocarbons |
| PCA | Principal component analysis |
| m/z | Mass-to-charge ratio |
| NTA | Non-targeted analysis |
| RT | Retention Time |
| SB | Snowbank |
| TCs | Tire Chemicals |
| TPs | Transformation products |
| WWTP | Wastewater treatment plant |

### Chemical Abbreviation

| | |
|---|---|
| BTHs | Benzothiazoles |

| | |
|---|---|
| BTH | Benzothiazole |
| BTs | Benzotriazoles |
| BZT | Benzotriazole |
| CPPD-Q | CPPD-Quinone |
| DCH | Dicyclohexalamine |
| DPG | Diphenylguanidine |
| DPG-228 | Diphenylguanidine-228 |
| DPG-210 | Diphenylguanidine-210 |
| DPG-136 | Diphenylguanidine-136 |
| DPPD-Q | DPPD-Quinone |
| HMMM | Hexamethoxymethylmelamine |
| CPPD | N-Cyclohexyl-N'-phenyl-p-phenylenediamine |
| DCMM | N,N Dicyclohexylmethylamine |
| DEET | N,N-diethyl-meta-toluamide |
| DPPD | N,N′-Diphenyl-1,4-phenylenediamine |
| 6PPD | N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine |
| 6PPD-Q | N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine-quinone |
| 1,3_DMBA | 1,3-dimethylbutylamine |
| DPU | 1,3-diphenylurea |
| DTG | 1,3-di-o-tolylguanidine |
| HBTH | 2-hydroxybenzothiazole |
| MBTH | 2-methylbenzothiazole |
| MTBTH | 2-(methylthio)-benzothiazole |
| TMQ | 2,2,4-trimethyl-1,2-dihydroquinoline |
| CHD | 3-Cyclohexyl-1,1-dimethylurea |

| | |
|---|---|
| DBH | 3,5-Di-tert-butyl-4-hydroxybenzaldehyde |
| 4_ADPA | 4-aminodiphenylamine |
| 4s_DPA | 4-nitrosodiphenylamine |
| 4_HDPA | 4-Hydroxydiphenylamine |
| MHBZT | 5-methyl-1-H-benzotriazole |

# Chapter 1 General Introduction

## 1.1 The Urban Area as a Repository of Organic Chemicals

Increasing urbanization brings an unprecedented spike in the release of organic contaminants to the immediate environment. One key factor responsible for such hike is the high population density of the urban area which dictates a huge demand for industrial and household materials. The production and usage of these materials may lead to a local discharge of organic chemicals and wastes in and around the city.[1] Many of these chemicals can be harmful, underscoring the urban area as a significant hub for chemical pollutants. Some of these pollutants eventually find their way into the aquatic ecosystem establishing a land-aquatic gradient of chemical contaminants.

Specific routes including roadway runoff, stormwater drain, and snow-melting represent critical pathways through which these chemicals are introduced to the aquatic media. Therefore, it can be inferred that elevated pollutant concentrations in the cities can lead to increased chemical exposures in aquatic systems. Hence, various monitoring campaigns have focused on deepening our understanding of the processes dictating the formation, release, and distribution of these contaminants.

To facilitate environmental monitoring of organic chemicals, different directories such as the Chemical Management Plan (CMP)[2] and National Pollutant Release Inventory (NPRI)[3] have been established. The NPRI, for instance, serves as a comprehensive national inventory of contaminants released from Canadian institutional sources into water, land, or air. It also includes substances that are either disposed of or transferred for treatment, or recycling.[4]Established under the 1999 Canadian Environmental Protection Act,[5,6] this inventory gathers data from industrial facilities, numbering over 7000 across more than 280 industrial sectors.[6]The collected data are then made publicly available[7] to keep the society abreast of pollutant emissions or disposal.

## 1.2 Sources of Chemical Emissions in the Urban Environment

Certain urban pollutants occur as point source chemicals[1] because they originate from a single discrete source. This includes substances consistently discharged from industrial facilities or power plants, as well as those resulting from events triggered by natural disaster. Chemicals listed on the NPRI best describe this category of pollutants. Others pollutant may stem from a range (non-point source) of anthropogenic activities including those of vehicular emissions occurring along transit pathways.[1]

Vehicular emissions especially those from tailpipes have received an age-long scientific attention due to their direct implication on air pollution.[8–12] These emissions contain many harmful pollutants and are deemed the mainspring of global warming.[13] But beyond tailpipe emissions, concerns arise due to non-exhaust, chemical deposition from wearing of tires, brakes, and road surfaces. These chemical sources in conjunction with the resuspension of road dust currently exceed tailpipe emissions in many municipalities.[14] Therefore, the scope of more recent studies encompasses investigations of non-exhaust chemicals.

Despite coordinated efforts invested in monitoring non-exhaust contaminants, there is limited knowledge on the factors driving their concentration and distribution in the urban area. An ongoing question, related to the origin of these chemicals, is the extent to which critical environmental pathways to their introduction into the aquatic systems have been assessed. Also, within the aquatic media, many of these chemicals can degrade into more toxic, bio-accumulative, and persistent forms and the impacts of these degradation products remain largely unknown.

## 1.3 Rubber and Tire Derived Chemicals as Important Non-exhaust Pollutants

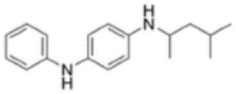
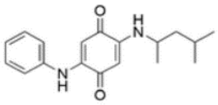
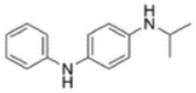
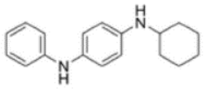
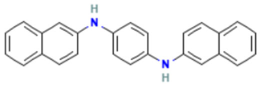
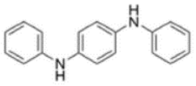
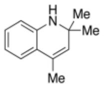
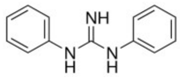
While rubber is the main component of most vehicle tires, it is crucial to acknowledge that tires are intricate products with compositions extending to various other materials. These materials may differ based on the tire's intended use and origin. The rubber tire of a typical passenger car is mainly composed of polymeric materials (butadiene for example), fillers (such as zinc oxide and carbon black), and over 100 chemical additives of varying proportions.[15–17] Three (3) important categories of tire additives exist according to their roles in tire manufacturing; protective, vulcanizing and processing additives.[16]

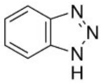
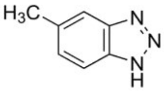
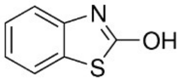
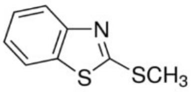
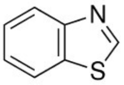
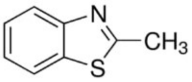
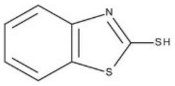
The protective additives function to extend the durability of rubber tires. They could be antioxidants (like secondary naphthylamines) functioning to protect against the effects of temperature and oxygen, or antiozonants which safeguard tires against ozone degradation. The most commonly used protective additives are the industrial antioxidants (IAs) (Table 1.1) especially the substituted para-phenylenediamines (PPDs) used as synthetic chemicals in several commercial and industrial products to inhibit oxidation reaction.[18] 2,2,4-trimethyl-1,2-dihydroquinoline (TMQ) is another prominent antioxidant that provides heat and anti-aging resistance to rubber materials.[19]

Vulcanizing additives represent chemical materials that speed up the vulcanization process. Vulcanization is the thermochemical process of rubber hardening necessary for the production of useful rubber materials. The process transforms the raw, sticky rubber into a more durable and usable material suitable for a wide range of applications, from tires to industrial products. It imparts several beneficial properties to the rubber products, including increased elasticity, strength, durability, and resistance to heat, abrasion, and aging. Important chemical agents necessary to bring about vulcanization (vulcanization accelerators, Table 1.1) include sulfur-containing compounds (thiazoles, thiurams, sulfonamides) and the nitrogen-containing additives (triazoles, phenylguanidines) that improve the mechanical and physical properties of the vulcanizates.[16]

Lastly, the processing additives include cross-linkers such as hexamethoxymethylmelamine (HMMM), homogenizers and plasticizers which enhanced the production efficiency and general quality of rubber materials.[16,20] In the rubber blend, plasticizers like oils and resins are incorporated to minimize friction within the tire. Representative of commercial plasticizers are the phthalates including dibutyl phthalate, diethyl phthalate, di(2-ethylhexyl)phthalate, and diisobutyl phthalate.[20] However, given the widespread use of phthalate in numerous other products including plastics, personal care products and lubricating oils, caution must be applied to not attribute their presence in the environments as solely tire-derived.

**Table 1.1 Chemical structures, leaching and production information of selected tire-derived chemicals (adapted from pubchem.ncbi.nlm.nih.gov).[49]**

| Class | Chemical Structure and Name | Abbreviation | Production Vol. | Solubility/Leaching Potential |
|---|---|---|---|---|
| Industrial Antioxidants |  $C_{18}H_{24}N_2$<br>N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine | 6PPD | China: 200,000 tons[23],<br>US: <100,000,000 lb[49] | 560 ± 200 µg/L[23] |
| |  $C_{18}H_{22}N_2O_2$<br>6PPD-quinone | 6PPD-Q | NA | 67 ± 5 µg/L[23] |
| |  $C_{15}H_{18}N_2$<br>N-isopropyl-N'-phenyl-1,4-phenylenediamine | IPPD | 10-15,000 tons[23] | NF |
| |  $C_{18}H_{22}N_2$<br>N-Cyclohexyl-N'-phenyl-p-phenylenediamine | CPPD | NF | NF |
| |  $C_{26}H_{20}N_2$<br>N,N'-di-2-naphthyl-p-phenylenediamine | DNPD | NF | NF |
| |  $C_{18}H_{16}N_2$<br>N,N'-Diphenyl-1,4-phenylenediamine | DPPD | US: <1,000,000 lb[49] | Less than 6PPD[6] |
| |  $C_{12}H_{15}N$<br>2,2,4-trimethyl-1,2-dihydroquinoline | TMQ | US:25,100 lb[49] | NF |
| |  $C_{13}H_{13}N_3$<br>Diphenylguanidine | DPG | US: <10,000,000 lb[49] | NF |

|  |  |  |  |  |
|---|---|---|---|---|
| Vulcanization Accelerators | <br>$C_6H_5N_3$<br>Benzotriazole | BZT | US: <10,000,000 lb[49] | NF |
|  | <br>$C_7H_7N_3$<br>5-methyl-1-H-benzotriazole | MHBZT | NF | NF |
|  | <br>$C_7H_5NOS$<br>Hydroxybenzothiazole | HBTH | NF | NF |
|  | <br>$C_8H_7NS_2$<br>2-(methylthio)-benzothiazole | MTBTH | NF | NF |
|  | <br>$C_7H_5NS$<br>Benzothiazole | BTH | US: 1,325,064 lb[49] | NF |
|  | <br>$C_8H_7NS$<br>2-methylbenzothiazole | MBTH | NF | NF |
|  | <br>$C_7H_5NS_2$<br>2-mercaptobenzothiazole | 2-SH-BTH | US: <10,000,000 lb[49] | NF |

NA=Not Applicable, NF=Not found.

## 1.4 Occurrence, Transformation, and Environmental Risks of Tire-derived Chemical

Evidence suggests that rubber tire additives easily partitions out of the rubber material during wear and tear because they are usually not covalently bonded to the polymer matrix.[20,21] As a result, these chemicals can leach into the immediate environment leading to their detection in different environmental media. Notable research showing the occurrence and toxicity of these chemicals exist.[16,22–27] In Canada, elevated levels of the vulcanization accelerator, 1,3-diphenylguanidine (DPG) in snowmelt,[28,29], HMMM in urban watershed,[30] IAs in surface water,[31] have been demonstrated. The concentration of some of these chemicals are reported in the μg/L range (more details in introductory section to chapter 3)

Tire chemicals (TCs) may undergo transformation either during their emission and retention on the road surface or even in the tire production process. Prominently, N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine-quinone (6PPD-Q) have been identified as an ozone-induced transformation product (TP) of the IA, N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD),[22,32] and similar pathway have been unravelled for related compounds.[26] Also the vulcanization agent, benzotriazole (BZT) can be readily degraded by ozone resulting in the formation of 1H-1,2,3-triazole-4,5-dicarbaldehyde.[33] Beside ozonation, photochemical transformation of 6PPD in the aquatic environment was recently reported.[34] The unravelling of these transformation pathways continue to provide insight on the potential underestimation of urban chemicals and associated ecological risks. Thus, environmental monitoring of tire substances must not be limited to parent compounds.

Tire leachates and their TPs continue to generate interest across the globe due to their pervasiveness and ecotoxicological risks to aquatic lives and mammals. Beside the widely reported lethality of 6PPD-Q observed in Coho salmons,[22] acute toxicity of 6PPD and 6PPD-Q affecting heart rate, swimming behavior, and oxygen consumption of zebrafish larvae in laboratory controlled experiments have been documented.[35] Studies have also demonstrated that benzothiazoles (BTHs) induce cytotoxicity and a transient rise in reactive oxygen species levels in cell lines of rainbow trout epithelium.[36] Similarly, sublethal effects, such as increased lipofuscin content in mussels exposed to tire rubber leachates have been attributed to chemicals including BTHs, phthalates, and polycyclic aromatic hydrocarbons (PAHs).[37] Given the ecological impact of tire additives on aquatic organisms, a systematic monitoring of tire derived compounds in urban aquatic systems has become critical.

In vivo assessments in rats demonstrate that TCs do not exhibit toxicity significant enough to affect pulmonary function but only results in minimal lung alterations.[38] However, in vitro analysis on human lung cells have revealed that exposure to TCs induces cell mortality, inflammatory responses, and nucleic acid damage and may lead to alterations in protein levels.[39,40] Consistently, exposing human macrophages to photoaged TCs resulted in notable increased secretion of inflammatory factors, decreased cell viability and heightened oxidative stress response.[41]

Environmental occurrence and the toxicities of TCs become particularly important when taking into account the number of vehicles on the road globally, estimated at over 1 billion and determined to reach 2 billion mark by 2040.[42] By implication, the ubiquity of tire-wear chemicals is believed to reach alarming levels with increasing urbanization

### 1.5 HRMS-based Targeted and Non-targeted Analysis of Chemicals of Environmental Concern

Chemicals that fall under established regulatory frameworks constitute a tiny fraction of the extensive body of known and unknown contaminants occurring in the urban environment. Therefore, current target analysis methods, in which chemicals are preselected, are limited because they cover a minute fraction of these environmental contaminants. This selection process can introduce bias and potentially exclude important chemicals.[43] Therefore, one of the most prominent trends in environmental monitoring involves the analysis of suspect chemicals, which are substances with basic information available (accurate mass of interest and unambiguous molecular formular), but no authentic standard.[43] This approach usually extends to non-target analysis (NTA), where there is no preselection of analyte. The NTA has now been recognized as a powerful method for capturing chemicals with varying physiochemical properties.[44] Moreso, it has emerged as an important tool for the comprehensive characterization of contaminants of emerging concerns (CEC), which are substances that are not currently regulated but can cause adverse human health and ecological risks.[45] To achieve a comprehensive risk assessment, it is essential to complement targeted and suspect approaches with non-targeted (figure 1.1) environmental monitoring based on high resolution mass spectrometry (HRMS). Such complementary approach has been employed in the analysis and monitoring of larger number of chemical stressors across different environmental media.[43,46,47]

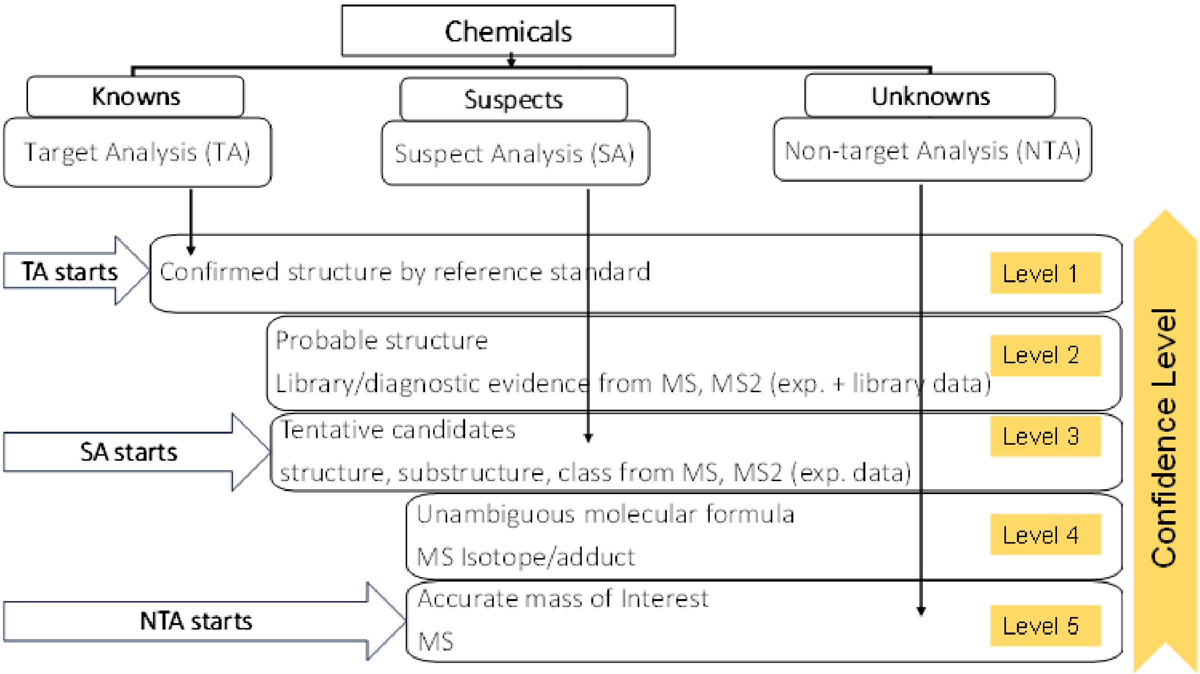


**Figure 1.1** **Chemical identification and characterization by targeted, suspect and non-targeted analyses as adapted from Schymanski et al., 2014.**

HRMS such as the orbitrap instrument has become a formidable tool in the characterization of environmental contaminants due to its high resolving power, excellent mass accuracy, speed and sensitivity. Whilst the resolving power measures the instrument's ability to distinguish two signals of similar mass-to-charge (m/z) ratio, the mass accuracy is a parameter that defines how close the measured m/z value is to the actual or experimental m/z value. The high mass accuracy and resolving power, with available acquisition modes for a wide m/z range make the HRMS the preferred tool for the analysis of tens of thousands of organic contaminants and their TPs in environmental matrices.[48]

## 1.6 Study Objectives

Whilst huge attention has been paid to pollutants listed under different regulatory directives, a significant number of lesser-known compounds occurring within the urban aquatic environment are yet to be unravelled. To assess environmental exposures and associated risk of these compounds, this study aims to:

- Investigate the occurrence of non-exhaust contaminants and a critical factor driving their mass loading in the urban environment. For this purpose, 15 TCs (Table 1.1) were selected based on their environmental importance. These chemicals are given priority over other contaminants of the same class because they are more widely reported as highlighted in section 1.4 above. These TCs also constitute the main tire additives used in rubber tire manufacturing as extensively discussed in sections 2.1 and 3.1.
- Identify and characterize lesser-known chemicals of environmental concern in environmental matrices (snow and surface water samples) through HRMS-based non-targeted approach.

This research has introduced novel quantitative chemical emission data that lays important foundation for improving public health and environmental policies. It has also identified lesser-known tire-derived chemicals of environmental concern and we believe these outcomes will have implications for a continued regulation of vehicle emissions standards and other transportation control measures.

# Chapter 2 Snow Outweighs Surface Water as an Indicator for Emissions and Aquatic Loadings of P-Phenylenediamine Antioxidants and Their Transformation Products in a Snow-Prone City

Joshua Onyeka Osagu[1*], Cassandra Johannessen[1], James Barnwell[1], Kevin Koueiki,[2] Tom Harner[3], Xianming Zhang[1*]

## Abstract

Chemical emissions from tire have become an emerging environmental concern because tires are made of a complex mixture of rubber and various chemical additives. As tires wear down, these compounds can be released into the environment. Some have toxic or harmful effects on organisms in aquatic ecosystems. To understand the role of road-side snow in trapping chemical tire additives and the corresponding influence of traffic intensity on concentration, we conducted a monitoring study to measure the concentrations of p-phenylenediamines (PPD) and related chemical accumulated in snow. PPDs in surface water were also measured. Using liquid chromatography (LC) and high-resolution mass spectrometry (HRMS), result across traffic categories showed that 6PPD and 6PPD-quinone (6PPD-Q) in snow were the foremost tire chemicals present. 6PPD and 6PPD-Q were at median concentrations of 364 ng/L and 975 ng/L for high traffic level, 238 ng/L and 641 ng/L for medium traffic level, 131 ng/L and 430 ng/L for low traffic level respectively. 6PPD and 6PPDQ levels in water were below MDL. Statistical analysis ($p < 0.05$) showed a clear dependence of concentration on traffic intensity. Other than 6PPD-Q, 4 predominant TPs of 6PPD were identified through suspect screening. Among these TPs, 1,3-dimethylbutylamine (1,3 DMBA) and 4-aminodiphenylamine (4ADPA), showed the highest (185 ng/L) and lowest (56 ng/L) mean concentrations respectively. Significant evidence indicated that the occurrence of these TPs was also driven by traffic volume. To evaluate the quantitative impact of traffic, a vehicular emission factor (VEF) was derived to estimate the mass loading of these substances into the environment. The findings revealed a significant load of 3000 ± 708 kg of 6PPD and 6PPDQ during a single winter season.

**Keywords**: traffic intensity, antioxidant, road snow, surface water, tire additive, p-phenylenediamines (PPD)

## 2.1 Introduction

The urban environment hosts numerous chemicals stemming from human activities and uses of commercial products. Among these chemical sources, traffic is a major contributor to urban chemical emissions and deterioration of various environmental media.[25,50] Beyond chemicals emitted from tailpipes, IAs originating from tire and rubber components of vehicles as non-exhaust emissions have become emerging environmental concern due to recently discovered environmental and health risks.[45,51]

The PPDs are IAs of high-production volume and used in the automobile industry for various rubber products such as seals, boots, hoses, and tires.[26,52,53] As one of the PPDs, 6PPD emerged as the most widely used antiozonant in the rubber industry during the early 2010s [54] and has been applied in approximately 2.3 billion tires globally.[32] Its annual production in 2020 reached 45,000 and 200,000 tons in the US and China, respectively.[18,55] Concern arises due to the toxicity of 6PPD-Q which is a transformation product of 6PPD reacted with ground-level ozone.[22] With various toxic effects, [27,56–58] 6PPD-Q has been identified as the primary toxin causing widespread pre-spawn coho salmon mortality.[22,59]

PPDs and their TPs enter receiving waters through different routes. These pathways include urban runoffs, wastewater discharge and snow melting.[25,28,60–63] Amongst these routes, snow melting is recognised as an important environmental pathway of different pollutants due to the snow's high capacity for these chemicals. [64–66] Some of these pollutants can volatilize back into the atmosphere, whilst others amass within the snow and are subsequently released into the urban aquatic media contributing up to 60% of the overall chemical load.[25,66] Recent studies have demonstrated the presence of PPDs and their TPs in snow.[28,62,65] However, due to low sample sizes, these studies did not comprehensively access factors such as traffic intensity that influence the abundance of the PPDs and their derivatives in snow.

To assess environmental exposure and associated risk, there is an urgent need to quantify emissions of PPDs from traffic. A recent study[67] found the total leachable 6PPD-Q accounts for 46% of 6PPD-Q present in tire wear particles (TWP), from which an emission factor of 5.2 μg of 6PPD-Q per gram of TWP is derived.[67] However, this emission quantification is only limited to 6PPD-Q and it does not apply to regions with freezing winter during which leaching of 6PPD-Q from TWP through water does not occur. Considering that TWPs loading onto road sides ranges between 95-99% of total emitted TWP,[68] in snowy days, vehicle-emitted tire additives should be largely captured by snow on roads. As such, we hypothesized that concentrations of the tire additives in roadside snow can be linked to the traffic intensity.

In this study, through judgement sampling and monitoring of PPDs and their TPs in surface water and roadside snow, we demonstrated that for a snowy city, roadside snow is a better environmental monitoring medium for assessing emissions and potential aquatic risk. We explored traffic intensity as an important factor controlling concentrations of PPDs and their TPs in roadside snow, from which we estimated urban emissions of the tire additives with emerging environmental concern. The results of this study provide a valuable reference for developing routine monitoring plan and model simulations in a snow-prone city for the chemical assessment and regulations.

## 2.2 Materials and Methods

### 2.2.1 Sample Collection and Processing

Sampling of surface water and snow were conducted in Montreal, Quebec, which is a main metropolitan area and represent the typical climate in Canada. Collection of surface water (42 sites) and snow (30 sites) samples were carried out in August 2022 and January 2023 respectively (Text S2.1). The water sampling was designed to cover influences from roadway runoffs, WWTP and stormwater drains. The snow samples were collected and covered a snow fall event over January 19-20, 2023. Twenty road snow samples were collected by pooling samples from different points of a road section with varying traffic intensities. Six snow samples were collected from natural parks (areas without traffic influence within 1 km radius) to serve as background references. In addition, two samples were obtained from snowbanks used as temporary dumping areas and two were collected from officially designated municipal snow dumping sites. The road snow were classified (Text S2.2) into high, medium and low road traffic samples according to available traffic information.[69] Both surface water and snow samples were transported on ice to the laboratory and processed using HLB Oasis solid-phase extraction (SPE) cartridges (6 mL, 200 mg, Sigma Millipore USA). For further details on sample collection, processing, and road classification, refer to Text S2.1, S2.2, Figure S2.1, and Table S2.1.

### 2.2.2 Chemical Analysis and Quantification with HPLC-MS

Chemicals analyzed with standards include 6PPD, 6PPD-Q, N-isopropyl-N’-phenyl-1,4-phenylenediamine (IPPD),N,N'-Di-2-naphthyl-p-phenylenediamine(DNPD),N-Cyclohexyl-N'-phenyl-p-phenylenediamine CPPD) and N,N′-Diphenyl-1,4-phenylenediamine (DPPD). Detailed information on the solvents and chemical standards used in this study are presented in Text S2.3 and Table S2.2a and b. A high-performance liquid chromatography (HPLC) (Agilent 1100 series LC system, equipped with a binary pump, de-gasser and an autosampler). coupled to an LTQ orbitrap velos mass spectrometer (MS) (Thermo Fisher Scientific, USA) was used for chemical analysis and the analytical method are detailed in Text S2.4. Briefly, full scan MS1 data were acquired with data-dependent MS/MS acquisition. Quantification was performed by the internal standard method with 7-point calibration curves (0.05–200 ng/ml, $R^2$ > 0.995). Quantitation relied on peak area response, employing an automated processing method with Genesis integration in the XcaliburTM software (Thermo Scientific version 3.0.).

The most intense ions of each quantified analyte were extracted using a ± 5 ppm window. Manual verification was performed for peak selection and integration.

### 2.2.3 Suspect Screening of PPDs and Their Transformation Products

Suspect screening of transformation products was performed using the Compound Discoverer (CD) software (version 3.3) based on exact masses (MS 1 spectra) and corresponding fragmentation information (MS 2 spectra). MS data were fed into the CD software using optimized workflow for suspect and untargeted environmental studies to generate chemical features. Hits were prioritized if they had a detection frequency of ≥ 50 % in all samples and peak area > 10,000. Subsequently, suspect search was performed for PPD-related chemicals using an in-house list that was generated mainly from the NORMAN exchange list (More information on Text S2.5, Table S2.3). Chemicals reaching a level 2 confidence[70] where a probable structure is generated by diagnostic evidence and library spectrum match, but lacking native standards were semi-quantified using the relative response factor (RRF, equation S2.1)

### 2.2.4 Quality Control

HPLC grade water was used as blanks (field, procedural) and was treated under the same conditions as samples during and after the sampling process. For every 10 samples, at least one field or procedure blanks and one samples of HPLC-grade water spiked with 50 ng of the targeted analytes were included for SPE processing and instrumental analysis. Method quantification limits (MQLs) were calculated as 3 times the standard deviation (SD) of the analyte concentration in the procedural blanks plus the average concentration of the analyte in the blanks. If the analyte was not detected in the blank, the MQL was established based on the concentration of standard that produces a peak in the matrix with a signal-to-noise (S/N) ratio of 10. The method detection limit (MDL) was taken as the calibration standard that produced a S/N of 3. MDLs, M QLs and Recoveries of the target compounds are presented in Table S2.4.

### 2.2.5 Data Analysis and Statistics

Statistical analysis was conducted using R-Studio 4.1.3. The normality and heteroscedasticity of the data were tested using Shapiro−Wilk and Bartlett's tests. Because the data set violated some of the normality and homogeneity of variance assumptions, the comparison of concentrations across different levels of traffic (Text S2.2, Table S2.5, S2.6, S2.7, S2.8) were performed using the non-parametric Kruskal−Wallis test followed by Wilcoxon post hoc test at a significant level of 0.05.

### 2.2.6 Emissions of PPDs and their Transformation Products from Road Traffic

Following statistics showing that traffic levels significantly influence concentrations of PPDs and their TPs in roadside snow, we derived a vehicular estimation factor (VEF) that estimates the average emissions of PPDs and their TPs per vehicle per kilometre. Because we took a snow sample by pooling 3-5 snow samples from the cross-section of the road, we can assume that the snow samples we took represent snow on the whole road section since the snow fall began at 10 PM the previous day. As such, VEF (μg/vehicle/km) can be calculated as C (ng/L) * 1000(L/$m^3$) * 0.001(μg/ng) * PQ(mm) * 0.001(m/mm) * W(m) * 1000(m/km) / N(vehicle), where C is the concentration of a PPD or its TP measured in snowmelt, PQ and N are the precipitation quantity[71] and the number of vehicle passing through the cross-section of the road from the start of the snow event to the sampling time, W is the width of the road where a snow sample was taken. PQ was determined based on hourly precipitation rate from weather data archived by Environment and Climate Change Canada.[71] W was determined using the Google Earth Pro (version 7.3). While we could retrieve daily averaged traffic data[72] for the road we sampled, we do not have hourly traffic data for each of our sampled roads to determine

N. Therefore, we used hourly traffic data available for a road within our sampling area in Montreal[72] (Figure S2.3) and assumed that the hourly traffic pattern applies to all the roads, which enable us to scale the daily average traffic to hourly traffic which is essential to determine N.

## 2.3 Results and Discussion

### 2.3.1 *p*-Phenylenediamines and Transformation Products in Snow and Surface Water

10 phenylenediamines (PPDs) were frequently detected (>50%) with concentrations between 3 and 1276 ng/L (Supporting Information, Table S2.6). The most frequently detected PPDs in snow were 6PPD and 6PPD-Q (Figure 2.1 A-B, 2.2A). Across traffic categories, 6PPD and 6PPD-Q were the dominant pollutants with median concentrations of 364 ng/L and 975 ng/L for high RTL, 238 ng/L and 641 ng/L for medium RTL, 131 ng/L and 430 ng/L for low RTL, respectively (Table S2.6, S2.7). Thus, there was a discernible dependence of concentration on traffic intensity (Figure 2.2C). This trend was true of all measured PPDs and related compounds with high road traffic levels (High RTL) showing significantly higher concentrations than Low RTL. The concentrations of all target analytes in the high RTL group were significantly higher ($p < 0.05$) than those of the low RTL in all sites except for DPPD and DPPD-Q (Table S2.8) further indicating the influence of traffic volume on chemical loading. However, there was no significant difference ($p > 0.05$) between high and medium RTL or between medium and low RTL. Clearly, the concentration of 6PPD in comparison to 6PPDQ is consistently lower across all sites (Figure 2.2B). Across the three road levels, 6PPDQ showed about 3 times higher median concentration than its parent compound. In contrast, this trend was not observed for CPPD and CPPD-Q with the later showing a lesser concentration range (3 - 27 ng/L) compared to its parent compound, CPPD (4 -355 ng/L) across all sites. 6PPD showed a median concentration (290 ng/L) approximately 2-fold higher than CPPD (134 ng/L),

a pattern that can be attributed to the higher production volume of the principal antioxidant (6PPD) in rubber. However, the unusual CPPD/CPPDQ (Figure 2.2B) ratio remains unclear since the quinone derivative (CPPDQ) is expected to show higher concentrations than the parent CPPD across all sampling sites.

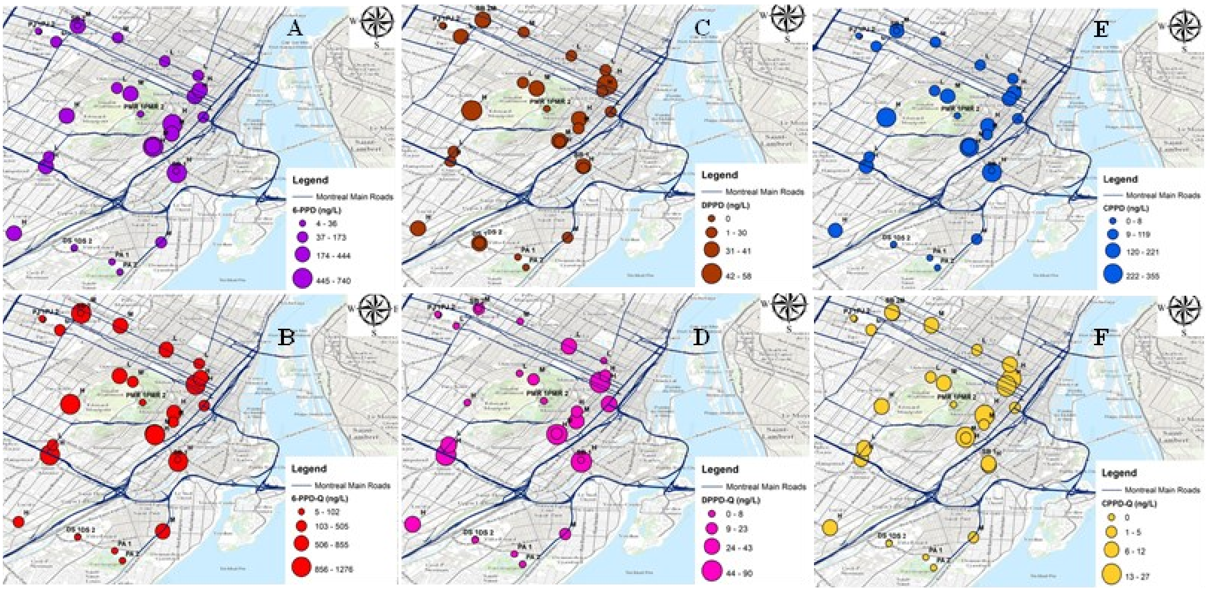


**Figure 2.1 Spatial distribution of detected p-phenylenediamine antioxidants and their quinone transformation products in road-side snow samples from Montreal, (A-B) 6PPD and 6PPD-Q, (C-D) DPPD and DPPD-Q, (E-F) CPPD and CPPD-Q**

The suspect screening analyses predominantly identified 4 compounds namely, 4-nitrosodiphenylamine (4s-DPA), 4-aminodiphenylamine (4ADPA), 4-Hydroxydiphenylamine (4HDPA) and 1,3-dimethylbutylamine (1,3 DMBA) in the snow samples. These PPDs related chemicals are also associated with tire rubber manufacturing and were recently demonstrated as TPs of 6PPDs resulting from amine and ring oxidation side cleavage of 6PPD.[23] These chemicals demonstrate excellent linear relationships with 6PPDs (Figure S2.4) and their pathways of occurrence have been elucidated in Figure 2.3. All reported TPs were consistently detected across all snow sampling sites with 1,3 DMBA (185 ng/L) and 4ADPA (56 ng/L) showing the highest and lowest mean concentrations respectively. This concentration pattern could be due to the unique amine oxidation side cleavage pathway generating 4-ADPA from

6PPD. 4-ADPA formed can be further oxidized to other TPs including 4s-DPA and another terminal stable product known as 4-nitrodiphenylamine (4NDPA).[23] Zhao et al.,[23] reports that this pathway (Figure 2.3) results in a 33 % loss by mass of the starting TP (4ADPA) whilst 1,3-DMBA when formed is not further oxidized.

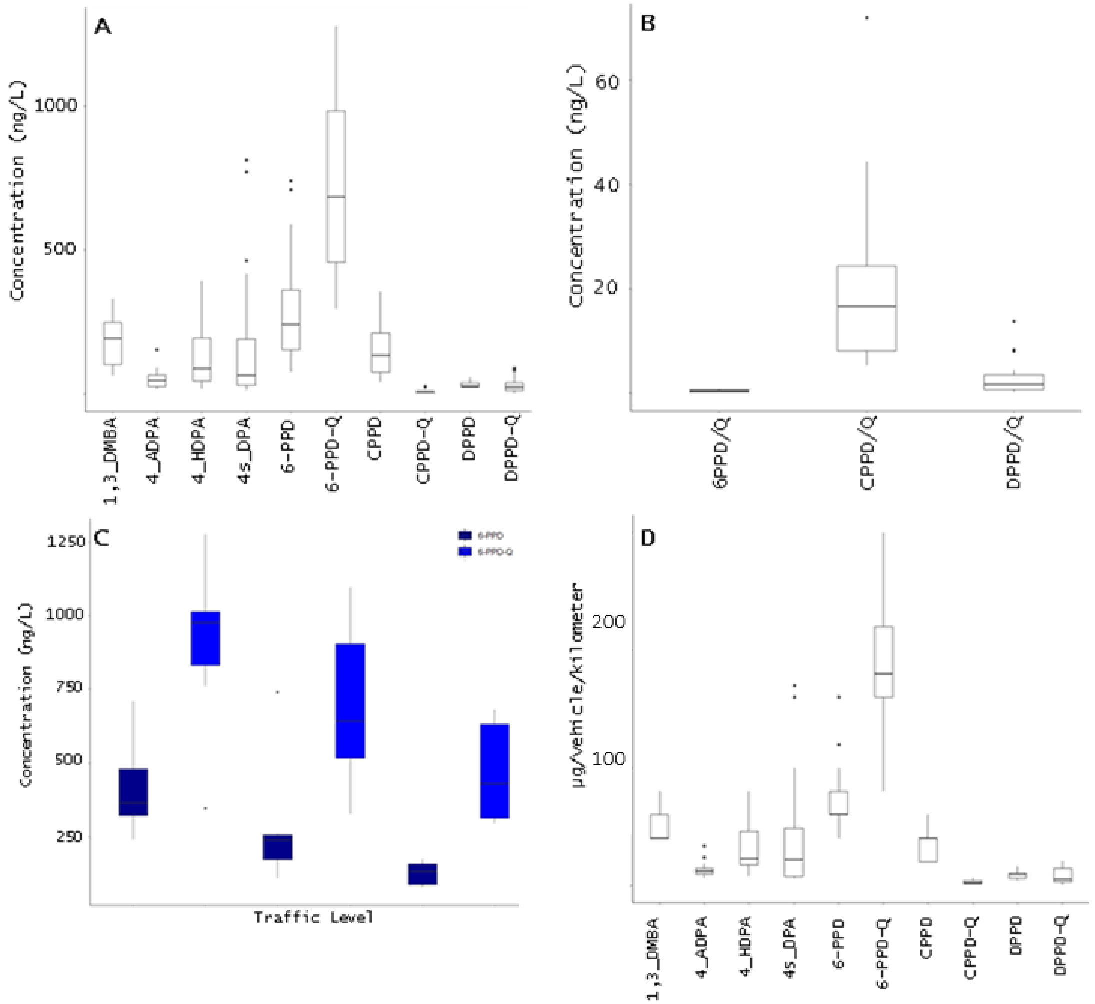


**Figure 2.2 p-Phenylenediamine antioxidants and their transformation products (TPs) in road-side snow samples. (A) Concentration of detected traffic derived PPDs and their TPs. (B) Comparison of parent analytes and their quinone derivative. (C) Traffic influence on the concentration pattern of 6PPD and 6PPD-Q (D) Mass loading of detected chemicals released per vehicle per kilometre road section in a typical winter season. Boxplots are defined as follows: center line, median; boxplot edges, 25th and 75th percentiles; and whiskers, 5th and 95th percentiles of the distribution.**

Concerning the background sample collected from all 3 natural parks, our findings showed that traffic related chemicals were largely non-detected. However, 6PPD and 6PPD-Q were detected at much lower concentrations (4-10 ng/L) compared to roadside snow which clearly demonstrates that these chemicals are released as a result of urban activities. On the other hand, samples from the snow dump sites showed much higher concentrations reaching 100 ng/L for two of the four dumping sites. Interestingly, the dumping sites were hypothesized to show elevated level of these chemicals, but the resulting concentrations were much lower than those of the road traffic arterials. This could be attributed to the impact of dilution of freshly precipitated snow. Also, the fresh snow could have covered a significant portion of the preexisting snowpack at the time of sampling.

Generally, the concentration of tire wear compounds especially 6PPD-Q in the present study is consistent with reported results globally. The mean concentration of snowmelt samples from Saskatoon was reported at 80−370 ng/L[28], although the authors indicated that these values might have been underestimated by a factor of 2 or more due to their quantitation approach. Similarly, traffic related chemicals recently identified in snow from Liepzig, Germany were in the range 1.3 ng/L to 75 $\mu$g/L.[25] What is of concern is that the highest recorded 6PPDQ value in our current study, which stands at 1276 ng/L, exceeds the $LC_{50}$ value (95 ng/L) of the compound[59] for coho salmon by approximately 13 times. This indicates that coho salmon and other susceptible aquatic organisms in the Montreal area could be facing a potential threat from this toxic quinone compound.

### 2.3.2 *p*-Phenylenediamines and Transformation Products in Surface Water

In contrast, the concentration of these compounds in surface water were found below MQL probably due to zero to light rainfall events prior to the sampling campaign. The closest heavy precipitation event (17.4 mm ) occurred 10 days to the sampling,[73] followed by multiple dry weather conditions. Given the unstable nature of some of these compounds[67], especially 6PPD, low detection is not unexpected. More so, the non-detects is also likely due to the grab sampling approach employed during the sampling campaign. Nonetheless, these results seem to align with the findings of Johannessen et al reporting very low concentration of tire-wear compounds (1.8 ng/L) during low precipitation (9 mm) periods.

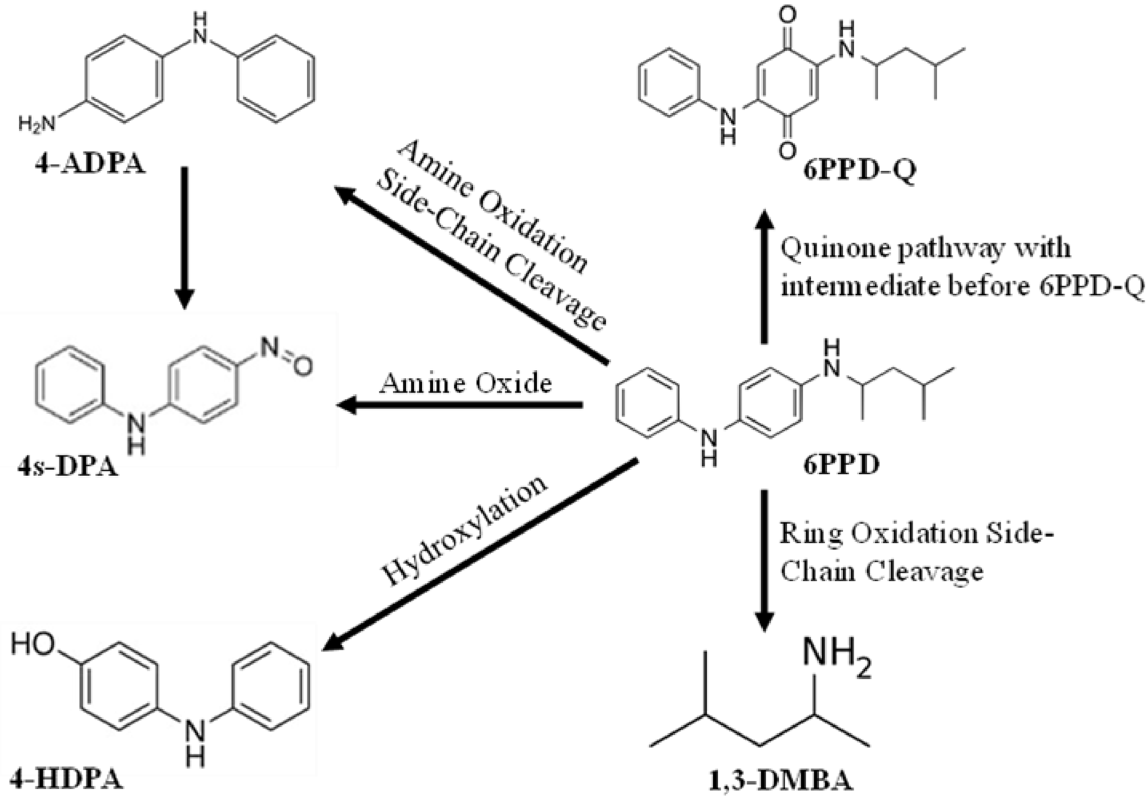


**Figure 2.3 Transformation pathways (as adapted from Zhao et al., 2023) for 6PPD along with chemical formulas, and structures for detected TPs. All TPs are identified with confidence levels 1.**

### 2.3.3 *p*-Phenylenediamine Loadings from Vehicular Emissions in Urban Roads

The hierarchical classification of urban roads is dictated by the traffic volume. Hence, the deposition of tire wear particles (TWPs) within a road section is hypothesized to be proportional to traffic intensity across local and main traffic corridors. The present result has proven a clear dependence of the concentration of PPDs on traffic density (Figure 2.2C). Using the vehicular emission factor (VEF) the chemical loading of this group of contaminants was estimated and the amount of PPDs deposited per vehicle per km in a typical winter season is hereby reported (Table S2.5).

Corresponding to concentration, 6PPD and 6PPD-Q released were found in highest amounts ranging from 36-125 and 73-267 µg/vehicle/km respectively (Figure 2.3D, Table S2.5). The average amount of 6PPD-Q emitted per vehicle/kilometre into the environment was 170 µg/g and it represents the highest amount of the reported chemical set in this study. Other analytes with high emission potentials include 6PPD, 4sDPA, 1,3-DMBA, CPPD, and 4HDPA with average emitted amounts 62, 37, 43, 33 and 29, µg//vehicle/km respectively. These values are likely to vary according to different seasons owing to the effect of temperatures on TWP deposition since it has been found that elevated temperatures lead to a higher production of tire wear particles, intensifying road surface wear, especially during the rapid increase in tread temperature.[74] The same report by Chang et al. shows that high temperatures significantly enhance the likelihood of larger particle deposition and also alters the mechanism of wear. These temperatures may cause the tire to deform more easily, leading to increased contact with the road surface and subsequent wear particle generation. Summing up the average emission rate for 6PPD and 6PPD-Q implies that 180 ± 59 ug of these chemicals is released per vehicle/km. Since the lifespan of a set of car tires is approximately 5 years[75] (6000 km/winter)[76] and given an estimated 2 million cars in Montreal,[77] we can deduce that approximately 2160 kg of 6PPD & 6PPDQ are loaded into the environment from Montreal over a given winter season.

Our study highlights the ubiquity of tire rubber-derived compounds in the Montreal area of Quebec, Canada. Distinctive and consistent patterns of chemicals related to traffic were detected in snow collected from roads, clearly distinguishing them from the background reference samples taken from natural parks. The concentration of these chemicals and their transformation products showed a discernible influence of traffic volume, with the relationship particularly evident in the case of 6PPD and its quinone derivative. However, the quantity emitted by individual vehicle per kilometre remained consistent across different traffic arterials. The present study not only offers valuable information about the occurrence and distribution patterns of tire-wear compounds, but of greater significance, it expands the limited emission and loading data of TWP-borne PPDs to the environment. This is critical for comprehending the quantitative introduction of these compounds to aquatic media and further highlights snowmelt as an important reservoir of this group of chemicals.

**Author Contribution**

**Joshua Osagu: Joshua Osagu:** Conceptualization, Snow and Surface Water Sampling, Sample Preparation, Investigation/Instrumental analysis, Writing – original draft, all data analysis except for PCA, Writing – review & editing final draft. **Kevin Koueiki**: Assisted in Snow Sampling. **Cassandra Johannessen:** Assisted to review the first draft and supplied most of the chemicals used for the project. **Xianming Zhang:** Conceptualization, Surface water sampling, reviewing & correcting the thesis drafts, data analysis-PCA, and general supervision. **Tom Harner:** Conceptualization.

**Note**

The authors declare that they have no known competing financial interests.

**Acknowledgement:**

The authors would like to thank Dr. Heng Jiang, the Mass Spectrometry Specialist of the Centre for Biological Applications of Mass Spectrometry (CBAMS), Concordia University, Montreal, QC, Canada for facilitating access and usage of the HRMS instrument used for this research.

# Chapter 3 Targeted and Non-Targeted Analysis of Rubber Additives and their Transformation Products in Urban Snow and Surface Water

Joshua Onyeka Osagu[1*], Cassandra Johannessen[1], Xianming Zhang[1*]

## Abstract

The ubiquity of non-exhaust pollutants (NEP) derived from tire emissions in urban aquatic environments raises concerns about their potential ecological and human health impacts. This study employs a comprehensive analytical approach to investigate both targeted and non-targeted tire-derived chemicals We apply liquid chromatography (LC) and high-resolution mass spectrometry (HRMS) for widely studied and lesser-known tire additives and their transformation product (TPs) in snowmelts and surface water in Montreal, Quebec, Canada. Targeted analysis highlights high concentrations of rubber additives, with 1,3-diphenylguanidine (DPG) as the most prominent chemical in road-side snow (4600 ng/L median concentration). Detected vulcanization accelerators such as benzothiazole (BTH) , 2-hydroxybenzothiazole (HBTH), and 5-methyl-1-H-benzotriazole (MHBZT) also showed elevated concentrations in snowmelts indicating 1650 ng/L, 851 ng/L and 816 ng/L median concentrations respectively. These chemicals also occurred in surface water but at comparatively lower levels. The median levels of BZT (359 ng/L) and BTH (1650 ng/L) for snow samples were approximately 9 and 4 times higher than in surface water respectively. Additionally, suspect and non-targeted analysis identified 11 lesser-known compounds, including Dicyclohexalamine (DCH), diphenylguinidine-136 (DPG-136,) and Hexa(methoxymethyl)melamine (HMMM) corresponding to 1641 ng/L ,527 ng/L and 1908 ng/L median concentrations in snow samples. To enhance the confidence in the identification of TPs, a specific strategy based on $H_2O_2$-induced transformation experiment was employed. This involved comparing the chromatographic and mass spectra information of experimental samples to field snow and surface water samples.

**Keywords:** tire-derived chemical, snowmelts, surface water, non-exhaust pollutants (NEP), vulcanization accelerator, rubber additive, liquid chromatography (LC)-high-resolution mass spectrometry (HRMS), targeted and non-targeted analysis.

## 3.1 Introduction

The widespread production and use of organic chemicals for industrial and commercial purposes has resulted in the introduction of man-made chemicals into the aquatic environment posing potential risks to both aquatic organisms and the human population. In urban environmental systems, a great portion of these chemicals originates from traffic emissions, particularly non-exhaust pollutants (NEPs). NEPs comprise a complex mixture of particulate matter derived from road surface erosion, brake and tire wear, road dust resuspension, and volatile components from solvent release and fuel evaporation.[14,78] NEPs are unregulated although they account for up to 90% of the total mass of particulate matter emitted from traffic-related sources.[78] Thus, there is a growing interest to expand the development of solid analytical framework for the detection and characterization of these chemicals as NEPs in the environment.

Amongst the components of NEP, tire-originated emission stands out as a prominent source of traffic related contamination. Many tire additives including industrial IAs and vulcanization accelerators have been associated with ecotoxicological hazards.[27,58,59,79–81] [16,27,59] IAs such as the PPDs are applied in the automobile industry to protect rubber materials including tires from deterioration caused by oxygen, heat or ozone.[26,52] Notable examples of PPDs are 6PPD and CPPD. Vulcanization accelerators, such as 1,3-diphenylguanidine (DPG), benzothiazoles (BTHs) benzotriazoles (BTs) and their derivatives also play crucial roles in tires by enhancing the thermochemical transformation of raw rubber materials into hardened, more durable and usable forms.[16] Due to their extensive use in tire industry, substantial amounts of these chemicals derived from tires can be released into different environmental media. Once released, these chemicals can undergo complex biotic and abiotic transformations, forming new toxic substances and further exacerbating the environmental concern associated with the chemicals used in tires.[62,79]

The prevalence of tire usage has resulted in the widespread occurrence of tire-derived chemicals and TPs in the environment, as documented in numerous studies.[16,22,26,60,79,82] Tire-derived chemicals have been detected in various environmental matrices, including stormwater, snowmelt, surface water, wastewater, and sediments. [28,60,80,83] Often, the concentrations of the tire-derived chemicals including their TPs exceed those of many other emerging contaminants. A study by Challis et al. [28] reported one of the highest average DPG concentrations at 60 µg/L and 1 µg/L in stormwater and snowmelt respectively. The research indicated that up to 15 kg of DPG can be loaded to aquatic environment from a single rain event. Further, over 80% detection frequency was reported of 6PPD-Q in snowmelt samples (80−370 ng/L).[28] Other tire additives such as BZT and 5-methyl-1-H-benzotriazole (MHBZT) also known as tolyltriazole have been detected in surface and wastewater samples at concentrations in the µg/L range.[83] Elevated levels of BTHs are also documented in WWTPs, urban runoffs and water receiving bodies.[80,84] Although, the presence of BTHs and BTs in surface water can be attributed to incomplete elimination from WWTPs (with removal rates ranging from 20-70%),[80,83,84] further evidence suggests that other pathways such as urban runoff can be more significant sources of these contaminants.[85]

Contaminants originated from rubber can cause adverse effects on humans and aquatic organisms. A notable effect is the mortality syndrome observed in coho salmon in the Pacific Northwest (USA), due to the toxicity of 6PPD-Q, present in stormwater runoff.[22] Another tire-derived compounds, DPG has also been reported to exhibit acute toxicity (48-hour $LC_{50}$) and (96-hour $LC_{50}$) for water fleas and bluegill fish respectively at moderate exposure concentration (0.5-10 mg/L).[86] Since the detection of benzothiazole (BTH) in human atherosclerotic aortas (10 ng/g, mean concentration),[87] several other studies have documented BTH-induced genotoxicity, cytotoxicity, and carcinogenicity.[88,89] More so, In vivo and in vitro investigations of these chemicals have revealed evidence of endocrine disruption..[90]

Despite extensive research on tire additives (especially vulcanization accelerators) in WWTPs, urban runoffs, stormwater, [83,84,91,92] knowledge of lesser-known chemicals originated from tire additives in critical pathways such as snow melting is limited. Considering evidence suggesting the exposure to these chemicals in urban area and the resulting adverse aquatic effects, it is imperative to explore major sources through which these substances are mobilized into the urban aquatic environment. To address the research needs in this study, we collected snow and surface water samples and analysed well-known and lesser-known compounds related to rubber additives using LC and HRMS-based targeted and non-targeted approaches. Oxidation experiment was conducted for the identification of TPs in environmental samples. The results of this study suggest that road snow is an environmental medium of concern for tire additives and their TPs in snow prone cities like Montreal and can be the medium for environmental monitoring for source tracking.

## 3.2 Materials and Methods

### 3.2.1 Sampling Sites, Sample Collection and Processing

Sampling and processing were carried out as described in chapter 2, section 2.2.1

### 3.2.2 Chemical Analysis and Quantification

Surface water and snow sample extracts were analysed using a LC (detail information in Text S2.4 and Table S2.2) coupled to orbitrap mass spectrometer (LTQ orbitrap velos, Thermo Fisher Scientific, USA) for targeted analysis of nine compounds known as rubber tire-derived additives, including DPG, BZT, MHBZT, BTH, 2-hydroxybenzothiazole (HBTH), 2-methylbenzothiazole (MBTH), 2-(methylthio)-benzothiazole (MTBTH), 2-mecaptobenzothiazole (2-SH-BTH) and TMQ. Detailed Information on the chemical standards and solvents used are presented in Text S2.3 and Table S3.1. For HRMS detection using orbitrap MS, a full scan (m/z 100−500, resolving power 100,000) was combined with data-dependent MS/MS acquisition (for details, see Text S2.4 and Table S2.2b). The internal standard method was used for quantitation with a 7-point calibration curves (0.1–1000 ng/ml, $R^2 > 0.995$). Other details are captured in section 2.2.2.

### 3.2.3 Quality Control

HPLC grade water (Fisher Scientific, Waltham, Massachusetts, USA). was used for solvent, field and procedural blanks. Blanks were treated under the same conditions as samples during and after the sampling process. More details in section 2.2.4

Recoveries (Table S3.3) were assessed in snowmelts and river water by comparing the differences between standard fortified blanks spiked before and after extraction. Recoveries represent losses incurred during sample processing including solid-phase extraction (SPE) and evaporation by nitrogen gas. Matrix effects (Table S3.4) were analysed using the isotopically labelled internal standard Dimethoate-D6 (for BTs and BTHs), N,N-diphenylguanidine-D10 (for DPG) and 6PPD-quinone-d5 (TMQ). This was achieved by spiking 7 random processed snowmelt samples and 7 Milli-Q water blank samples at the same concentration of 50 ng/mL.

### 3.2.4 Suspect Screening and Non-target Analysis

Analysis of lesser-known compounds and TPs was performed using the Compound Discoverer software (version 3.3) with an optimized workflow for suspect and untargeted environmental studies (details on software parameters are in Table S3.6). Chemical features, i.e. extracted chromatographic peaks with given retention times (RT), masses and intensities, were retrieved and prioritized with detection frequency of ≥ 50% across the water or snow samples and a peak area greater than or equal to 10,000. Identification of compounds was based on information generated from MS1 and MS2, isotope pattern matching and relevant chemical databases. Suspect searches were initially performed for mass to charge ratios (m/z) of rubber tire compounds within the prioritized list. Firstly, the optimized suspect screening workflow was validated with all target compounds (BTHs, BTs, DPG and TMQ) treated as synthetic suspects. The only preliminary information available was the exact mass of the protonated species $[M + H]^+$ deduced from the chemical formulae. The chemicals were tentatively identified using a combination of data sources including the mzCloud, Massbank and Chemspider information. Validated workflow was applied to ''real'' suspect compounds and suggested hits were primarily ranked based on accuracy of exact m/z, fragmentation match and application/uses of the chemical. Chemicals with a level 2a identification,[70] i.e., a likely structure is proposed based on diagnostic evidence and a match with library spectra but lacking native standards, were semi-quantified using relative response factors (details in S2.1). Reference standards for selected compounds were used to help compound identification by comparing the RT, m/z values, and MS2 spectra (Supplementary figure S3.4)

### 3.2.5 Transformation Experiment

Batch transformation experiments of model analytes including DPG, BZT and BTH were performed in 15 ml quartz flat bottom vials containing 10mM hydrogen peroxide ($H_2O_2$) and 5uM of individual analyte. An aliquot of 2 ml samples was collected and stored as 0 h control. The flasks were immediately exposed to ultra-violet (UV) radiation in a multi-lamp photochemical reactor for 2 and 8 hours. Other controls were set up to investigate the effect of $H_2O_2$ on DPG without UV influence as well as the influence of UV radiation in the absence of the oxidizing agent, $H_2O_2$. Blank control was prepared at the same concentration, stored, and protected from light until instrumental analysis.

### 3.2.6 Data Analysis

Statistical analysis was conducted using R-Studio 4.1.3. The normality and heteroscedasticity of the data were tested using Shapiro−Wilk and Bartlett's tests. Because the data set violated some of the normality and homogeneity of variance assumptions, the comparison of concentrations across and within sample matrices (Table S3.5) were achieved using the non-parametric Kruskal−Wallis test followed by Wilcoxon post hoc test at a significant level of 0.05.

Using the R package of *rplos* (v 1.28.2), principal component analysis (PCA) was conducted to explore the influence of traffic on the chemical compositions of snow samples.

## 3.3 Results and Discussion

### 3.3.1 Target Analysis of Vulcanization Accelerators in Snowmelts and Surface Water

In the snow samples, eight of the nine target analytes were detected (Figure 3.1 and Table S3.7). The distribution of the chemicals varied significantly within the sample matrix. DPG, BZT, MHBZT, HBTH, MTBTH, and BTH were detected in every sample while TMQ and MBTH were detected in over 65 % of the samples. DPG had a median concentration of 4600 ng/L, followed by BTH (1650 ng/L), HBTH (851 ng/L), and MHBZT (816 ng/L). The occurrence of MHBZT and HBTH exhibited a similar trend, suggesting a common pattern of usage in tire manufacturing.

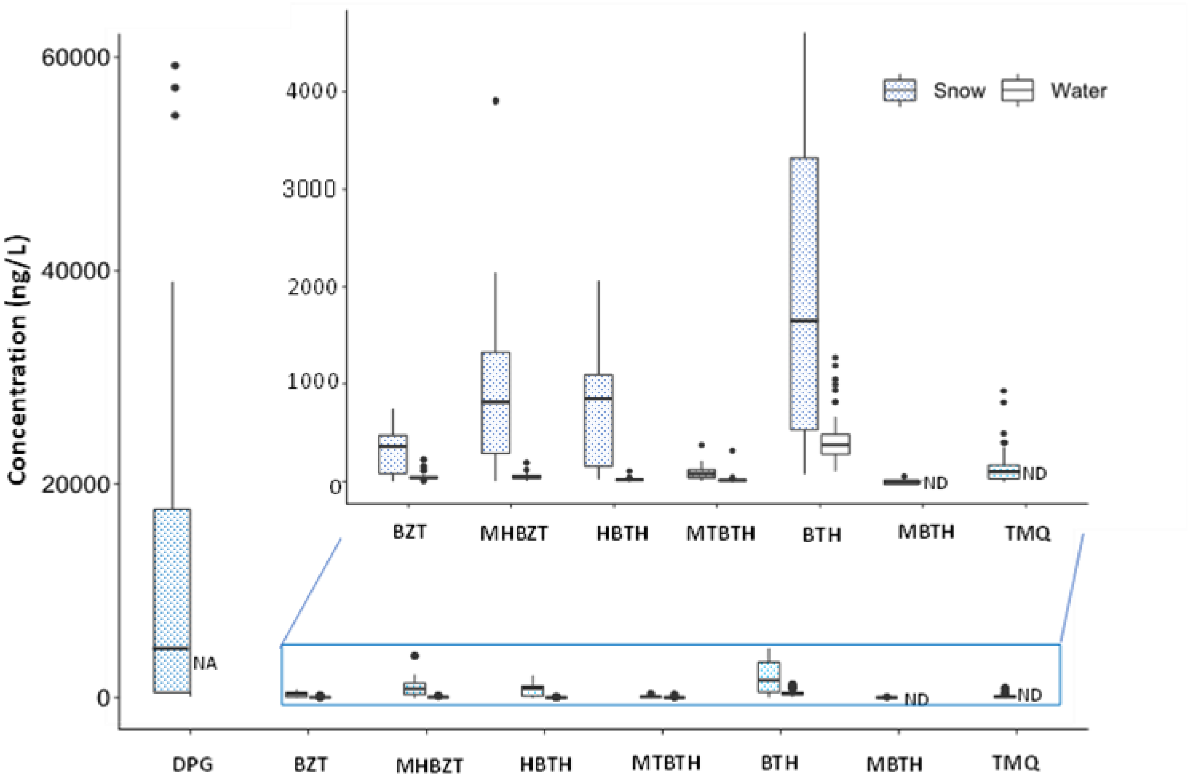


**Figure 3.1 Concentrations of Diphenylguanidine (DPG), Benzotriazoles (BTs), Benzothiazoles (BTHs), and 2,2,4-trimethyl-1,2-dihydroquinoline (TMQ) in snowmelt and surface water samples.**

In the surface water, the chemical distribution profile was more uniform except for BTH (378 ng/L, median value) which was one order of magnitude higher than other detected compounds. Other analytes including BZT, MHBZT, HBTH, and MTBTH, had median concentration in the range of 10 to 45 ng/L. However, there were significant differences in concentration between all four compounds except BZT (Table S3.5).

The concentrations of tire additives were consistently higher in snowmelts than surface water samples. This difference was particularly pronounced for DPG, with a maximum concentration of 59,200 ng/L in snowmelts, which was four orders of magnitude greater than the MDL in surface water samples. Similarly, the concentrations of BTs and BTHs (Figure 3.1) were significantly higher in snowmelts than in surface water ($p<0.05$) (Table S3.5). The median BZT concentration in snow (359 ng/L) was approximately nine times higher than in surface water, while the median BTH concentration in snow (1650 ng/L) was four times higher. This pattern holds true for TMQ with a maximum concentration of 928 ng/L in snowmelts but below the MDL in surface water.

The reference snow background samples collected from the three natural parks indicated that BTs, BTHs, and DPG were largely present but at generally lower concentrations than those from traffic routes (Table S3.7b). This suggests that the emissions of these compounds are largely due to urban influences. TMQ concentrations were below its MDL in samples from natural parks except for one sample site, PA 2, (Table S3.7b). The closeness of the sample location to vehicular tracts within the park could be responsible for the detection in the sample.

DPG concentration in snow analyzed in the present study had a range of <1-59.2 µg/L, while that of Leipzig, Germany, was 3-14 µg/L[25] and Saskatoon, Canada, ranged <1-8.7 µg/L. [28] Comparing maximum detected concentrations, the present work is approximately 4-fold greater than in Leipzig snow and 7-fold higher than that of Saskatoon. Such result approximately match the differences in the population of the three cities (Montreal ≈1, 800,000,[93] Leipzig ≈600,000[94] and Saskatoon≈270,000.[95]) and the population of a city is generally correlated with the average traffic intensities.

The concentration of DPG in surface water samples measured in this study was below the MDL. This is in contrast to DPG reported in surface water at concentrations of 8.14–188 ng/L[16] in Southern China, above 9.8 ng/L[29] in Ontario Canada, and 5 –540 ng/L in the US.[96] Reasons for these differences may relate to a complex interplay of several factors, including drought, occasional or intense rainfall events preceding sampling campaigns, road conditions, fluctuating temperatures, and humidity, which potentially influence the deposition/emission of these chemicals during wear and tear. These results also suggest that the tire-derived chemicals in surface water are highly episodic. Thus, snap-shot sampling might not be good in capturing the contamination condition and aquatic exposures to these chemicals. Time integrated sampling is merited if a routine water monitoring campaign is to be implemented for monitoring this group of contaminants in aquatic systems.

Different from DPG, which was only detected in the snow but not in the surface water, BTHs were detected in both media with concentrations of 110-1271 ng/L and 76-4606 ng/L (Table S3.7a and b) in surface water and snow respectively. The concentration range of BTHs in surface water were in similar range to measurements conducted in China (30-1465 ng/L),[97] and (2-1993 ng/L)[16]. Our measured BTHs concentrations in snow were also within the previously reported range in Germany (40- 50000 ng/L).[25] The detection of BTHs but no detection of DPGs in surface water suggests that there are sources of BTHs in urban environment other than rubber tire in which BTHs are used as vulcanization accelerators. Various BTHs are used as corrosion inhibitors or biocides[84] so in addition to emission from vehicle tires, other urban sources can predominantly contribute their presence in the surface water.

### 3.3.2 Non-Target Chemicals: Classification and Prioritization

Through filtering of the detected chemical features based on detection frequency and peak abundance, an initial of 17,000 (surface water) and over 30,000 (snow) features generated by the HRMS analysis and non-targeted data processing were reduced to 183 and 2005 respectively. Figure 3.2 shows the data analysis of the chemical features using principal component analysis (PCA). The PCA score plot (Figure 3.2) shows the clustering pattern of snow samples and field blanks only. PC1, accounting for 55% of the data variability, separate the samples with traffic influence from background sites and field blanks. This suggests that PC1 can be interpreted as traffic influenced. The chemical profiles in samples from the background sites are closer to the field blanks, in which chemicals detected can be from the sample containers, solvents used and instrument background. PC2 and PC3 accounts for only 9% and 8% of the data variability respectively. Snow samples from roads of varying traffic intensity had no clear separation on the dimensions defined by PC2 and PC3. This suggests that the samples from varying traffic sites had little difference in the chemical profiles despite differences in absolute concentrations. Thus, traffic can be the major contributor for the chemicals in all the snow samples collected from the roads.

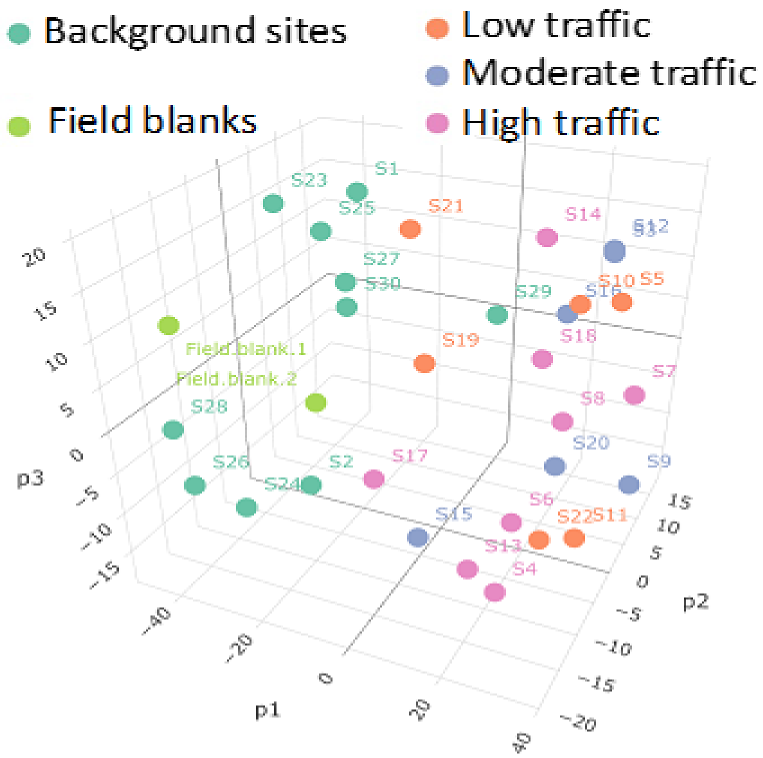


**Figure 3.2 Principal component analysis (PCA, Q2 = 0.72) based on the internal standard normalized abundance of chemical features extracted and prioritized using the non-targeted workflow. PCA score plot shows snow and blank samples with site colored based on the traffic influence classification.**

### 3.3.3 Identification of Prioritized Pollutants

Efforts were made to identify the chemicals prioritized based on their abundances and detection frequencies in the snow and surface water matrices. Figure 3.3, S3.1, S3.2 and S3.3 shows the profile of chemicals identified with a level 1 or 2 confidence (Table S3.8) based on the Schymanski classification scheme.[70]

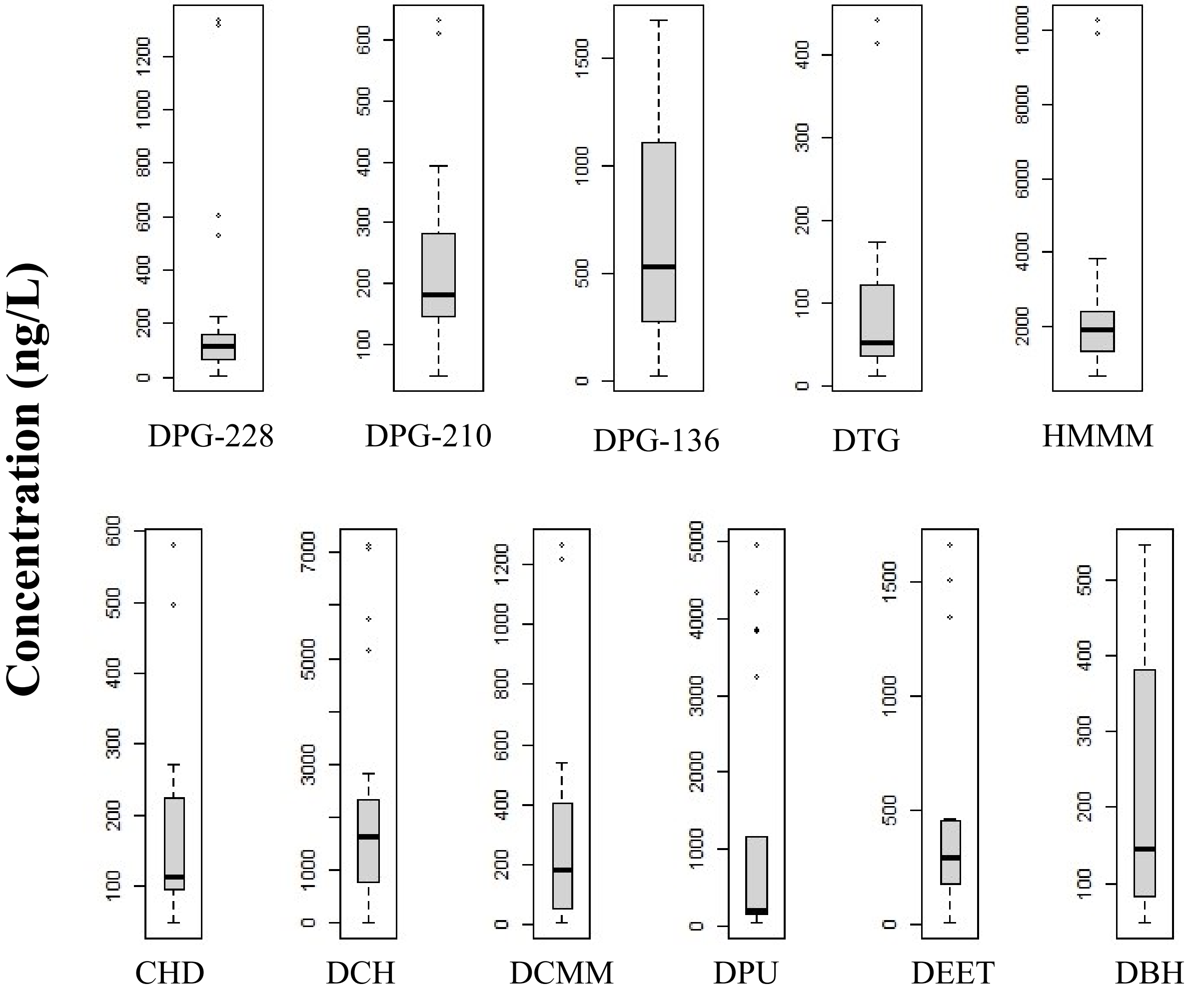


**Figure 3.3 Concentrations of identified chemicals in snow matrix based on available native standard and transformation experiments. Concentrations of these compounds were quantified based on the relative response factors (RRF) described in SI text S2.1.**

Figure S3.2 shows the mass spectra of one of the identified chemicals, N,N Dicyclohexylmethylamine (DCMM), a rubber tire additive detected with high frequency of 82 % in surface water and 76 % in snowmelts. The MS2 in ESI(+) gave a good match with the spectrum generated from MassBank database. This procedure was applied to other level 2a chemicals (Figure 3.3) for identification in conjunction with other observable analytical patterns. This approach facilitated the identification of dicyclohexalamine (DCH) through direct comparison with its derivative, DCMM (Figure S3.3). HMMM was unambiguously identified and confirmed with the authentic standard and matching the RT, MS1 and MS2 to reach a level 1 confidence (Figure S3.4). Most of the tentatively identified compounds had mass spectral information on mass library however, further identification of other compounds with missing library information have been designed to follow manual deduction of loss of common MS2 fragments and the possibilities of their environmental occurrence based on applications. Apart from the manual deduction of the MS/MS spectra, additional experiment was conducted to identify TPs of well-known chemicals. Overall, 37 substances were tentatively identified (Figure 3.3 and more details on table S3.8), 11 with MS2 spectra evidence and 14 without such evidence, but with supporting information including excellent chromatographic peak shape and reasonable RT supporting their likelihood of occurrence. 12 chemicals were rejected based the absence of relevant supporting evidence including invalid MS2 and RT information. In the end, 4 of the 11 chemicals shown in Figure 3.3 above have been confirmed and thus, designated as level 1 compounds. These includes, HMMM (Figure S3.4), diphenylguanidine-228 (DPG-228, Fig. 3.4a-b), diphenylguinidine-210 (DPG-210, Fig. 3.4c-d) and diphenylguinidine-136 (DPG-136, Fig. 3.4e-f). Other chemicals of the set yet to be confirmed include DCH, 1,3-di-o-tolylguanidine (DTG), 3-Cyclohexyl-1,1-dimethylurea (CHD), N'N Dicyclohexylmethylamine (DCMM),, 1,3-Diphenylurea (DPU), 3,5-Di-tert-butyl-4-hydroxybenzaldehyde (DBH) and N,N-diethyl-meta-toluamide (DEET)-the only tentatively identified chemical belonging to the pesticide class. Amongst these, HMMM, DCH and DPG-136 were prominent with median concentrations, 1908 ng/L, 1641 ng/L and 527 ng/L respectively. The confidence in the quantitation of these chemicals is further strengthen by their zero to minimal occurrence in blanks except for DPG (Figure S3.6) for which blank subtraction was performed. This study confirm the presence of abundant species of rubber chemicals and it is consistent with the snowmelt assessment recently conducted in Germany.[25] This work identified the antioxidant DBH that to the best of our knowledge has not been reported in road-sides snow.

### 3.3.4 Identification by Transformation Studies

A simple strategy based on the suspect screening of expected TPs (Table S3.9) were applied for identification. Firstly, chromatographic peaks, full-scan-MSI and MS2 data of DPG, BZT, and BTH exposed to $H_2O_2$ under UV-radiation in a photochemical reactor were obtained by LC-HRMS-analysis. A suspect search of accurate m/z was conducted for predicted TPs (Table S3.9). Experimental hits were compared with field samples to examine analytical information match including RT and fragmentation patterns. Based on the interpretation of the chromatography and mass spectra evidence, DPG showed three prominent TPs (Figure 3.4) in the snow samples; DPG-228, DPG 210 and DPG-136. A fourth TP was also identified but the detection frequency (3 %) was way less than the minimum threshold of $\geq 50$ % set for this study. Since the parent DPG was not detected in surface water, the absence of its TPs in the matrix was not unexpected. The result of the top 3 TPs of DPG showed excellent RT match between the experimental and field samples. The elution order of the 3 chemicals follows LC chromatographic principle with DPG-210 (resulting from the dehydroxylation of the parent compound) eluting last being the most hydrophobic (figure 4c-d). Also, dephenylation product, DPG-136 (Figure 4e-f) as the most hydrophilic was only retained in the LC column for an average of 3.49 minutes with consistent mass spectra information between field and experimental samples. However, the spectral match between the field and experimental DPG-210 is quite poor based on the relative intensity of the two most prominent fragments- 168 and 192 as shown in Figure 3.4c-d. Moreso, the chromatogram of the field samples is suggestive of possible co-eluting substances present in the sample. Further analysis (Figure S3.5) was performed to evaluate the relationship between these TPs and their parent compound, DPG.

The results showed a non-linear relationship, and this may relate to a combination of factors including the transformation rate of TPs and non-point source influence.

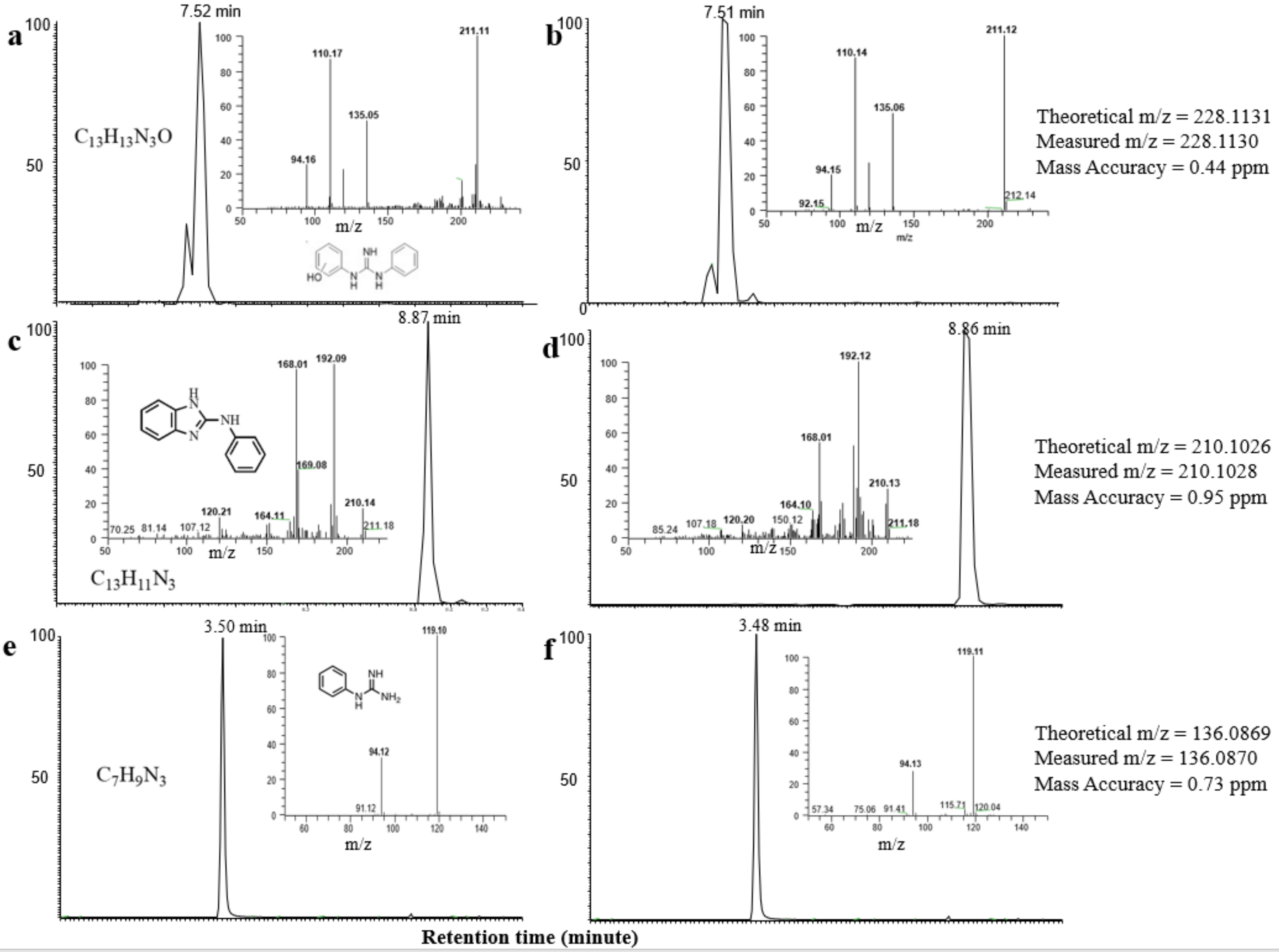


**Figure 3.4 Transformation Experiment of DPG. (a) DPG-288 resulting from the oxidation of DPG-Experimental and (b) field sample (c) DPG-210 from dihydroxylation of DPG-experimental sample and (d) field sample (e) DPG-136 from dephenylation-experimental and (f) field sample**

The same approach of identification was performed for BTH and BZT, however, the results were either a zero or low detection frequency hits except for hydroxybenzothiazole ($C_7H_5NOS$) which is also one of the target analytes in this study. The reason for these zero observations is unclear but might not be unrelated to instability of compounds.

These findings highlight the presence of important tire additives detected in surface water and snow matrices through HRMS-based targeted and non-targeted approach. To the best of our knowledge, the results of this work represent the first elucidation of traffic-driven occurrence of NEP in the Quebec region of Canada. It further shows the importance of snow as a critical environmental reservoir from which harmful organic substances can be released into the environment. Some of the chemicals detected showed elevated levels especially DPG, BTH, HMMM, DCH and DPG-136. Given the impact of previously reported additives in different species of organisms, it is pertinent to further evaluate the fate and toxicity of some of the lesser- known compounds reported in this work.

## Author Contribution

**Joshua Osagu: Joshua Osagu:** Conceptualization, Snow and Surface Water Sampling, Sample Preparation, Investigation/Instrumental analysis, Writing – original draft, all data analysis except for PCA, Writing – review & editing final draft. **Cassandra Johannessen:** Supplied most of chemicals used for the project. **Xianming Zhang:** Conceptualization, Surface water sampling, reviewing & correcting the thesis drafts, data analysis-PCA, and general supervision**.**

## Note

The authors declare that they have no known competing financial interests.

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## Acknowledgement:

The authors would like to thank Dr. Heng Jiang, the Mass Spectrometry Specialist of the Centre for Biological Applications of Mass Spectrometry (CBAMS), Concordia University, Montreal, QC, Canada for assisting in the analysis of the samples used for this research.

The authors would like to thank Tom Haner of Environment and Climate Change Canada for contributing conceptual ideas that were instrumental in actualizing the goals of this work.

# Chapter 4 Conclusions and Recommendations

The main objective of this research was to examine the emissions and fate of tire-derived chemicals and TPs in the urban environment. This study not only captured the occurrence of well-known non-exhaust contaminants but also identified and characterized lesser-known chemical analogues of environmental concern in urban snow and surface water samples through HRMS-based targeted and non-targeted approach. The research design centred around two major categories of rubber tire additives, IAs and vulcanization accelerators and offers insight for source-tracking and advancement of environmental policies. In the first part of the research, the quantitative emission rate of industrial antioxidants and TPs was studied while the second part involved the investigation of snow as a critical reservoir of lesser-known pollutants in the environment. The current chapter highlights and summarizes key results of both aspects as well as limitations and recommendations for future work.

## 4.1. Measurement of Industrial Antioxidant in Urban Snow and Surface Water

Concentrations of IAs and TPs were measured in 30 snow and 42 surface water samples in the Montreal region of Quebec, Canada. Snow sampling was designed along road traffic and non-traffic areas to capture point and non-point sources influences while surface water samples included source contributions from wastewater treatment plants (WWTP), stormwater drains, urban runoffs and snow melting. Measurement of analytes in both matrices was conducted using targeted and suspect screening approaches based on HRMS. Target compounds quantified includes, 6PPD, 6PPD-Q, CPPD, and DPPD while the suspect screening revealed the presence of TPs such as CPPD-Q, DPPD-Q, 4_HDPA, 4_ADPA, and 1,3_DMBA. These compounds were only detected and quantified in snow but were below MDLs in surface water. Quantified compounds had median concentration range of 3-1276 ng/L. Chemical analysis of snow and surface water samples revealed the presence of various rubber tire additives. 6PPD and 6PPD-Q were the most prominent analytes across the samples of the snow and showed 100 % detection frequencies. Their concentrations were significantly influenced by traffic intensity. The high dependence of chemical concentrations on traffic intensities was largely highlighted by the low detections in natural parks. 6PPD-Q in road snow was over a 100-fold greater in concentrations than that in snow samples from local parks. This trend holds for other analytes. The high dependence of chemical concentrations on traffic intensities highlights the need to estimate the mass loading of these chemicals into the environment. The result showed that an alarming 2160 ± 480 kg of 6PPD & 6PPDQ are loaded into the environment from Montreal over a given winter season.

## 4.2 Targeted and Non-Targeted Analysis of Lesser-known Rubber Tire Additives

Whilst the goal of the first part of this study was centred on targeted analysis, the second aspect of the research extended to captured lesser-known contamination through non-targeted analysis (NTA). Target compounds included in this part of the study were the BTHs, BTs, DPG and TMQ. These analytes also served as performance compounds for the validation of the NTA workflow. Quantification of target compounds achieved through the internal standard calibration method revealed high intensity of rubber chemicals in both sample matrices.

DPG exhibited the highest concentration (median concentration of 4600 ng/L) in snowmelt. The vulcanization accelerator, BTH was the second most abundant analyte in snowmelt (1650 ng/L median concentration) and was detected in every sample. The analyte was also detected in surface water at approximately 4-fold lower concentrations than median concentration in snow melt. The ubiquity of other vulcanization accelerators (e.g. HBTH; 851 ng/Lmedian snowmelt concentration) was observed.

Suspect and non-targeted analysis of rubber tire substances in snow and surface water matrices using the orbitrap HRMS instrument detected over 17000 features in surface water and greater than 30000 in snow samples. Peak filtering and prioritization based on detection frequency (≥ 50%) and peak intensity (≥ 10,000) reduced the number of features to 183 and 2005 in surface water and snowmelt, respectively. Statistical analysis using OPLS-DA, was further used to prioritize chemical hits based on differences in the chemical profiles between snow and surface water. Among the top 50 chemical features with significantly higher abundance in snow, 37 were identified through both the suspect and NTA approach. Among these, 11 (classified as level 2 compounds) exhibited credible MS2 spectra evidence, while 14 lacked such MS2 evidence but contain other diagnostic information, such as well-defined chromatographic peak shapes and reasonable RT supporting the presence of suggested compounds. Ultimately, 4 out of the 11 chemicals were confirmed through either a native standard (HMMM) or transformation experiment (DPG-228, DPG-210, DPG-136), thus earning designations as level 1 compounds.

### 4.3 Summary, Limitation and Recommendations for Further Study

The results of this study highlight the presence of tire rubber-derived compounds in Montreal, Quebec, Canada, underscoring the impact of traffic on PPD+PPDQ concentrations. This study contributes novel quantitative emission data obtained through the VEF deduction, which considered the snowpack depth, contaminant's mass in the snow, and traffic volume experienced by the road. However, accurately estimating traffic volume proved challenging due to the absence of comprehensive hourly data for all sampled roads. To address this, percentage normalized hourly traffic data from a major road in Montreal[72] was used, assuming a similar distribution for other local and medium road arterials. Determining the VEF also took into account certain assumptions including (a) uniform vehicle types in urban road traffic, (cars and trucks) (b) consistent tire abrasion rates, and (c) analytes following the same leaching kinetics from tire wear particles. Considering the VEF's reliance on traffic volume, strategic sampling of the region becomes essential to include roads with available hourly traffic data for a more accurate insight of organic chemical mass loading. Additionally, it may be beneficial to incorporate specific truck routes in the analysis to compare emissions between trucks and cars whilst increasing the number of replicates per sampling points of the sampling region. Besides, sampling could include days without snow events to the circumvent the impact of fresh snow precipitation on dumping sites and better capture non-point source influence. The VEF was extended to estimate 6PPD and 6PPDQ loading taking into account the lifespan of winter tires at 5 years[75] (30000-50000 km).[76] Taking the lower limit, it implies that approximately 6000 km is covered per winter season. This information combined with the number of cars in Montreal resulted in the emission and subsequent mobilization of about 2160 kg of 6PPD & 6PPDQ into the environment. Although this mathematical approach provides a good insight of the contaminants' loading estimate, the result may be influenced by factors including tread depth, weather conditions, driving habits amongst others.

The quantitation of analytes in this work utilized the internal standard method. However, not all quantified analytes had available internal standards. Therefore, the quantification of analytes lacking internal standards was performed using alternative internal standards with close retention times or belonging to the same chemical group. In principle, the selection of internal standard in chemical analyses such as those performed in this work follows that standards are chemically similar to analyte of interest, stable under experimental conditions, soluble in analytic solvent of choice, absent in sample under analysis and exhibits detector response per unit concentration similar to that analyte of interest. Although the responses of all the internal standards used match with those of their respective analytes in terms of RTs and validation study assessment, (recovery, Table S3.3), it would be advantageous to broaden the internal standard list to ensure representation for all chemical groups.

The overall findings of this research are not only critical in advancing our understanding of snow as a reservoir of chemical additives, but also further highlights the importance of mass loading of PPDs from snow melt into the aquatic media. Our research also suggests that road snow is an environmental medium of concern for tire additives and their TPs. It may play a more crucial role in the mobilization of organic chemicals into the urban aquatic environment compared to sources that are more commonly reported. Thus, this work readily provides an important framework for advancing environmental monitoring and policy making related to regulatory measures. Further research is needed to understand the environmental fate and cumulative effects of detected chemicals and their TPs. Monitoring of these substances along the snow melting pathway should be a future research priority.

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# Appendix A

## Supporting Information for Chapter 2

Supplementary Text on Materials and Methods
S2.1: Sample Collection and Processing
S2.2: Road Classifications
S2.3: Analytical Standards and Solvents
S2.4: Instrument Method and Data Analysis
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Supplementary Figures
S2.1: Snow and surface water sampling points across Greater Montreal
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S2.4: Relationship between the concentration of 6PPD and its quinone derivative

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S2.2: Summary of Chemical Standards and LC-MS Parameters
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S2.4: Recoveries, MDLs and MQLs of target contaminants
S2.5: Vehicular Emission of PPDs
S2.6: Concentration of PDDs Detected in Snow Samples
S2.7: Summary Concentration Data
S2.8: Non-Parametric Comparison Across Traffic Levels

### Text S2.1 Sample Collection and Processing

A surface water sampling campaign was conducted across forty-two sites in August 2022. Surface water samples were collected at a depth of 1-2 m in precleaned, borosilicate glass bottles using a stainless-steel deep-water sampler and surface water sampling pole (6 m long) on August 19 and 20, 2022. Sampling sites were located in the north-western region of Quebec between the islands of Montreal and Laval (45.74° N, 73.88°W−45.41°N, 74.00° W). Sampling sites were selected to capture influences from a variety of point-sources and non-point sources such as municipal wastewater treatment plants, roadway runoffs, local roads, and storm water drains. Similarly, a snow sampling campaign was conducted during a snow fall event on January 20, 2023, from thirty (30) sampling points across different natural parks (n = 6), snow dumping sites (n= 2), temporal snowbanks (n = 2), and varying road categories (n=20) in Montreal (45.53° N, 73.56°W−45.44°N, 74.64° W). To create a representative sample, metallic scoops were utilized to collect piled and roadside snow from various surfaces, sides, and depths (5-10 cm for snow piles). Aggregate samples collected from different points within a sample location were pooled into 1 L polypropylene bottles. Both the surface water and snow samples were processed using solid-phase extraction (SPE) with Oasis hydrophilic-lipophilic balance (HLB) cartridges (6 mL, 200 mg, Sigma Millipore USA). Initially, a mixture of Isotopically labelled internal standard was spiked into approximately 200 mL samples to achieve a concentration of 50 ng/mL. HLB Cartridges were pre-conditioned with 5 mL of methanol, then rinsed with 5 mL LC-MS grade water (3 mL/min). Extraction (100-200 mL per samples) utilized a SPE vacuum manifold (Supelco 12-port model, Milipore Sigma, MA, USA) at a maximum flow rate of 3 mL/min. Rinsing of sample bottles at the end of extraction was deemed unnecessary as each bottle contained volumes exceeding the required samples. After extraction, SPE cartridges were air-dried for 3 minutes, eluted with 2 * 5 mL aliquots of methanol, and concentrated under a gentle stream of nitrogen gas to approximately 0.5 mL. The resulting extracts were then transferred into 1.5-mL HPLC vials and diluted 2-10 times (depending on sample type) prior to LC-MS analysis.

### Text S2.2 Road Classification: High, Medium and Low Traffic.

The snow samples were collected from roads that have varying daily average traffic densities. In order to investigate the relationship between the volume of traffic and chemical loading in the snow samples on road surfaces, traffic information was extracted from the Canadian traffic flow dashboard[69] and used to classify each road into different categories. Traffic information obtained for the sampling locations indicated that the least dense traffic road had a traffic volume of 1226 vehicles per day while the densest traffic road had a daily traffic volume of 6031 (Table S1). To simplify the classification, a non-overlapping histogram (Figure S2) was employed to categorize the sampled roads into three distinct groups. The first group consisted of average daily vehicular volume ≤ 2000 and was tagged as low Road Traffic Level (RTL), the second category as medium RTL (2000 < x ≤ 4000), while the third group with volume > 4000 was represented as high RTL. Table S1 presents the information on exact location, sampling time and number of replicates in each category.

### Text S2.3 Analytical standards, Solvents and Additive

The *p*-phenylenediamines (PPDs) and 6PPD-quinone were purchased as analytical grade (98 % purity) from Sigma Aldrich (Atlanta, GA, USA) and the internal standard 6PPD-quinone-D5 was purchased from Toronto Research Chemicals (Toronto, ON, Canada). All solid standards were stored in airtight bags at either 4 °C or −20 °C according to manufacturer's recommendation, whilst dissolved standards (stock and sub-stock solutions) were stored at −20 °C. LC-MS grade methanol and water were purchased from Fisher Scientific (Waltham, Massachusetts USA). Formic acid (98% purity) and acetonitrile (≥ 99.9 % purity) were purchased from Honeywell and Sigma-Aldrich respectively.

### Text S2.4 Instrumental Method: High-Resolution Mass Spectrometry

The Orbitrap was operated in positive mode with electrospray ionization source (ESI). The mass analyser was utilized in the full scan mode (m/z 100−500, mass resolution, 100,000) and data-dependent acquisition (collision induced dissociation, collision energy =35 eV) mode. Optimized electrospray ionization (ESI) parameters which includes capillary temperature, capillary voltage, source heater temperature, and sheath gas flow, were 275 °C, 4 kV in the positive ion mode, 300 °C, and 10 arbitrary units, respectively. Liquid chromatographic separation was performed on a reverse phase C-18 phenomenex column (3.0 μm, 3 × 100 mm) with mobile phases consisting of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile by volume. The flow rate of the mobile phase was optimized at 0.3 mL/min. The elution process followed a gradient profile: starting with 5 % B (0−1 min), increased to 98 % B (1−19 min), held for 3 min (19−21 min), then back to 5 % B, and re-equilibrated for 3 min. (See Table S2.2b for a comprehensive summary).

### Text S2.5 In-House Suspect list

An in-house suspect list was generated mainly from the NORMAN 2022 suspect list. Compounds ending with -N, $-N_2$, $-N_2O_2$, and -NO were searched, filtered and subsquently fed into the compound discoverer software. Hits were prioritized based on full chemical formulae, existing literature information and general uses.

### Equation S2.1 Relative Response Factor

$RRF_{6PPD\text{-}Q} = ((C_{6PPD\text{-}Q})/C_{IS})/((A_{6PPD\text{-}Q})/(A_{IS}))$........................................................1

$RRF_{6PPD\text{-}Q} = (C_x/C_{IS})/(A_x/A_{IS})$…………………………………………………….…..2

Where $C_{6PPD\text{-}Q}$ and $A_{6PPD\text{-}Q}$ are the concentrations and peak areas of 6PPD-Q respectively. $C_{IS}$ and $A_{IS}$ are internal standard concentration and its corresponding peak area while $A_x$ and $C_x$ are the peak area and the unknown concentration of the suspect analytes.

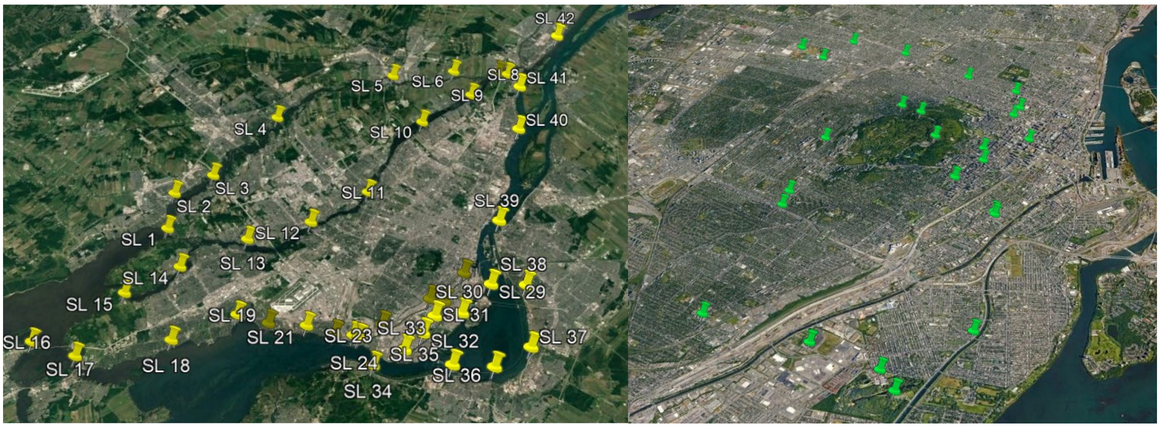


**Figure S2.1 Snow and surface water sampling points across Greater Montreal (a) Surface water spatial map and sampling activity (b) Spatial map of urban snow and sampling activity**

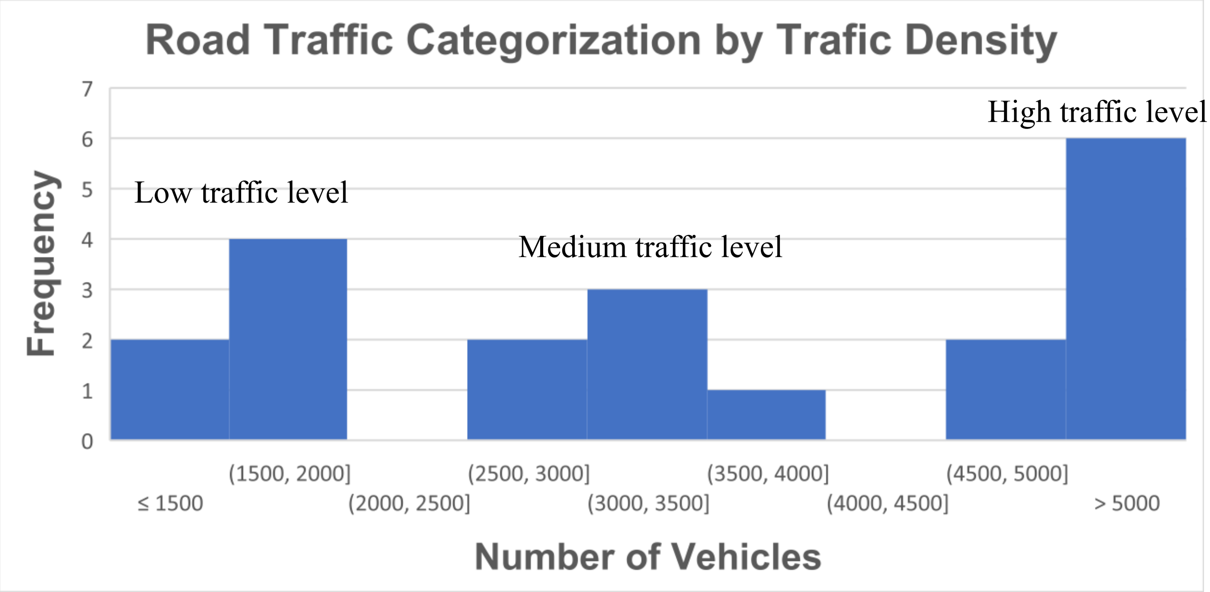


**Figure S2.2 Road traffic levels separated into three distinct categories based on traffic information on sampling day.**

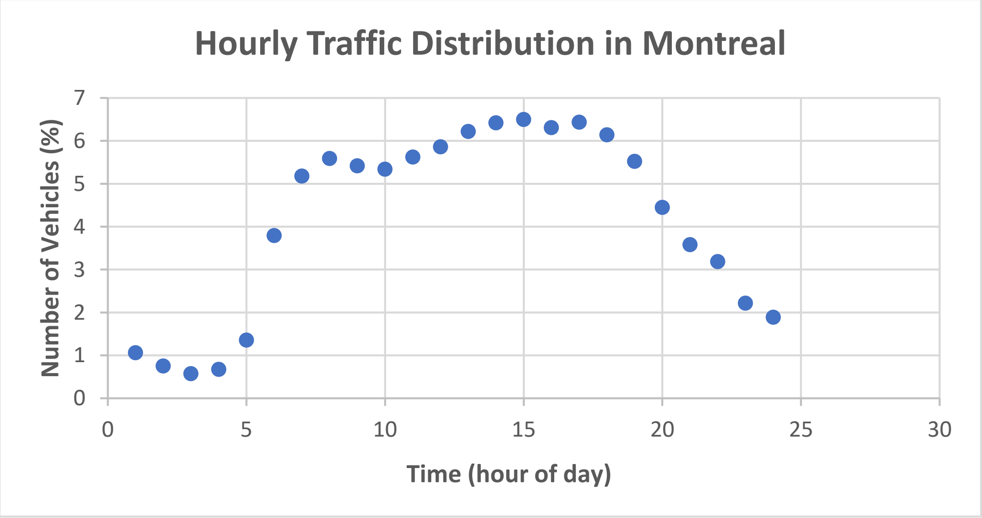


**Figure S2.3 A typical hourly traffic distribution of a high traffic arterial in Montreal, QC, Bd Décarie during the winter season**[72].

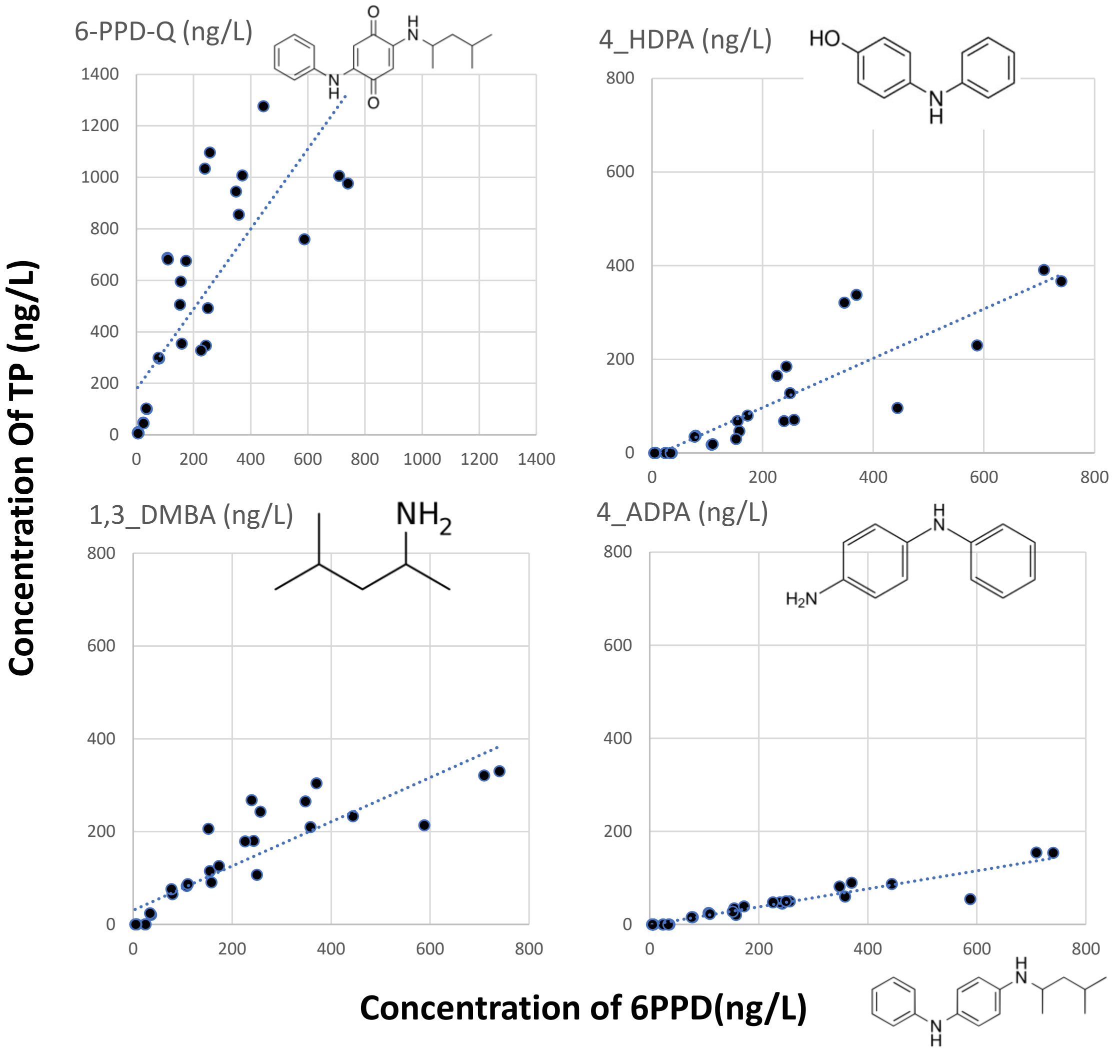


**Figure S2.4 A Linear fit for the relationship between the concentration of 6PPD and its quinone derivative**

**Table S2.1 Location and traffic information of sampling points**

| Water Sample (August 19 and 20, 2022) | | |
|---|---|---|
| Site Tag | Latitude | Longitude |
| SL_1 | 45.523661 | -73.883400 |
| SL_2 | 45.558516 | -73.881698 |
| SL_3 | 45.577770 | -73.841010 |
| SL_4 | 45.641522 | -73.774237 |
| SL_5 | 45.692678 | -73.637835 |
| SL_6 | 45.699629 | -73.563534 |
| SL_7 | 45.699235 | -73.509219 |
| SL_8 | 45.697756 | -73.497924 |
| SL_9 | 45.672456 | -73.543372 |
| SL_10 | 45.640479 | -73.602598 |
| SL_11 | 45.564547 | -73.664186 |
| SL_12 | 45.533368 | -73.726330 |
| SL_13 | 45.515363 | -73.794432 |
| SL_14 | 45.487863 | -73.863560 |
| SL_15 | 45.462948 | -73.918258 |
| SL_16 | 45.418505 | -74.004446 |
| SL_17 | 45.407630 | -73.956951 |
| SL_18 | 45.423341 | -73.861720 |
| SL_19 | 45.446539 | -73.796844 |
| SL_20 | 45.439820 | -73.763490 |
| SL_21 | 45.438774 | -73.723824 |
| SL_22 | 45.432478 | -73.694428 |
| SL_23 | 45.431606 | -73.675232 |
| SL_24 | 45.429987 | -73.669117 |
| SL_25 | 45.441319 | -73.645583 |
| SL_26 | 45.464920 | -73.595907 |
| SL_27 | 45.463275 | -73.580685 |
| SL_28 | 45.488959 | -73.559232 |
| SL_29 | 45.473642 | -73.536797 |
| SL_30 | 45.447528 | -73.568679 |
| SL_31 | 45.444335 | -73.596871 |
| SL_32 | 45.429431 | -73.609260 |
| SL_33 | 45.414992 | -73.628376 |
| SL_34 | 45.407094 | -73.654109 |
| SL_35 | 45.403680 | -73.579698 |
| SL_36 | 45.402799 | -73.537457 |
| SL_37 | 45.419965 | -73.499377 |
| SL_38 | 45.473620 | -73.499904 |
| SL_39 | 45.533308 | -73.524423 |
| SL_40 | 45.628809 | -73.495408 |
| SL_41 | 45.676819 | -73.490539 |
| SL_42 | 45.739256 | -73.438622 |

| Snow Smaple (Janaury 20, 2022) | | | | |
|---|---|---|---|---|
| Site Tag | Latitude | Longitude | Sampling time | Daily Traffic Volume |
| RTL 1A | 45.512500 | -73.568333 | 13:41 | 5663 |
| RTL 1B | 45.456944 | -73.640277 | 10:02 | 5073 |
| RTL 1C | 45.490800 | -73.586552 | 12:33 | 5500 |
| RTL 1D | 45.502478 | -73.619786 | 16:31 | 4570 |
| RTL 1E | 45.480333 | -73.577222 | 11:28 | 5346 |
| RTL 1F | 45.499166 | -73.578888 | 12:58 | 6031 |
| RTL 1G | 45.510000 | -73.570278 | 13:33 | 5615 |
| RTL 1H | 45.482878 | -73.627778 | 17:33 | 5028 |
| RTL 2A | 45.532777 | -73.600000 | 14:21 | 3876 |
| RTL 2B | 45.537500 | -73.615555 | 14:33 | 3163 |
| RTL 2C | 45.511105 | -73.595111 | 15:44 | 3267 |
| RTL 2D | 45.490555 | -73.586388 | 11:53 | 3047 |
| RTL 2E | 45.495555 | -73.579166 | 12:49 | 2635 |
| RTL 2F | 45.453333 | -73.583333 | 16:39 | 3876 |
| RTL 3A | 45.486667 | -73.626667 | 11:18 | 1773 |
| RTL 3B | 45.518113 | -73.568969 | 13:48 | 1389 |
| RTL 3C | 45.531111 | -73.623888 | 15:37 | 1226 |
| RTL 3D | 45.513333 | -73.600278 | 15:56 | 1773 |
| RTL 3E | 45.523611 | -73.581944 | 14:06 | 1844 |
| RTL 3F | 45.501944 | -73.566944 | 13:14 | 1741 |
| PMR 1 | 45.503147 | -73.591252 | 16:09 | NA |
| PMR 2 | 45.503147 | -73.591252 | 16:12 | NA |
| PJ 1 | 45.535277 | -73.630555 | 15:14 | NA |
| PJ 2 | 45.535452 | -73.630838 | 15:18 | NA |
| DS 1 | 45.451286 | -73.616861 | 10:21 | NA |
| DS 2 | 45.451111 | -73.616944 | 10:25 | NA |
| SB 1 | 45.481036 | -73.577383 | 11:41 | NA |
| SB 2 | 45.537630 | -73.615677 | 14:49 | NA |
| PA 1 | 45.445833 | -73.602222 | 10:54 | NA |
| PA 2 | 45.441944 | -73.599166 | 10:59 | NA |

## Table S2.2a Summary of Chemical Standards

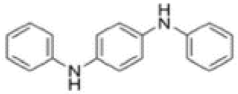
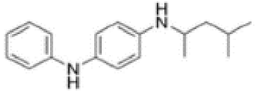
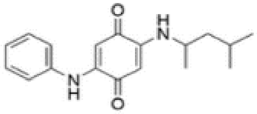
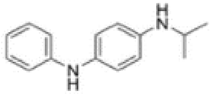
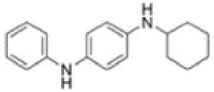
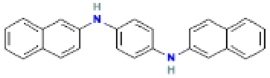
| Name | Abbreviation | Formula | Structure | m/z | RT |
|---|---|---|---|---|---|
| N,N′-Diphenyl-1,4-phenylenediamine | DPPD | $C_{18}H_{16}N_2$ |  | 261.1386 | 18.77 |
| N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine | 6PPD | $C_{18}H_{24}N_2$ |  | 269.2012 | 11.43 |
| 6PPD-quinone | 6PPD-Q | $C_{18}H_{22}N_2O_2$ |  | 299.1754 | 18.51 |
| N-isopropyl-N′-phenyl-1,4-phenylenediamine | IPPD | $C_{15}H_{18}N_2$ |  | 227.1542 | 9.66 |
| N-Cyclohexyl-N'-phenyl-p-phenylenediamine | CPPD | $C_{18}H_{22}N_2$ |  | 267.1886 | 11.51 |
| N,N′-di-2-naphthyl-p-phenylenediamine | DNPD | $C_{26}H_{20}N_2$ |  | 361.1699 | 20.98 |

**Table S2.2b LC-MS Parameters**

| LC gradient using a Phenomenex C18 (3.0 x 100 mm, 3.0µm) equipped with a guard column; T: Room Temp; injection volume: 5 µL | | | |
|---|---|---|---|
| Time (min) | %A (0.1% formic acid in LC-MS water) | %B (0.1% formic acid in acetonitrile) | Flow Rate (mL/min) |
| 0 | 95 | 5 | 0.3 |
| 1 | 95 | 5 | 0.3 |
| 19 | 2 | 98 | 0.3 |
| 21 | 2 | 98 | 0.3 |
| 21 | 95 | 5 | 0.3 |
| 32 | 95 | 5 | 0.3 |

| Mass Spectrometer | |
|---|---|
| LTQ orbitrap velos.<br>ESI in positive mode<br>MS1 mass range = 100-500, Resolution = 100, 000<br>MS2- Acquisition mode: DDA MS/MS of 5 top ions, CID (linear ion trap = low resolution)<br>Dynamic exclusion: time= 25 s,  no charge state selection, repeated MS/MS acquisition= 2x | |
| **Other Parameters** | **Value** |
| Capillary Temperature °C | 275 |
| Source Heat Temperature °C | 300 |
| Sheath Gas Flow (AU) | 10 |
| Aux Gas Flow (AU) | 10 |
| Ion spray voltage (4kV) | 4 |
| Source Current (uA) | 100 |
| S-Lens RF Level (AU) | 60 |

### Table S2.3 PPD Suspect List

| Name | Monoisotopic Mass |
|---|---|
| N,N-Dicyclohexyl-2-benzothiazolesulfenamide | 346.1537 |
| Diphenylamine | 169.0886 |
| Methenamine | 140.1056 |
| N,N'-1,3-Phenylenedimaleimide | 268.0478 |
| Triisopropanolamine | 191.1516 |
| 1,4-Benzenediamine, N-(1,4-dimethylpentyl)-N'-phenyl- | 282.2090 |
| 2-Methylundecanal | 184.1822 |
| 4-Hydroxydiphenylamine | 185.0835 |
| 4-aminodiphenylamine | 184.0995 |
| 4-nitrodiphenylamine | 214.0737 |
| 4-nitrosodiphenylamine | 198.0788 |
| 1,3-dimethylbutylamine | 101.1199 |
| N,N-Diphenyl-1,4-phenylenediamine | 260.1308 |
| N,N-Diphenyl-1,4-phenylenediamine | 290.1050 |
| N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine | 268.1934 |
| N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone | 298.1676 |
| N-isopropyl-N’-phenyl-1,4-phenylenediamine | 226.1465 |
| N-isopropyl-N’-phenyl-1,4-phenylenediamine-quinone | 256.1206 |
| N-Cyclohexyl-N'-phenyl-p-phenylenediamine | 266.1778 |
| N-Cyclohexyl-N'-phenyl-p-phenylenediamine- quinone | 296.1519 |
| Hexa(methoxymethyl)melamine | 390.2221 |
| N,N’-ditolyl-p-phenylenediamine | 288.1621 |
| N,N’-ditolyl-p-phenylenediamine-quinone | 318.1363 |
| N,N’-di-2-naphthyl-p-phenylenediamine | 360.1621 |
| N,N’-di-2-naphthyl-p-phenylenediamine-quinone | 390.1363 |
| N,N’-Diphenyl-1,4-phenylenediamine | 260.1308 |
| N,N’-Diphenyl-1,4-phenylenediamine | 290.1050 |

### Table S2.4 Recoveries, MDLs and MQLs of target contaminants (Snowmelts)

| Name | Abbreviation | Recovery (%) 1 | Recovery (%) 2 | Recovery (%) 3 | Avg. Recovery (%) | RSD (%) | MDL (ng/mL) | MQL (ng/mL) |
|---|---|---|---|---|---|---|---|---|
| N,N’-Diphenyl-1,4-phenylenediamine | DPPD | 67 | 82 | 69 | 73 | 11 | 3.9 | 14.8 |
| N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine | 6PPD | 89 | 77 | 68 | 78 | 14 | 1.0 | 3.9 |
| 6PPD-quinone | 6PPD-Q | 81 | 86 | 74 | 80 | 8 | 0.9 | 3.6 |
| N-isopropyl-N’-phenyl-1,4-phenylenediamine | IPPD | 78 | 64 | 85 | 76 | 14 | 0.8 | 3.2 |
| N-Cyclohexyl-N'-phenyl-p-phenylenediamine | CPPD | 92 | 86 | 73 | 84 | 12 | 0.4 | 1.5 |
| N,N’-di-2-naphthyl-p-phenylenediamine | DNPD | 80 | 66 | 69 | 72 | 10 | 4.4 | 16.6 |

## Table S2.5 Vehicular Emission of PPDs

| | | | | | Emission Factor (µg/vehicle/kilometre) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Sample Location** | **Site Tag** | **Sampling time** | **Snow Volume/km (dm3)** | **6-PPD** | **6-PPD-Q** | **DPPD** | **DPPD-Q** | **CPPD** | **CPPD-Q** | **4_HDPA** | **4_ADPA** | **4s_DPA** | **1,3_DMBA** |
| 1 | Road Traffic Level 1 | RTL 1A | 13:41 | 42000 | 72 | 172 | 12 | 3 | 45 | 5 | 21 | 12 | 17 | 42 |
| 2 | 1 | RTL 1B | 10:02 | 40600 | 65 | 93 | 11 | 10 | 41 | 2 | 50 | 12 | 18 | 48 |
| 3 | 1 | RTL 1C | 11:33 | 41300 | 58 | 250 | 10 | 3 | 40 | 6 | 16 | 12 | 7 | 65 |
| 4 | 1 | RTL 1D | 14:51 | 40600 | 70 | 201 | 9 | 1 | 48 | 1 | 15 | 14 | 7 | 37 |
| 5 | 1 | RTL 1E | 17:28 | 50820 | 89 | 126 | 4 | 11 | 45 | 1 | 49 | 20 | 52 | 40 |
| 6 | 1 | RTL 1F | 15:58 | 46800 | 80 | 104 | 5 | 3 | 19 | 4 | 31 | 8 | 39 | 29 |
| 7 | 1 | RTL 1G | 13:33 | 41300 | 69 | 187 | 4 | 17 | 41 | 4 | 63 | 17 | 151 | 56 |
| 8 | 1 | RTL 1H | 13:03 | 41300 | 72 | 196 | 5 | 16 | 44 | 2 | 67 | 17 | 160 | 55 |
| 9 | Road Traffic Level 2 | RTL 2A | 12:01 | 41650 | 40 | 257 | 9 | 1 | 25 | 4 | 7 | 9 | 6 | 31 |
| 10 | 2 | RTL 2B | 14:33 | 34300 | 65 | 276 | 10 | 4 | 40 | 3 | 18 | 13 | 7 | 61 |
| 11 | 2 | RTL 2C | 15:44 | 38400 | 50 | 73 | 9 | 5 | 29 | 2 | 37 | 11 | 14 | 40 |
| 12 | 2 | RTL 2D | 16:53 | 47880 | 125 | 165 | 5 | 14 | 49 | 1 | 62 | 26 | 78 | 56 |
| 13 | 2 | RTL 2E | 12:49 | 40600 | 77 | 151 | 9 | 8 | 37 | 2 | 39 | 15 | 50 | 33 |
| 14 | 2 | RTL 2F | 16:39 | 41160 | 36 | 137 | 5 | 4 | 18 | 1 | 16 | 8 | 34 | 27 |
| 15 | Road Traffic Level 3 | RTL 3A | 11:18 | 32900 | 47 | 177 | 14 | 20 | 27 | 5 | 22 | 10 | 26 | 39 |
| 16 | 3 | RTL 3B | 13:48 | 32200 | 50 | 111 | 7 | 2 | 23 | 3 | 15 | 7 | 10 | 29 |
| 17 | 3 | RTL 3C | 15:37 | 36800 | 44 | 145 | 10 | 2 | 26 | 1 | 9 | 8 | 4 | 59 |
| 18 | 3 | RTL 3D | 15:56 | 36400 | 36 | 225 | 8 | 1 | 21 | 1 | 6 | 8 | 5 | 29 |
| 19 | 3 | RTL 3E | 14:06 | 31850 | 45 | 176 | 6 | 9 | 26 | 1 | 21 | 10 | 39 | 33 |
| 20 | 3 | RTL 3F | 10:14 | 32200 | 48 | 186 | 16 | 18 | 25 | 2 | 22 | 10 | 25 | 47 |

## Table S2.6 Concentration of PDDs Detected in Snow Samples

| Sample Location | 6-PPD (ng/L) | 6-PPD-Q (ng/L) | DPPD (ng/L) | DPPD-Q (ng/L) | CPPD (ng/L) | CPPD-Q (ng/L) | 4_HDPA (ng/L) | _ADPA (ng/ | 4s_DPA (ng/L) | 1,3_DMBA (ng/L) |
|---|---|---|---|---|---|---|---|---|---|---|
| RTL 1A | 358 | 855 | 58 | 14 | 221 | 27 | 102 | 60 | 83 | 210 |
| RTL 1B | 243 | 346 | 41 | 37 | 152 | 9 | 185 | 45 | 66 | 180 |
| RTL 1C | 239 | 1033 | 40 | 14 | 167 | 25 | 68 | 48 | 28 | 268 |
| RTL 1D | 444 | 1276 | 55 | 4 | 305 | 9 | 96 | 87 | 47 | 233 |
| RTL 1E | 709 | 1005 | 29 | 88 | 355 | 8 | 391 | 155 | 416 | 321 |
| RTL 1F | 588 | 759 | 40 | 21 | 137 | 26 | 230 | 55 | 285 | 214 |
| RTL 1G | 370 | 1007 | 24 | 90 | 220 | 24 | 338 | 90 | 812 | 304 |
| RTL 1H | 348 | 945 | 24 | 76 | 210 | 9 | 321 | 82 | 771 | 265 |
| RTL 2A | 108 | 687 | 25 | 3 | 66 | 10 | 18 | 25 | 15 | 83 |
| RTL 2B | 257 | 1096 | 40 | 16 | 160 | 12 | 71 | 50 | 28 | 243 |
| RTL 2C | 226 | 327 | 40 | 23 | 130 | 8 | 165 | 48 | 64 | 179 |
| RTL 2D | 740 | 976 | 27 | 82 | 288 | 4 | 367 | 154 | 463 | 330 |
| RTL 2E | 250 | 491 | 29 | 27 | 119 | 5 | 128 | 50 | 161 | 107 |
| RTL 2F | 155 | 595 | 23 | 16 | 78 | 3 | 68 | 35 | 147 | 115 |
| RTL 3A | 79 | 296 | 24 | 43 | 45 | 8 | 37 | 16 | 43 | 65 |
| RTL 3B | 158 | 354 | 23 | 7 | 73 | 9 | 47 | 21 | 33 | 91 |
| RTL 3C | 152 | 505 | 35 | 8 | 91 | 5 | 30 | 28 | 15 | 206 |
| RTL 3D | 110 | 681 | 24 | 3 | 63 | 3 | 19 | 23 | 15 | 87 |
| RTL 3E | 173 | 675 | 24 | 33 | 98 | 3 | 80 | 39 | 151 | 126 |
| RTL 3F | 77 | 299 | 25 | 29 | 41 | 3 | 35 | 16 | 40 | 76 |
| Park Mount Royal 1 | 7 | 6 | nd | nd | nd | nd | nd | nd | nd | nd |
| Park Mount Royal 2 | 6 | 7 | nd | nd | nd | nd | nd | nd | nd | nd |
| Park Jarry 1 | 5 | 10 | nd | nd | nd | nd | nd | nd | nd | nd |
| Park Jarry 2 | 4 | 7 | nd | nd | nd | nd | nd | nd | nd | nd |
| Dumping Site 1 | 24 | 47 | 30 | nd | 4 | nd | nd | nd | nd | nd |
| Dumping Site 2 | 25 | 44 | 34 | nd | 6 | nd | nd | nd | nd | nd |
| Snowbank 1 | 36 | 100 | 39 | nd | 6 | 7 | nd | nd | nd | 21 |
| Snowbank 2 | 34 | 102 | 40 | nd | 8 | 8 | nd | nd | nd | 24 |
| Park Angrignon 1 | 6 | 7 | nd | nd | nd | nd | nd | nd | nd | nd |
| Park Angrignon 2 | 5 | 5 | nd | nd | nd | nd | nd | nd | nd | nd |

## Table S2.7 Sample Concentrations and Emission Summary

| Median Sample Concentration | 6-PPD | 6-PPD-Q | DPPD | DPPD-Q | CPPD | CPPD-Q | 4_HDPA | 4_ADPA | 4s_DPA | 1,3_DMBA |
|---|---|---|---|---|---|---|---|---|---|---|
| High Traffic Level (ng/L) | 364 | 975 | 40 | 29 | 215 | 17 | 208 | 71 | 184 | 249 |
| Medium Traffic Level (ng/L) | 238 | 641 | 28 | 20 | 125 | 7 | 100 | 49 | 86 | 147 |
| Low Traffic Level (ng/L) | 131 | 430 | 24 | 19 | 68 | 4 | 36 | 22 | 37 | 89 |
| Parks - Background Samples (ng/L) | 6 | 7 | nd | nd | nd | nd | nd | nd | nd | nd |
| Dumping Sites (ng/L) | 30 | 74 | 37 | nd | 6 | 8 | nd | nd | nd | 23 |
| MIN. Concentration (ng/L) | 5 | 5 | 23 | 3 | 4 | 3 | 18 | 16 | 15 | 21 |
| MAX. Concentration (ng/L) | 740 | 1276 | 58 | 90 | 355 | 27 | 391 | 155 | 471 | 330 |

| Emission Rate | 6-PPD | 6-PPD-Q | DPPD | DPPD-Q | CPPD | CPPD-Q | 4_HDPA | 4_ADPA | 4s_DPA | 1,3_DMBA |
|---|---|---|---|---|---|---|---|---|---|---|
| Maximum (µg/vehicle/kilometre) | 125 | 276 | 16 | 20 | 49 | 6 | 67 | 26 | 160 | 65 |
| Minimum (µg/vehicle/kilometre) | 36 | 73 | 4 | 1 | 18 | 1 | 6 | 7 | 4 | 27 |
| Average (µg/vehicle/kilometre) | 62 | 170 | 8 | 8 | 33 | 3 | 29 | 12 | 37 | 43 |

## Table S2.8 Non-Parametric Statistical Comparison Across Traffic Levels (<0.05 = significant )

| Name | Abbreviation | Kruskal Wallis test (P-Value) | Pairwise comparison (Post Hoc) |
|---|---|---|---|
| 4-Hydroxydiphenylamine | 4-HDPA | 0.0120 | High RTL Sig diff from Low RTL |
| 4-aminodiphenylamine | 4-ADPA | 0.0029 | High RTL Sig diff from Low RTL |
| 4-nitrosodiphenylamine | 4s DPA | 0.0921 | N/A |
| 1,3-dimethylbutylamine | 1,3 DMBA | 0.0096 | High RTL Sig diff from Low RTL |
| N,N′-Diphenyl-1,4-phenylenediamine | DPPD | 0.0674 | N/A |
| N,N′-Diphenyl-1,4-phenylenediamine-quinone | DPPD-Q | 0.3064 | N/A |
| N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine | 6PPD | 0.0045 | High RTL Sig diff from Low RTL |
| N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine - quinone | 6PPD-Q | 0.0270 | High RTL Sig diff from Low RTL |
| N-Cyclohexyl-N'-phenyl-p-phenylenediamine | CPPD | 0.0022 | High RTL Sig diff from Low RTL |
| N-Cyclohexyl-N'-phenyl-p-phenylenediamine - quinone | CPPD-Q | 0.0213 | High RTL Sig diff from Low RTL |

## Appendix B

## Supporting Information for Chapter 3

Supplementary Text on Materials and Methods
S3.1: Analytical Standards and Solvents
S3.2: Instrument Method and Data Analysis
S3.3: Detection Limits, Recoveries and Matrix Effects
S3.4: Data Preprocessing and Reduction

**Supplementary Figures**
S3.1: Concentrations distribution of non-targeted chemicals in surface water
S3.2: Mass Spectra of N,N dicyclohexylmethylamine (DCMM)
S3.3: Analytical Information of dicyclohexalamine and N,N dicyclohexylmethylamine (DCMM) Compared.
S3.4: Confirmation of Hexa(methoxymethyl)melamine (HMMM) by RT and MS information of native standard
S3.5: Relationship between DPG and its Transformation Products
S3.6. Chromatographic profile of blank samples showing non-detection of target analytes except for diphenylguanidine.

**Supplementary Tables**
S3.1: Target Analyte Information
S3.2 Internal Standards Peak Areas and Relative Standard Deviations (Surface Water)
S3.3: Method Detection Limit (MDL), Recovery Matrix, Linearity, and Internal Standard
S3.4: Internal Standards and Matrix Effect
S3.5: Statistical Testing: Non-Parametric test
S3.6: Compound Discoverer Software Parameters
S3.7a: Concentration of Target Analytes (Surface water)
S3.7b: Concentration of Target Analytes (Snow)
S3.8: Summary of Analytes by Suspect and Non-Targeted Approach
S3.9: Expected Transformation Products of Diphenylguanidine, Benzotriazole and Benzothiazole

### Text S3.1 Analytical standards, Solvents and Additives

The benzotriazoles (BTs), benzothiazoles (BTHs) and 6PPD-quinone were purchased as analytical grade (98 % purity) from Sigma Aldrich (Atlanta, GA, USA) and the internal standard 6PPD-quinone-D5 was purchased from Toronto Research Chemicals (Toronto, ON, Canada). All solid standards were stored in airtight bags at either 4 °C or −20 °C according to manufacturer's recommendation, whilst dissolved standards (stock and sub-stock solutions) were stored at −20 °C. LC-MS grade methanol and water were purchased from Fisher Scientific

(Waltham, Massachusetts, USA). Formic acid (98% purity) and acetonitrile (≥ 99.9 % purity) were purchased from Sigma-Aldrich (Atlanta, GA, USA) respectively.

### Text S3.2 LC-HRMS Analysis and Parameters

Text S2.4 and Table S2.2 summarize the LC-MS information.

### Text S3.3 Detection Limits, Recoveries and Matrix Effects

Method quantification limits (MQLs) were calculated as 10 times the standard deviation (SD) of the analyte concentration in the procedural blanks plus the average concentration of the analyte in the blanks. If the analyte was not detected in the blank, the MQL was established based on the concentration of standard that produces a peak in the matrix with a signal-to-noise (S/N) ratio of 10. The method detection limit (MDL) was taken as the calibration standard that produced a S/N of 3. Recoveries, matrix effect, MDLs and MQLs of the target compounds are presented in Table S4.

Recoveries (Table S4a) were assessed in snowmelts and river water as (Pre-extraction addition/Post extraction addition) * 100. Absolute recoveries for snow samples ranged from 67 – 92%

Matrix effects were analysed using the isotopically labelled internal standard Dimethoate-D6 (for benzothiazoles and triazoles), N,N-Diphenylguanidine-D10 (for diphenyl guanidine) and 6PPD-quinone-d5 (for 2,2,4-trimethyl-1,2-dihydroquinoline). This was done by spiking 7 random processed snowmelt samples and 7 Milli-Q water blank samples at the same concentration 50 ng/mL. Peak areas in snowmelt matrix were on average approximately 50 % of the peak areas in the water matrix (Table S4b). The results of matrix effect assessment suggest that without the use of internal standards, the quantification of target compounds may lead to a 2-fold underestimation of analytes' concentrations.

### Text S3.4 Data Preprocessing and Reduction

Initial steps of data pretreatment involved log scaling the snow and surface water data to promote normal distribution and reduce skewness of variables. Row-wise scaling through total area normalization, was also performed to diminish systematic variations inherent in the experimental process. Finally, column-wise mean-centering was applied to emphasize variability by removing overall shift or offsets, focus fluctuating feature intensities around zero and facilitate data interpretation. An Additional autoscaling step was implemented during the Principal Component Analysis (PCA).

The PCA as an exploratory method, was applied to examine the relationship between the varying traffic levels of snow samples. The PCA score plot served for both data visualization and to select site-specific markers.

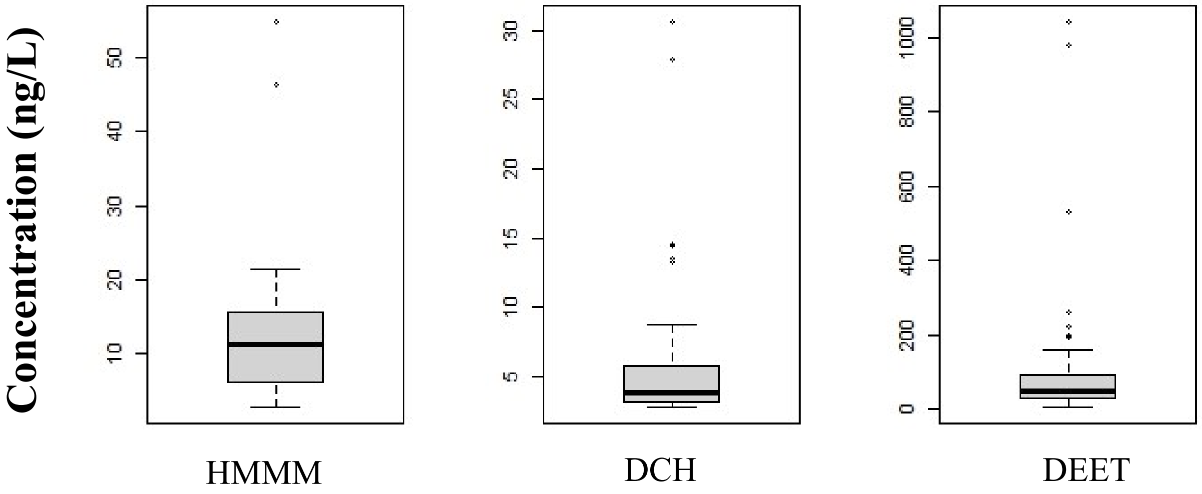


**Figure S3.1 Non-targeted approach target chemicals in surface water**

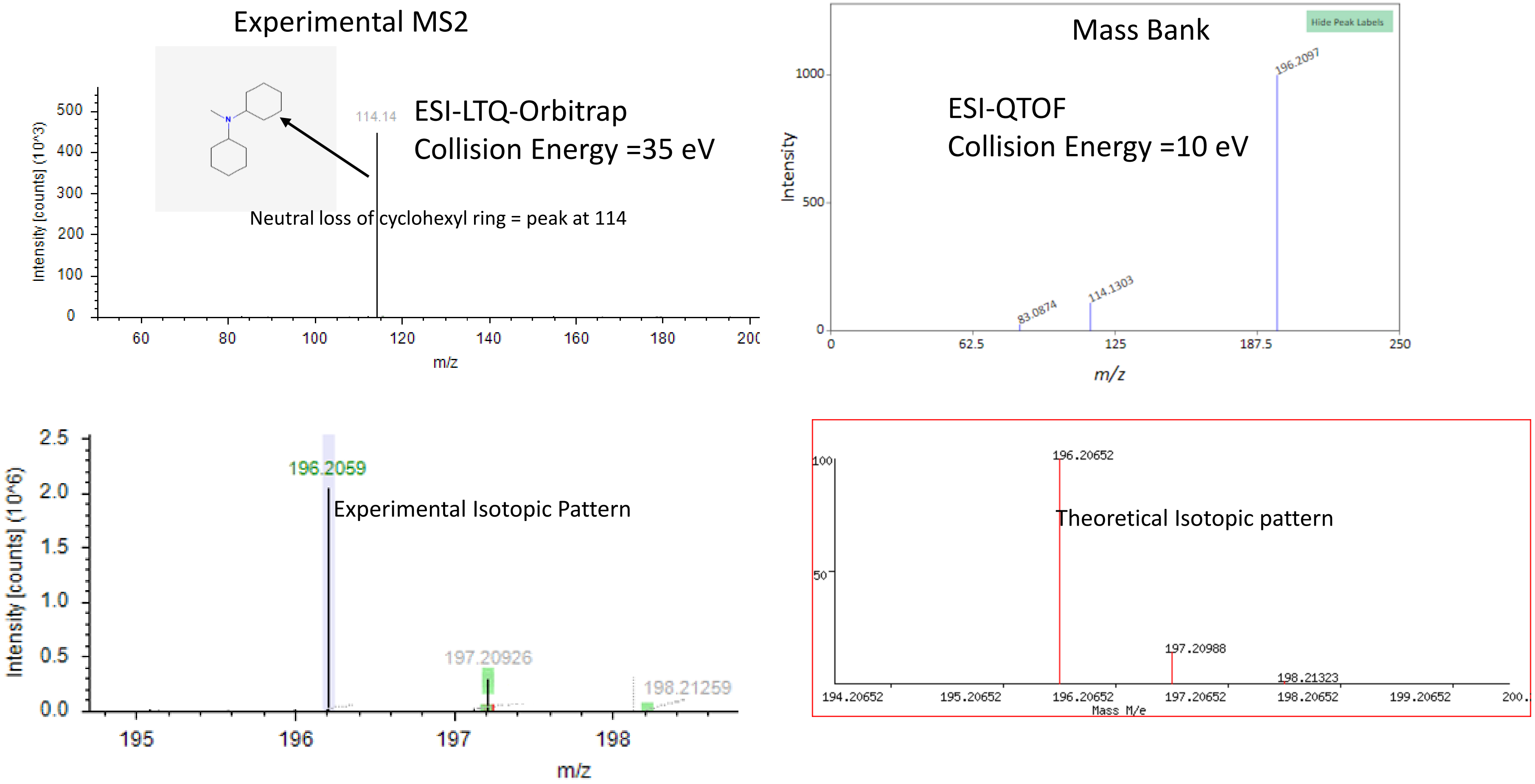


**Figure S3.2 Experimental vs Library Mass Spectra of N,N Dicyclohexylmethylamine (DCMM)**

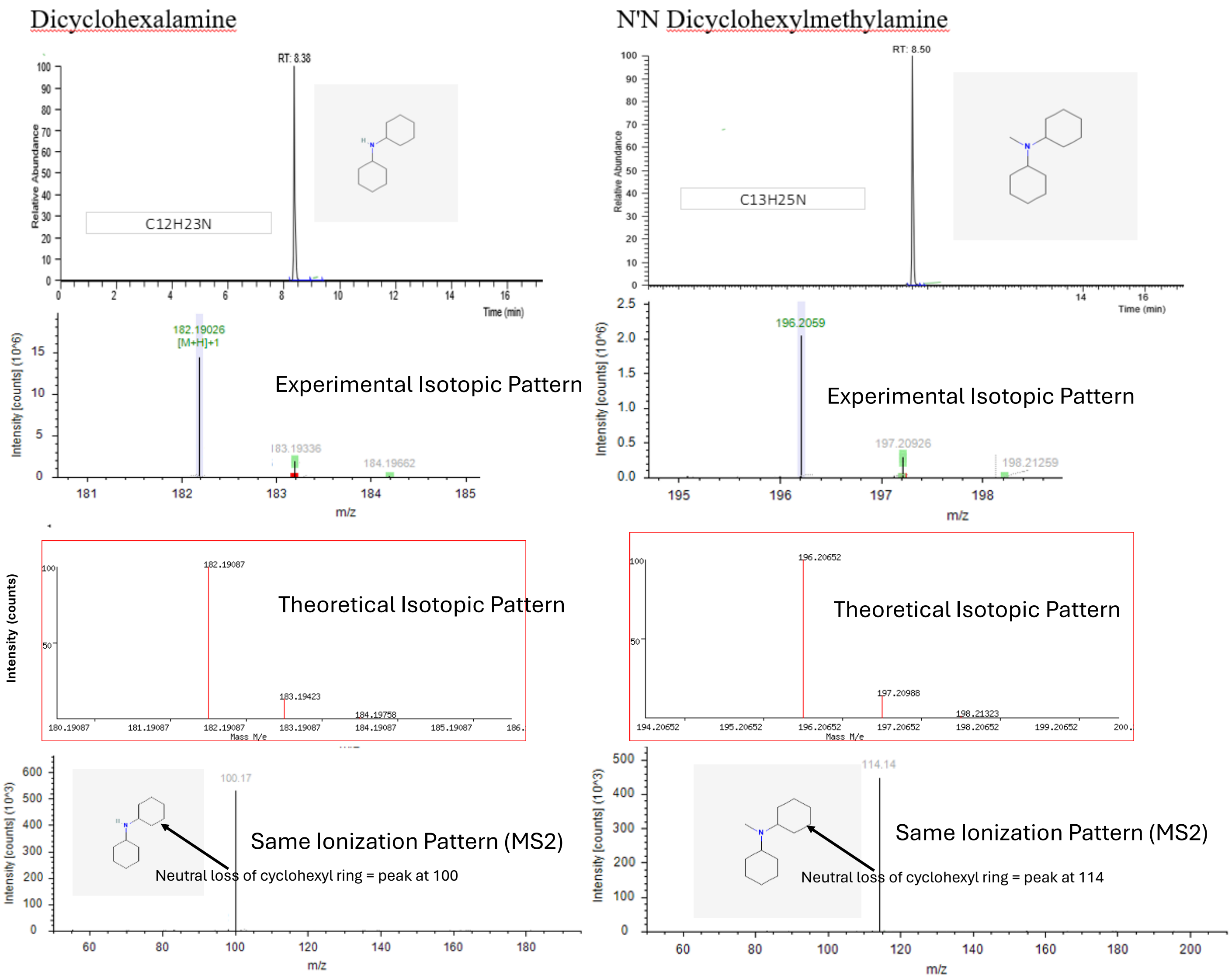


**Figure S3.3** **Analytical Information of Dicyclohexalamine and N,N Dicyclohexylmethylamine (DCMM) Compared**

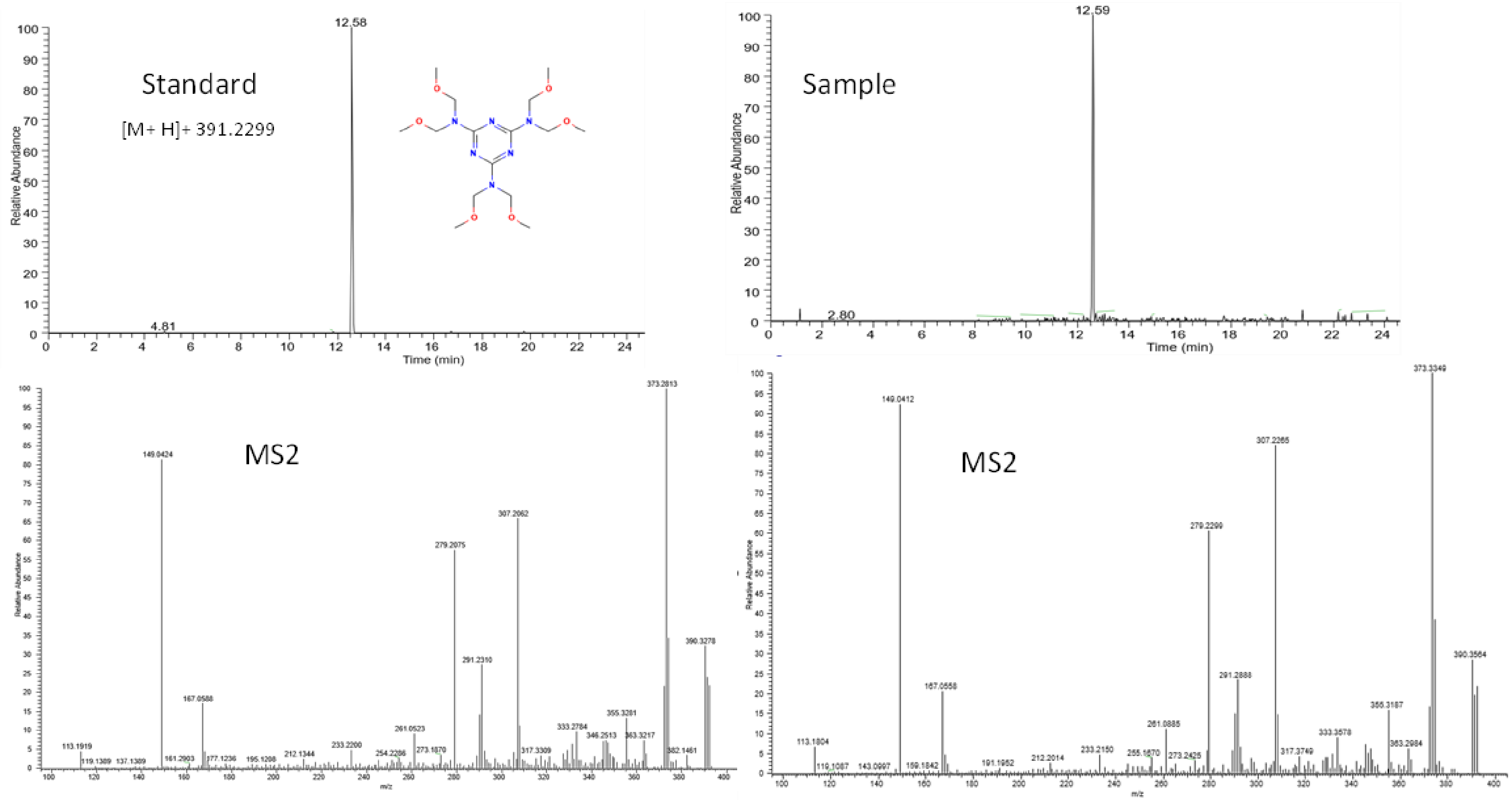


**Figure S3.4** Confirmation of Hexa(methoxymethyl)melamine HMMM by RT and MS information of native standard

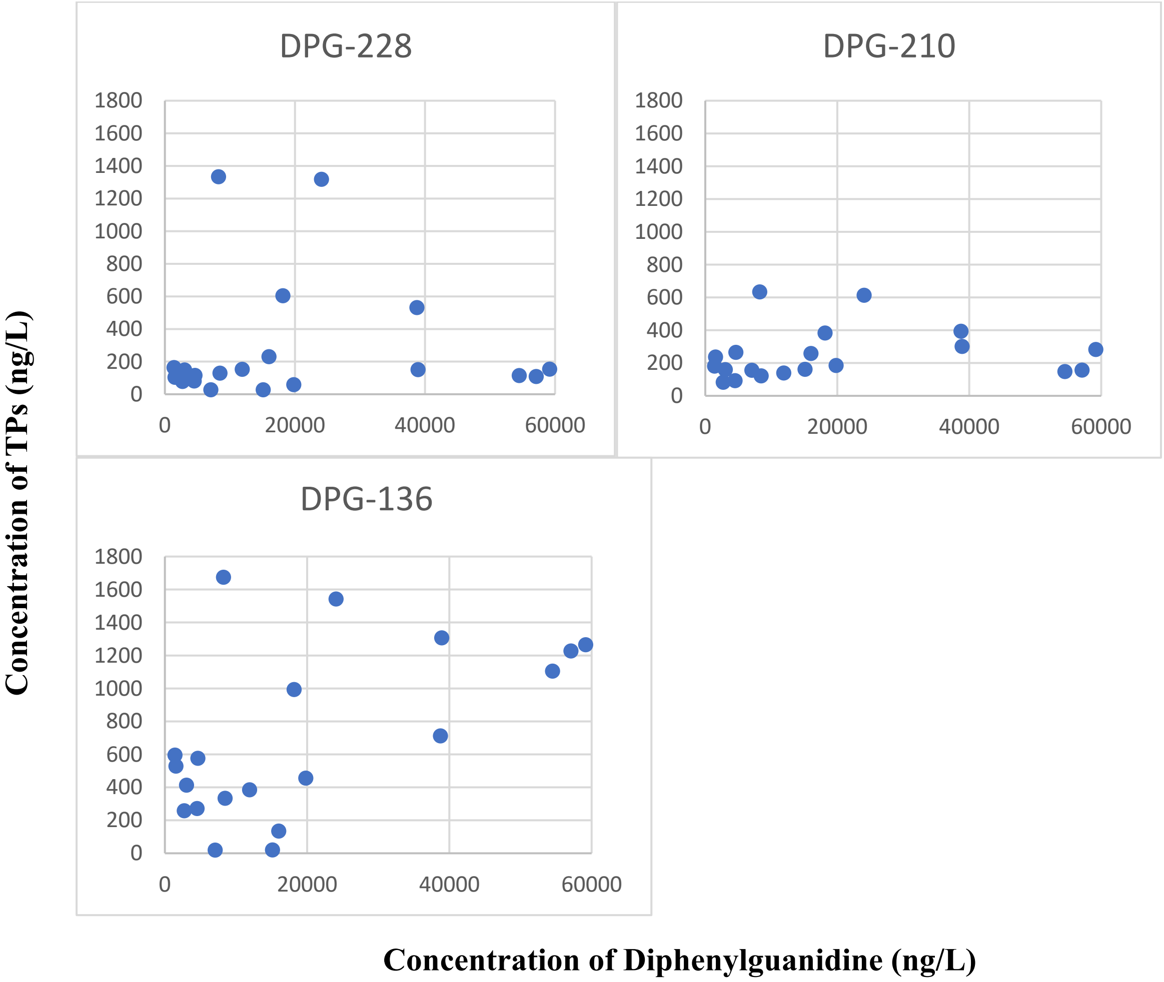
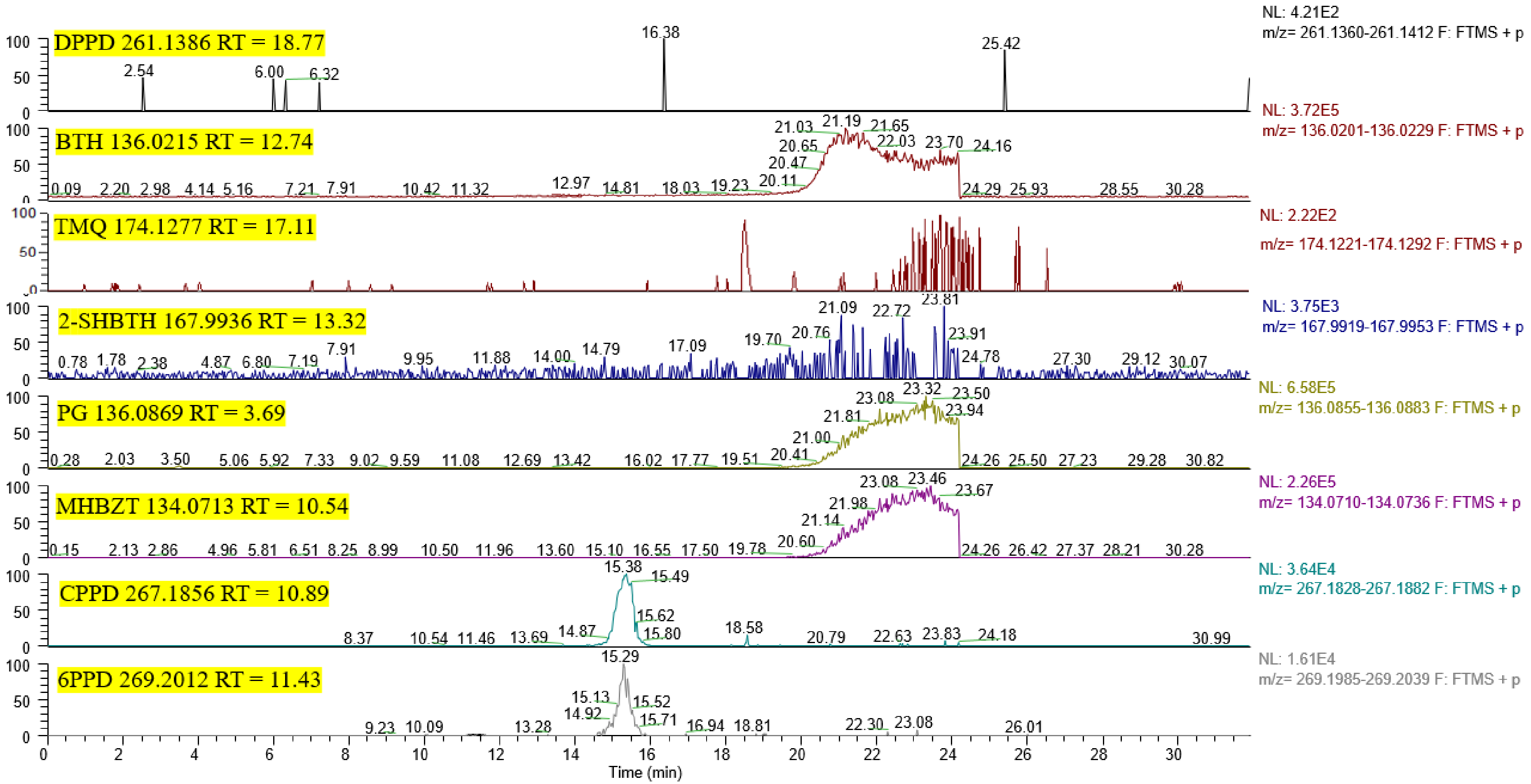


**Figure S3.5 Relationship between DPG and its Transformation Products: Concentrations of Transformation Products (TPs) does seem to show a linear relationship with the parent compound, Diphenyl guanidine (DPG)**


DPPD 261.1386 RT = 18.77
NL: 4.21E2
m/z= 261.1360-261.1412 F: FTMS + p
2.54
6.00
6.32
16.38
25.42
BTH 136.0215 RT = 12.74
NL: 3.72E5
m/z= 136.0201-136.0229 F: FTMS + p
0.09 2.20 2.98 4.14 5.16 7.21 7.91 10.42 11.32 12.97 14.81 18.03 19.23 20.11 20.47 20.65 21.03 21.19 21.65 22.03 23.70 24.16 24.29 25.93 28.55 30.28
TMQ 174.1277 RT = 17.11
NL: 2.22E2
m/z= 174.1221-174.1292 F: FTMS + p
2-SHBTH 167.9936 RT = 13.32
NL: 3.75E3
m/z= 167.9919-167.9953 F: FTMS + p
0.78 1.78 2.38 4.87 6.80 7.19 7.91 9.95 11.88 14.00 14.79 17.09 19.70 20.76 21.09 22.72 23.81 23.91 24.78 27.30 29.12 30.07
PG 136.0869 RT = 3.69
NL: 6.58E5
m/z= 136.0855-136.0883 F: FTMS + p
0.28 2.03 3.50 5.06 5.92 7.33 9.02 9.59 11.08 12.69 13.42 16.02 17.77 19.51 20.41 21.00 21.81 23.08 23.32 23.50 23.94 24.26 25.50 27.23 29.28 30.82
MHBZT 134.0713 RT = 10.54
NL: 2.26E5
m/z= 134.0710-134.0736 F: FTMS + p
0.15 2.13 2.86 4.96 5.81 6.51 8.25 8.99 10.50 11.96 13.60 15.10 16.55 17.50 19.78 20.60 21.14 21.98 23.08 23.46 23.67 24.26 26.42 27.37 28.21 30.28
CPPD 267.1856 RT = 10.89
NL: 3.64E4
m/z= 267.1828-267.1882 F: FTMS + p
8.37 10.54 11.46 13.69 14.87 15.38 15.49 15.62 15.80 18.58 20.79 22.63 23.83 24.18 30.99
6PPD 269.2012 RT = 11.43
NL: 1.61E4
m/z= 269.1985-269.2039 F: FTMS + p
9.23 10.09 13.28 14.92 15.13 15.29 15.52 15.71 16.94 18.81 22.30 23.08 26.01
100
50
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
Time (min)

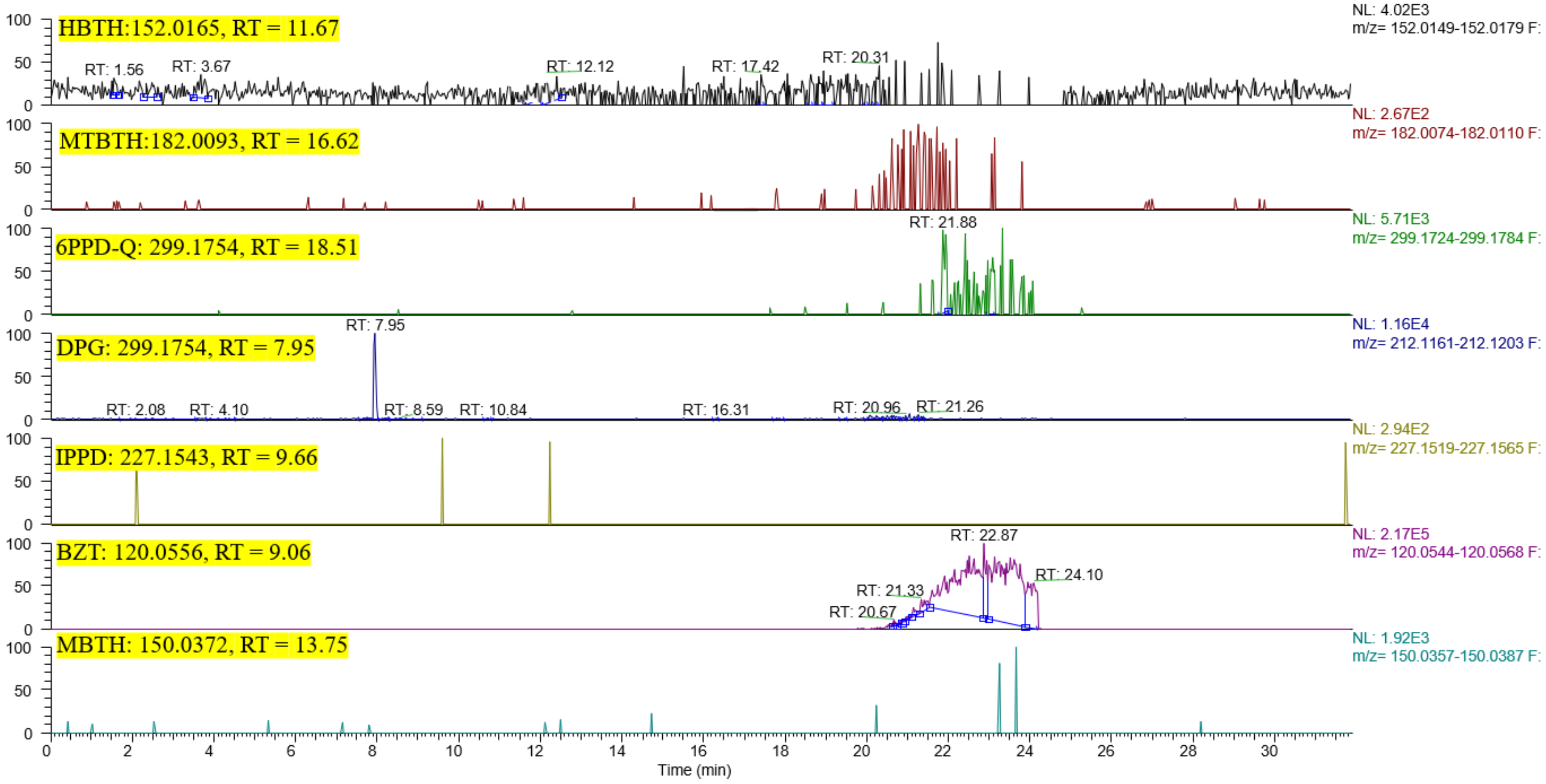


**Figure S3.6** **Chromatographic profile of blank samples showing non-detection of target analytes except for diphenylguanidine.**

## Table S3.1 Target Analytes Information

| Compound | Manufacturer | Purity |
|---|---|---|
| Diphenylguanidine (DPG) | Sigma-Aldrich (Oakville, ON) | 97% |
| Benzothiazole (BTH) | | 96% |
| 2-hydroxybenzothiazole (HBTH) | | 98% |
| 2-(methylthio)-benzothiazole (MTBTH) | | 97% |
| 2-mercaptobenzothiazole (2-SH-BTH) | | 97% |
| 2-methylbenzothiazole (MBTH) | | 99% |
| Benzotriazole (BZT) | | 99% |
| 5-methyl-1H-benzotriazole (MHBZT) | | 98% |
| 2,2,4-trimethyl-1,2-dihydroquinoline (TMQ) | Toronto Research Chemicals (Toronto, ON) | >85% |

## Table S3.2 Internal Standards Peak Areas and Relative Standard Deviation (Surface Water)

| Sample No | 6PPD D5 | DPG D10 | DTH D6 | Sample No | 6PPD D5 | DPG D10 | DTH D6 |
|---|---|---|---|---|---|---|---|
| 1 | 6839489 | 13729692 | 1432457 | 22 | 7501570 | 13788835 | 1583158 |
| 2 | 8027698 | 16670560 | 1897406 | 23 | 6880234 | 5317149.14 | 1649814 |
| 3 | 6022203 | 14436207 | 1648415 | 24 | 8205097 | 12586057.3 | 1999716 |
| 4 | 10353248 | 16500733 | 2106045 | 25 | 7035052 | 16778859.6 | 2146869 |
| 5 | 5378524 | 14322266 | 1731091 | 26 | 7809693 | 17535870.5 | 2107140 |
| 6 | 8282991 | 15121016 | 1871453 | 27 | 6373874 | 11865182.7 | 1235147 |
| 7 | 7102370 | 16233327 | 1842810 | 28 | 9519493 | 18544799.9 | 2205239 |
| 8 | 7806585 | 12996892 | 1533297 | 29 | 5662491 | 12390254.2 | 1805259 |
| 9 | 6220138 | 16175425 | 1701040 | 30 | 6596058 | 17412585.8 | 1772627 |
| 10 | 6075422 | 13774311 | 1536312 | 31 | 8074732 | 15036687.7 | 1666749 |
| 11 | 6875628 | 11012873 | 1469487 | 32 | 6006392 | 16830464.7 | 1992915 |
| 12 | 7454601 | 12717504 | 1778426 | 33 | 5489731 | 10825886.5 | 1571221 |
| 13 | 7396972 | 17722097 | 2040055 | 34 | 5060826 | 10639021.1 | 1739253 |
| 14 | 6007775 | 12956604 | 1523609 | 35 | 7093152 | 13646898.9 | 1428067 |
| 15 | 6980081 | 17520156 | 1911319 | 36 | 5808234 | 9551577.31 | 1984671 |
| 16 | 5723483 | 11556503 | 1573489 | 37 | 5633850 | 10820495.1 | 1832772 |
| 17 | 7908155 | 14523840 | 2157796 | 38 | 6300804 | 13015990.7 | 1672915 |
| 18 | 8303401 | 16801595 | 2561410 | 39 | 5394403 | 14793383.2 | 1744381 |
| 19 | 7469908 | 17169968 | 2188062 | 40 | 5329524 | 11670073.7 | 1446290 |
| 20 | 6605722 | 14572835 | 1750135 | 41 | 6527595 | 15648514.9 | 1780585 |
| 21 | 7876110 | 16907131 | 2035460 | 42 | 6722226 | 12572477 | 1999125 |

| | 6PPD D5 | DPG D10 | DTH D6 |
|---|---|---|---|
| Average | 6898465 | 14159347.6 | 1801273 |
| SD | 1154142 | 2727081.5 | 267055.4 |
| RSD | **17%** | **19%** | **15%** |

| | |
|---|---|
| **6PPD D5** | 6PPD-quinone-D5 |
| **DPG D10** | N,N-diphenylguanidine-D10 |
| **DTH D6** | Dimethoate-D6 |

**Table S3.3 Method Detection Limit (MDL), Recovery Matrix, Linearity, and Internal Standard**

| Compound | Abbreviation | Surface Water | | Snowmelt | |
|---|---|---|---|---|---|
| | | Recovery (%) | MDL (ng/ml) | Recovery (%) | MDL (ng/ml) |
| Benzotriazole | BZT | 79 | 4.0 | 86 | 2.9 |
| 5-methyl-1-hydroxyl-benzotriazole | MHBZT | 74 | 0.1 | 67 | 1.6 |
| Hydroxybenzothiazole | HBTH | 88 | 0.5 | 92 | 4.1 |
| 2-(methylthio)-benzothiazole | MTBTH | 81 | 0.1 | 78 | 1.4 |
| Benzothiazole | BTH | 88 | 2.4 | 85 | 6.9 |
| 2-methylbenzothiazole | MBTH | 84 | 1.5 | 78 | 1.2 |
| Diphenylguanidine | DPG | 90 | 14.4 | 78 | 35.2 |
| 2,2,4-trimethyl-1,2-dihydroquinoline | TMQ | 84 | 0.9 | 80 | 1.1 |

| Compound | Abbreviation | linearity ($R^2$) | Internal Standard |
|---|---|---|---|
| Benzotriazole | BZT | >0.9991 | Dimethoate-D6 |
| 5-methyl-1-hydroxyl-benzotriazole | MHBZT | >0.9992 | Dimethoate-D6 |
| Hydroxybenzothiazole | HBTH | >0.9987 | Dimethoate-D6 |
| 2-(methylthio)-benzothiazole | MTBTH | >0.9981 | Dimethoate-D6 |
| Benzothiazole | BTH | >0.9996 | Dimethoate-D6 |
| 2-methylbenzothiazole | MBTH | >0.9993 | Dimethoate-D6 |
| Diphenylguanidine | DPG | >0.9984 | N,N-Diphenylguanidine-D10 |
| 2,2,4-trimethyl-1,2-dihydroquinoline | TMQ | >0.9979 | 6PPD-quinone-D5 |

## Table S3.4 Internal Standards and Matrix Effect

| Peak areas of Dimethoate-D6 internal standard spiked at 50 ng/mL in Milli-Q Water and snowmelt processed samples. | | |
|---|---|---|
| Sample No | Peak Area (Milli-Q Water) | Peak Area (Snowmelt) |
| 1 | 4788192 | 1670629 |
| 2 | 5107700 | 1587167 |
| 3 | 4911298 | 1587167 |
| 4 | 4798424 | 1414084 |
| 5 | 4786994 | 1544257 |
| 6 | 4659131 | 1531299 |
| 7 | 4537158 | 1724088 |
| Average | **4798414** | **1579813** |
| Matrix Effect | | 33% |

| Peak areas of 6PPD-quinone-d5 internal standard spiked at 50 ng/mL in Milli-Q Water and snowmelt processed samples. | | |
|---|---|---|
| Sample No | Peak Area (Milli-Q Water) | Peak Area (Snowmelt) |
| 1 | 1845378 | 1340947 |
| 2 | 1944649 | 1098380 |
| 3 | 1919332 | 1025717 |
| 4 | 1836371 | 1050611 |
| 5 | 1866298 | 1243208 |
| 6 | 1856776 | 1128632 |
| 7 | 1798522 | 1371681 |
| 8 | **1866761** | **1179882** |
| | | 63% |

| Peak areas of N,N-Diphenylguanidine-D10 internal standard spiked at 50 ng/mL in Milli-Q Water and snow samples. | | |
|---|---|---|
| Sample No | Peak Area (Milli-Q Water) | Peak Area (Snowmelt) |
| 1 | 51541396 | 39127021 |
| 2 | 52125407 | 38713200 |
| 3 | 46986732 | 13646217 |
| 4 | 47882266 | 12214435 |
| 5 | 47501859 | 11790692 |
| 6 | 45246823 | 11082213 |
| 7 | 49526040 | 34024317 |
| 8 | **48687218** | **22942585** |
| | | 47% |

## Table S3.5 Non-Parametric test: Comparison between Snow and Water (<0.05 = significant)

| Name | Abbreviation | Kruskal Wallis test (P-Value) | Remark |
|---|---|---|---|
| Benzotriazole | BZT | 3.18E-06 | Significant |
| Benzothiazole | BTH | 6.41E-06 | Significant |
| 5-methyl-1-hydroxyl-benzotriazole | MHBZT | 1.19E-06 | Significant |
| Hydroxybenzothiazole | HBTH | 8.35E-13 | Significant |
| 2-(methylthio)-benzothiazole | MTBTH | 6.69E-10 | Significant |

**Within Water Comparison (<0.05 = significant)**

|  | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 2 | 0.28 |  |  |  |
| 3 | 6.7E-15 | 4.2E-16 |  |  |
| 4 | 1.7E-15 | < 2E-16 | 3.1E-10 |  |
| 5 | < 2E-16 | < 2E-16 | < 2E-16 | < 2E-16 |

**Within Snow Comparison (<0.05 = significant)**

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| 2 | 0.02474 |  |  |  |  |  |  |
| 3 | 0.02474 | 1.00000 |  |  |  |  |  |
| 4 | 0.00984 | 0.00028 | 3.40E-05 |  |  |  |  |
| 5 | 1.40E-05 | 0.02497 | 0.00984 | 4.20E-12 |  |  |  |
| 6 | 3.40E-05 | 0.00984 | 0.00489 | 3.20E-13 | 0.09582 |  |  |
| 7 | 0.00054 | 0.00051 | 1.20E-10 | 1.70E-05 | 7.10E-12 | 7.10E-12 |  |
| 8 | 0.26043 | 0.01836 | 0.01982 | 0.02497 | 3.50E-07 | 8.70E-08 | 1.60E-09 |

|  |  |  |
|---|---|---|
| 1 | Benzotriazole | BZT |
| 2 | 5-methyl-1-hydroxyl-benzotriazole | MHBZT |
| 3 | Hydroxybenzothiazole | HBTH |
| 4 | 2-(methylthio)-benzothiazole | MTBTH |
| 5 | Benzothiazole | BTH |
| 6 | Diphenyl guanidine | DPG |
| 7 | 2-methylbenzothiazole | MBTH |
| 8 | 2,2,4-trimethyl-1,2-dihydroquinoline | TMQ |

## Table S3.6 Compound Discoverer Software Parameters

| | | |
|---|---|---|
| 1 | **Select Spectra** | |
| | Lower RT Limit: | 3 |
| | Upper RT Limit: | 26 |
| | First Scan: 0 | 0 |
| | Last Scan: 0 | 0 |
| | Lowest Charge State: | 0 |
| | Highest Charge State: | 0 |
| | Min. Precursor Mass: | 100 Da |
| | Max. Precursor Mass: | 500 Da |
| | Total Intensity Threshold: | 0 |
| | Minimum Peak Count: | 1 |
| 2 | **Align Retention Times** | |
| | Suitable reference file | |
| 3 | **Detect Compounds** | |
| | Mass Tolerance [ppm]: | 5 |
| | Min. Peak Intensity: | 10000 |
| | Min. # Scans per Peak: | 5 |
| | Use Most Intense Isotope Only: | FALSE |
| | **Peak Detection** | |
| | Chromatographic S/N Threshold: | 1.5 |
| | Remove Baseline: | Fasle |
| | **Isotope Pattern Detection:** | |
| | Group Isotopes for: Br; Cl | Br, Cl |
| | Filter out Features with Bad Peaks Only: | TRUE |
| | Zig-Zag Index Threshold: | 0.2 |
| | Jaggedness Threshold: | 0.4 |
| | Modality Threshold: | 0.9 |
| | Remove Potentially False Positive Isotopes: | FALSE |
| | **Compound Detection** | |
| | Ions: | [M+2H]+2; [M+ACN+H]+1; [M+H]+1; [M+H+K]+2 |
| | Base Ions: | [M+H]+1; [M+NH4]+1 |
| 4 | **Group Compound Node** | |
| | Mass Tolerance | 5 ppm |
| | RT Tolerance [min]: | 0.1 |
| | Align Peaks: False | FALSE |
| | Preferred Ions: | [M+H]+1 |
| | Area Integration | Most Common Ion |
| | **Peak Rating Contributions** | |
| | Area Contribution: | 3 |
| | CV Contribution: | 10 |
| | FWHM to Base Contribution: | 5 |
| | Jaggedness Contribution: | 5 |
| | Modality Contribution: | 5 |
| | Zig-Zag Index Contribution: | 5 |
| 5 | **Fill Gaps** | |
| | Mass Tolerance: | 5 ppm |
| | S/N Threshold: | 1.5 |
| | Use Real Peak Detection: | TRUE |
| | Apply Restrictive Gap Filling: | TRUE |
| 6 | **Mark Background Compounds** | |
| | Max. Sample/Blank: | 5 |
| | Max. Blank/Sample: | 0 |
| | Hide Background: | FALSE |
| 7 | **Assign Compound Annotations** | |
| | Mass Tolerance: | 5 ppm |
| | **Data Sources:** | |
| | Data Source #1: | mzCloud Search |
| | Data Source #2: | mzVault Search |
| | Data Source #3: | MassList Search |
| | Data Source #4: | Predicted Compositions |
| | Data Source #5: | ChemSpider Search |
| | **Scoring Rules:** | |
| | Use mzLogic: | TRUE |

| | | |
|---|---|---|
| 8 | **Search mzCloud** | |
| | Compound Classes | All |
| | Precursor Mass Tolerance: | 10 ppm |
| | FT Fragment Mass Tolerance: | 10 ppm |
| | IT Fragment Mass Tolerance: | 0.4 Da |
| | Library: Autoprocessed; | Reference |
| | Post Processing | Recalibrated |
| | Max. # Results: | 10 |
| | Annotate Matching Fragments: | TRUE |
| | Search MSn Tree: | False |
| | **DDA Search:** | |
| | Identity Search: | HighChem DP |
| | Match Activation Type: | TRUE |
| | Match Activation Energy | Match with Tolerance |
| | Activation Energy Tolerance: | 20 |
| | Apply Intensity Threshold: | TRUE |
| | Similarity Search | Similarity Forward |
| | Match Factor Threshold: | 50 |
| 9 | **Predict Compositions** | |
| | Mass Tolerance: | 5 ppm |
| | Min. Element Counts: | C H |
| | Max. Element Counts: | C90 H190 Br3 Cl8 F18 N10 O18 P3 S5 |
| | Min. RDBE: | 0 |
| | Max. RDBE: | 40 |
| | Min. H/C: | 0.1 |
| | Max. H/C: | 3.5 |
| | Max. # Candidates: | 10 |
| | Max. # Internal Candidates: | 500 |
| | **Pattern Matching:** | |
| | Intensity Tolerance [%]: | 30 |
| | Intensity Threshold [%]: | 0.1 |
| | S/N Threshold: | 3 |
| | Min. Spectral Fit [%]: | 30 |
| | Min. Pattern Cov. [%]: | 80 |
| | Use Dynamic Recalibration: | TRUE |
| | Fragments Matching: | |
| | Use Fragments Matching: | TRUE |
| | Mass Tolerance: | 5 ppm |
| | S/N Threshold: | 3 |
| 10 | **Search ChemSpider** | |
| | Database(s): | ACToR: Aggregated Computational Toxicology Resource<br>DrugBank<br>EAWAG Biocatalysis/Biodegradation Database<br>EPA DSSTox<br>EPA Toxcast<br>FDA UNII - NLM |
| | Search Mode: | By Formula or Mass |
| | Mass Tolerance: | 5 ppm |
| | Max. # of results per compound: | 20 |
| 11 | **Apply mzLogic:**Search Settings: | |
| | FT Fragment Mass Tolerance: | 10 ppm |
| | IT Fragment Mass Tolerance: | 0.4 Da |
| | Match Factor Threshold: | 30 |
| 12 | **Search Mass Lists**: Search Settings | |
| | Mass Lists: | In-house |
| | Mass Tolerance: | 5 ppm |
| | Use Retention Time: | TRUE |
| | RT Tolerance [min]: | 0.5 |
| 13 | **Merge Features:** Peak Consolidation | |
| | Mass Tolerance: 5 ppm | 5 ppm |
| | RT Tolerance [min]: | 0.1 |
| 14 | **Post Processing node:** | |
| | Update Peak Rating: | TRUE |

## Table S3.7a Concentration of Target Analytes (Surface water)

| Surface Water (ng/L) | | | | | |
|---|---|---|---|---|---|
| SITE | Benzotriazole (BZT) | 5-methyl-1H-benzotriazole (MHBZT) | Hydroxybenzothiazole (HBTH) | 2-(methylthio)-benzothiazole (MTBTH) | Benzothiazole (BTH) |
| 1 | 37 | 31 | 18 | 14 | 438 |
| 2 | 9 | 27 | 27 | 10 | 305 |
| 3 | 45 | 32 | 17 | 9 | 382 |
| 4 | 106 | 70 | 11 | 10 | 162 |
| 5 | 140 | 95 | 19 | 16 | 658 |
| 6 | 161 | 118 | 26 | 14 | 423 |
| 7 | 35 | 31 | 14 | 14 | 562 |
| 8 | 39 | 33 | 14 | 10 | 273 |
| 9 | 33 | 32 | 35 | 19 | 993 |
| 10 | 38 | 34 | 17 | 10 | 436 |
| 11 | 40 | 33 | 16 | 10 | 541 |
| 12 | 40 | 34 | 16 | 14 | 936 |
| 13 | 38 | 35 | 17 | 13 | 467 |
| 14 | 30 | 25 | 14 | 8 | 372 |
| 15 | 35 | 31 | 16 | 9 | 295 |
| 16 | 26 | 24 | 17 | 8 | 364 |
| 17 | 224 | 192 | 17 | 10 | 219 |
| 18 | 35 | 33 | 19 | 8 | 222 |
| 19 | 31 | 42 | 34 | 10 | 429 |
| 20 | 37 | 37 | 19 | 20 | 816 |
| 21 | 37 | 46 | 16 | 9 | 123 |
| 22 | 48 | 84 | 14 | 10 | 375 |
| 23 | 32 | 48 | 23 | 9 | 388 |
| 24 | 38 | 42 | 12 | 9 | 353 |
| 25 | 42 | 44 | 9 | 9 | 165 |
| 26 | 37 | 40 | 12 | 9 | 293 |
| 27 | 40 | 51 | 20 | 10 | 472 |
| 28 | 39 | 56 | 28 | 25 | 1046 |
| 29 | 44 | 60 | 16 | 8 | 176 |
| 30 | 35 | 43 | 22 | 7 | 222 |
| 31 | 6 | 8 | 21 | 14 | 814 |
| 32 | 49 | 53 | 13 | 8 | 323 |
| 33 | 52 | 58 | 15 | 7 | 110 |
| 34 | 52 | 58 | 19 | 8 | 315 |
| 35 | 48 | 54 | 32 | 9 | 536 |
| 36 | 64 | 87 | 19 | 17 | 371 |
| 37 | 19 | 67 | 105 | 315 | 1271 |
| 38 | 53 | 63 | 16 | 10 | 398 |
| 39 | 46 | 60 | 20 | 42 | 1190 |
| 40 | 46 | 56 | 17 | 10 | 405 |
| 41 | 37 | 70 | 15 | 8 | 255 |
| 42 | 114 | 77 | 14 | 10 | 228 |
| Minimum Concentration | 224 | 192 | 105 | 315 | 1271 |
| Maximum Concentration | 6 | 8 | 9 | 7 | 110 |
| Median Value | **39** | **45** | **17** | **10** | **378** |

## Table S3.7b Concentration of Target Analytes (Snow)

| Snowmelt (ng/L) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| SITE TAG | Benzotriazole (BZT) | 5-methyl-1H-benzotriazole (MHBZT) | Hydroxybenzothiazole (HBTH) | 2-(methylthio)-benzothiazole (MTBTH) | Benzothiazole (BTH) | 2-methylbenzothiazole (MBTH) | 2,2,4-trimethyl-1,2-dihydroquinoline (TMQ) | Diphenylguanidine (DPG) |
| HRT 1 | 587 | 1889 | 1717 | 87 | 3163 | <MDL | 928 | 54518 |
| HRT 2 | 584 | 1886 | 1802 | 87 | 3197 | <MDL | 812 | 57111 |
| HRT 3 | 663 | 1416 | 1197 | 208 | 4606 | <MDL | 107 | 7082 |
| HRT 4 | 391 | 847 | 964 | 140 | 4036 | <MDL | 51 | 19841 |
| HRT 5 | 363 | 926 | 806 | 95 | 3366 | <MDL | 109 | 15142 |
| HRT 6 | 742 | 2143 | 1715 | 164 | 4108 | 13.7 | 183 | 11923 |
| HRT 7 | 370 | 1582 | 2060 | 374 | 1187 | <MDL | 180 | 59197 |
| HRT 8 | 471 | 3898 | 1734 | 165 | 3999 | 10.8 | 493 | 38945 |
| MRT 1 | 204 | 524 | 684 | 72 | 1513 | <MDL | 123 | 8488 |
| MRT 2 | 355 | 892 | 949 | 96 | 3418 | 16.6 | 93 | 16032 |
| MRT 3 | 368 | 784 | 990 | 133 | 3536 | 14.7 | 44 | 18182 |
| MRT 4 | 477 | 3918 | 1611 | 167 | 3964 | 12.5 | 399 | 38771 |
| MRT 5 | 428 | 864 | 902 | 84 | 1502 | 9.5 | 351 | 1433 |
| MRT 6 | 503 | 1250 | 935 | 76 | 1788 | <MDL | 107 | 4657 |
| LRT 1 | 430 | 859 | 896 | 85 | 1385 | <MDL | 399 | 1571 |
| LRT 2 | 516 | 1325 | 961 | 73 | 1837 | <MDL | 103 | 4542 |
| LRT 3 | 251 | 565 | 475 | 79 | 1804 | 9.8 | 41 | 3062 |
| LRT 4 | 127 | 287 | 346 | 57 | 1787 | 14.6 | 87 | 2740 |
| LRT 5 | 200 | 500 | 621 | 65 | 1464 | <MDL | 106 | 8265 |
| LRT 6 | 530 | 1324 | 1130 | 132 | 2325 | 10.9 | 102 | 24097 |
| PMR 1 | 7 | 8 | 27 | 13 | 258 | 9.2 | <MDL | 140 |
| PMR 2 | 8 | 9 | 36 | 14 | 341 | 10.7 | <MDL | 179 |
| PJ 1 | 8 | 9 | 33 | 15 | 410 | 9.8 | <MDL | 191 |
| PJ 2 | 8 | 8 | 40 | 14 | 383 | <MDL | <MDL | 237 |
| DS 1 | 54 | 294 | 113 | 15 | 280 | 14.0 | <MDL | 483 |
| DS 2 | 56 | 296 | 126 | 15 | 359 | 13.7 | <MDL | 472 |
| SB 1 | 300 | 281 | 242 | 39 | 891 | 16.4 | 57 | 404 |
| SB 2 | 296 | 290 | 257 | 37 | 893 | 16.0 | 54 | 424 |
| PA 1 | 64 | 72 | 87 | 48 | 76 | 50.7 | 93 | 144 |
| PA 2 | 5 | 7 | 26 | 9 | 140 | 6.8 | <MDL | 578 |
| Minimum Concentration | 5 | 7 | 26 | 9 | 76 | 7 | 41 | 140 |
| Maximum Concentration | 742 | 3918 | 2060 | 374 | 4606 | 51 | 928 | 59197 |
| Median Value | **359** | **816** | **851** | **78** | **1650** | **13** | **107** | **4600** |

## Table S3.8 Summary of Analytes Identified and Quantified by Suspect and Non-Targeted Approach

Table S8: Summary of Analytes Identified and Quantified by Suspect and Non-Targeted Approach

| No | Analyte | RT [min] | Formula | m/z | Approach | Concentration Range (Snow) ng/L | Median Concentration (ng/L) | Internal Standard | Status |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Diphenylguanidine-228 (DPG-228) | 7.26 | $C_{13}H_{13}N_3O$ | 228.1130 | Non-Targeted | 3.6-1333 | 115 | N,N-Diphenylguanidine-D10 | Confirmed |
| 2 | Diphenylguanidine-210 (DPG-210) | 8.84 | $C_{13}H_{11}N_3$ | 210.1026 | Non-Targeted | 47-632 | 180 | N,N-Diphenylguanidine-D10 | Confirmed |
| 3 | Diphenylguanidine-136 (DPD-136) | 3.49 | $C_7H_7N_3$ | 136.0869 | Non-Targeted | 18-527 | 1673 | N,N-Diphenylguanidine-D10 | Confirmed |
| 4 | 1,3-di-o-tolylguanidine (DTG) | 8.95 | $C_{15}H_{17}N_3$ | 240.1495 | Suspect | 10-441 | 51 | N,N-Diphenylguanidine-D10 | Level 2a |
| 5 | Hexa(methoxymethyl)melamine (HMMM) | 12.66 | $C_{15}H_{30}N_6O_6$ | 391.2300 | Suspect | 665-10258 | 1908 | 6PPD-quinone-D5 | Confirmed |
| 6 | 3-Cyclohexyl-1,1-dimethylurea (CHD) | 11.20 | $C_9H_{18}N_2O$ | 171.1492 | Non-Targeted | 47-580 | 112 | 6PPD-quinone-D5 | Level 2a |
| 7 | Dicyclohexalamine (DCH) | 8.37 | $C_{12}H_{23}N$ | 182.1903 | Non-Targeted | 4-7136 | 1641 | N,N-Diphenylguanidine-D10 | Level 2a |
| 8 | N,N Dicyclohexylmethylamine (DCMM) | 8.50 | $C_{13}H_{25}N$ | 196.2060 | Non-Targeted | 6-1263 | 181 | N,N-Diphenylguanidine-D10 | Level 2a |
| 9 | 1,3-Diphenylurea (DPU) | 13.40 | $C_{13}H_{12}N_2O$ | 213.1023 | Non-Targeted | 35-4952 | 191 | 6PPD-quinone-D5 | Level 2a |
| 10 | N,N-diethyl-meta-toluamide (DEET) | 14.10 | $C_{12}H_{17}NO$ | 192.1383 | Suspect | 3-1664 | 288 | 6PPD-quinone-D5 | Level 2a |
| 11 | 3,5-Di-tert-butyl-4-hydroxybenzaldehyde (DBH) | 18.90 | $C_{15}H_{22}O_2$ | 235.1693 | Suspect | 47-545 | 144 | 6PPD-quinone-D5 | Level 2a |

| Analyte | Concentration Range (Water) ng/L | Median Concentration (ng/L) |
|---|---|---|
| HMMM | 2.8-54 | 11 |
| DCH | 2.7-31 | 3.8 |
| DEET | 4-1042 | 49 |

**Table S3.9 Expected Transformation Product of Diphenylguanidine, Benzotriazole and Benzothiazole**

| Transformation Product | [M+H]+ | Formula |
|---|---|---|
| DPG-228 | 228.1131 | $C_{13}H_{13}N_3O$ |
| DPG-210 | 210.1026 | $C_{13}H_{11}N_3$ |
| DPG-244 | 244.1081 | $C_{13}H_{13}N_3O_2$ |
| DPG-136 | 136.0869 | $C_7H_9N_3$ |
| DPG-134 | 134.0712 | $C_7H_7N_3$ |
| BZT-136 | 136.0505 | $C_6H_5N_3O$ |
| BZT-152 | 152.0455 | $C_6H_5N_3O_2$ |
| BTH-152 | 152.0165 | $C_7H_5NOS$ |
| BTH-168 | 168.0114 | $C_7H_5NO_2S$ |