| Table 5-2. Summary of sample collection and analytical method information for studies of 6PPD |
| --- |
| Matrix | Detection Limit | Container and Storage | Internal or Surrogate Standards | Sample/Pretreatment, Extraction, and Cleanup | Instrumental Analysis | LC or GC | MS | Quantitation Ion | Confirmation Ion | Reference |
| Water, Fish Bile, and Plasma | MDL: Plasma: 0.0075–0.025 ng/mLBile: 0.015–0.05 ng/ml | Glass vials and bottles | Surrogate: DPPD-d14Internal: progesterone‑d9 | Water: SPE or dilution in MeOH, analysisBile and plasma: Water dilution, SPE, analysis | Water, bile, plasma: LC-MS/MS | Phenomenex Kinetex C18 EVO (100×2.1 mm, 1.7 µm particle size) | ESI+ (MRM) | 268>211 | 268 >167 | Bile/plasma: (da Silva et al. In preparation) |
| Groundwater, Stormwater, and Surface Water | MRL: 2 ng/L | Collection: amber glass bottles, no headspace. Storage: Analyzed within 72 hours of collection or frozen until analysis  | Surrogate internal standard: D5-6PPD-q | 0.7-micron GFF | UPLC-MS/MS | Waters ACQUITY UPLC BEH C18 (1.7 µm, 2.1×50 mm) plus Vanguard pre‐column (1.7 µm, 2.1×5 mm) column with 0.1% formic acid in water and ACN mobile phase | ESI+ | 269.1/184.1 | 269.1/93.0 | (Lane et al. 2024) |
| Influent and Effluent of WWTP | MDL: 60 ng/L | Large volume SPE | Not available as of the publication date | Filtered using a Sartopure GF + Midicap, 0.65 μm deep filter and extracted using cartridges filled with 10 g of Chromabond HR-X, eluted with 5 mL of ethyl acetate, 5 mL of methanol, 5 mL of methanol containing 1% of formic acid, 5 mL of methanol containing 2% of 7 N ammonia in methanol, evaporated under a nitrogen to near dryness, brought to 1 mL with methanol | LC-HRMS | Kinetex C18 EVO column (50×2.1 mm, 2.6 µm particle size, 83 Phenomenex, pre-column 4×2.1 mm, and in-line filter 0.2 µm) and a gradient elution with 0.1% of formic acid and methanol containing 0.1% of formic acid mobile phase | Ion modeESI+ | M+H= 269.2012 |  | (Maurer et al. 2023) |
| Snow from Urban Street | LOQ: 100 ng/L | 250 mL glass bottles | Not available as of the publication date |  | UPLC-TOF-MS | HSS T3 column; 100×2.1 mm, 1.7 μm, and Atlantis T3 (3 µm, 2.1×100 mm); (A) water with 0.1% formic acid (v/v) and (B) methanol with 0.1% formic acid (v/v) mobile phase | ESI(+) modeMRM | 269.1/184.0 | 269.1/106.9 | (Seiwert et al. 2022) |
| Influent and Effluent WWTP during Snow Melt, Rain, And dry Conditions | LOQ: 100 ng/L | 250 mL glass bottles | Not available as of the publication date |  | UPLC-TOF-MS | HSS T3 column; 100×2.1 mm, 1.7 μm, and Atlantis T3 (3 µm, 2.1×100 mm); (A) water with 0.1% formic acid (v/v) and (B) methanol with 0.1% formic acid (v/v) mobile phase | ESI(+) modeMRM | 269.1/184.0 | 269.1/106.9 | (Seiwert et al. 2022) |
| Air from 18 Major Cities That Comprise the GAPS Network | Instrument LOQ: 1.95 ng/mLMethod LOQ: 2.71 pg/m3 | PUF disk samplers collecting both gas- and particle-phase chemicals | Not available as of the publication date | ASE extraction with petroleum ether and acetone (83/17, v/v), rotary evaporation, reconstituted with iso-octane, silica column cleanup | UPLC-HRMS | Phenomenex (Torrance, CA, USA) Kinetex C18 column (2.6 μm in particle size, 50×4.6 mm in length and inner diameter); HPLC-grade methanol, with 0.1% of formic acid in both, were used as the mobile phase | Positive ionization mode with a HESI source (HESI-II probe)PRM | 269.2012/ 185.1068 | Not available as of the publication date | (Johannessen, Saini, et al. 2022) |
| Fine Particulate Matter (PM2.5) | MQL: 0.25 pg/m3MDL: 0.07 pg/m3 | Quartz fiber filter | Surrogate standard: diphenylamine-d10 Internal standard: D5‑6PPD-q | Serial ultrasonication with dichloromethane and ACN, concentrated with nitrogen in ACN and PTFE filtered | HPLC-HRMS | Waters acquity HSS T3 column (1.8 μm, 2.1×100 mm) with 0.1% formic acid in water and 0.1% formic acid in ACN mobile phase | Data-dependent MS2 modeMRM | 269.2/93.1 | 269.2/184.1269.2/212.1 | (Wang et al. 2022) |
| Fine Particulate Matter (PM2.5) from Megacity | LOD: 1 pg/mL | Whatman medium-volume quartz fiber filters | pyrene-d10 and benzophenone-d10 | Ultrasonication ACN and dichloromethane/hexane, taken to near dryness with nitrogen, redissolved in methanol and filtered with PTFE membrane | UHPLC-MS/MS | A Waters ACQUITY UPLC C18 column (1.7μm, 2.1 mm×100 mm) with 0.4 mM ammonium acetate (A)/ MeOH (B) mobile phase | ESI(+) modeMRM | 269/184 | 269/107 | (Y. Zhang et al. 2022) |
| Airborne Particulate Matter along a Highway in Mississippi, USA | LOD: 2.13 ng/L | Airborne particulate matter was collected using Sigma-2 passive samplers  | Not available as of the publication date | Methanol and hexane extraction shaker table, filtered with polycarbonate gold-coated filters, rotary evaporation to 3 mL, nitrogen evaporation to near dryness and redissolved in 66% methanol | UHPLC-HRMS | Online filter cartridge with a 2.1 mm ID×0.2 μm porosity stainless-steel filter, an Eclipse Plus C18 RRHD (5 mm×2.1 mm ID; 1.8 μm) guard column followed by the analytical column with the same stationary; 1 mM ammonium formate and 0.1% formic acid (A) and methanol 0.1% formic acid (B) mobile phase  | HESI mode; data-dependent product scan | Not available as of the publication date | Not available as of the publication date | (Olubusoye et al. 2023) |
| Dust: Road Dust, Interior Car Dust , Parking Lot Dust , Indoor Dust from Homes Near E-waste Dismantling Area  | LOQ: 0.11 ng/g | Precleaned nylon bag (pore size of 25 μm) | ISTD: Coumaphos-d10SSTD: Benzophenone‑d10 | Serial sonication with ACN and 1:1 dichloromethane:hexane; concentrated by nitrogen into methanol and filtered | HPLC-MS/MS | HPLC: C18 column (100×2 mm, Luna 3 μm, Phenomenex) with 0.3 g/L ammonium acetate (A) and methanol (B) mobile phase | ESI(+) modeMRM | 269.0/184.1 | 269.0/212.4269.0/92.8 | (Huang et al. 2021) |
| Sediments across Urban Rivers, Estuaries, Coasts, and Deep-Sea Regions | MDL: 0.043 ng/g | Sediment packed in aluminum foil and stored in polypropylene tubes; freeze-dried and 1.0 mm mesh screened | D5-6PPD | Transferred to glass tube, ultrasonicated with ACN, concentrated and filtered with PTFE membrane | LC−MS/MS | C8 column (Waters Xbridge BEH, 2.5μm,2.1 mm×100 mm) 0.1%formic acid in water and (B) methanol mobile phase at a flowrate of 0.3 mL/min | ESI(+) modeMRM | 269.2/184.1 | 269.2/212.1 | (Zeng et al. 2023) |
| Fish  | LOD: 0.00025 mg/kgLOQ: 0.00043 mg/kg | Homogenized by the electric blender, frozen until extraction in centrifuge tube | Not available as of the publication date | Modified QuEChERS  | HPLC-MS/MS | Athena C18-WP chromatographic column (2.1 mm×50 mm, 3.0 μm)Mobile Phase: MeOH: Deionized water/90:10  | ESI(+) modeMRM | 269.3/184.1 | 269.3/211.0 | (Ji, Li, et al. 2022)  |
| Larval Zebrafish and Water | LOQ: 0.1 ng/mL | Glass beakers | Not available as of the publication date | QuEChERS | HPLC/MS-MS | Luna Omega C18, 100×2.1 mm, 1.7 μmMobile phase: 5 mmol/L ammonium formate solution (A), methanol (B) | ESI(+) modeMRM | 269.40 > 184.25 | 269.40 > 107.15 | (Fang et al. 2023)  |
| Zebrafish Embryo | LOD: 0.130 ng/mLLOQ: 0.638 ng/mL | Glass trays | Not available as of the publication date | FastPrep homogenizer, sonication, and centrifuging | HPLC/MS-MS | Atlantis T3 C18-phase column (2.1 mm×50 mm, 3μm; Waters) with an Atlantis T3 Security Guard column (2.1×10 mm, Waters). Mobile phase of 0.1% formic acid in MilliQ water (A) and 0.1% formic acid in methanol (B) | ESI(+) modeMRM | 269.079/ 106.90 | 269.079/184.0 | (Grasse et al. 2023) |
| Embryonic Zebrafish | LOD: 1 pg/mL | Well plate exposures | 6PPD‑q-13C6  | Homogenization, polyfiltration, sonication, concentrated to near dryness, and redissolved in methanol and filtrated through a 0.22 µm poly (ether sulfone) membrane | UPLC/MS-MS | A Waters ACQUITY UPLC C18 column (1.7μm, 2.1 mm×100 mm) with 0.4 mM CH3COONH4 (A)/ MeOH (B) | ESI(+) modeMRM | 269/184 | 269/107 | (S.-Y. Zhang, Gan, Shen, Jiang, et al. 2023) (Y. Zhang et al. 2022) |
| Rainbow Trout Tissue and Exposure Water Samples | MDLs: 0.1–0.6 ng/g in tissue | Plastic and glass | D5-6PPD-q  | Whole fish body was homogenized into Eppendorf tube, serial sonication with ACN and centrifuged | UHPLC-HRMS | Hypersil GOLD C18 column (50×2.1 mm, 1.5 μm). Mobile phase of 0.1% formic acid in ultrapure water (A) and 0.1% formic acid in methanol (B) | ESI(+/-)Full-scan mode | Not available as of the publication date | Not available as of the publication date | (Nair et al. 2023) |
| Lumpfish blood | LOD: 0.5 pg | Not available as of the publication date | Internal: D5-6PPD-qRecovery: 6PPD‑q-13C6 | Vortex-sonication and centrifuged | HRGC/HRMS | TG-5SILMS column (30 m, 0.25 mm ID, film thickness—0.25 μm) | Nontargeted screeningFull-scan mode | Not available as of the publication date | Not available as of the publication date | (Hägg et al. 2023) |
| Human Urine from General Adults, Children, and Pregnant Women | MDL: 0.012 ng/mL | Urine immediately transferred to the laboratory, stored at −40°C until analysis; glass used during laboratory extractions | 6PPD‑q-13C6 | Salting-out assisted liquid−liquid extraction, concentrated with nitrogen and 0.22 μm filtered | LC-MS/MS | Ultra-Fast LC: Waters XBridge C8 column (2.1 mm×100 mm, 2.5μm) column with 0.1% formic acid in water and 0.1% formic acid in methanol mobile phase | ESI(+) modeMRM | 269.2/184.1 | 268.2/107.1 | (Du et al. 2022)  |
| Honey | LOD: 0.0003 mg/kgLOQ: 0.0001 mg/kg | Stored at room temp until extraction in centrifuge tube | Not available as of the publication date | Modified QuEChERS  | HPLC-MS/MS | Athena C18-WP chromatographic column (2.1 mm×50 mm, 3.0 μm)Mobile Phase: MeOH: Deionized water/90:10  | ESI(+) modeMRM | 269.3/184.1 | 269.3/211.0 | (Ji, Li, et al. 2022) |
| Lettuce (*Valerianella locusta*) Plant and Roots and TWPs in Nutrient Solution | Not available as of the publication date | Glass vials | Not available as of the publication date | Plant: serial bead beater with stainless-steel beads and ACN extraction, centrifuged, filtered with nylon filterRoots: Freeze-dried roots, suspended in nutrient solution, reciprocal shaker, centrifuge, nylon syringe filterNutrient Solution: serial liquid−liquid extraction, nylon syringe filter | UPLC-MS/MS | C18 column (Waters ACQUITY HSS T3, 1.8 µm, Waters), ultrapure water (phase A) and ACN (phase B), both containing 0.1% formic acid mobile phase | ESI(+) modeMRM | 269/184 | 269/107269/93 | (Castan et al. 2023) |
| Soil, Water, Atmospheric Particles; Urban Runoff Water Samples Were Collected in a Dense Traffic Urban Area | IQL: 0.035 ng/mL | Soil: stainless-steel shovel, transported to lab within 2 hours, freeze-dried, homogenized, sieved through a 60 meshAtmospheric particle: collect on quartz fiber filters and stored at -20C Water: 200 mL collected in Teflon tubes, glass microfiber filter, acidified with 2% formic acid | Internal: diphenylamine-d10 Surrogate: D5-6PPD-q | Soil: serial ultrasonication with ACN, concentrated to dryness with nitrogen, redissolved in methanol and 0.45 μm nylon filteredAtmospheric particles: serial ultrasonication with dichloromethane and ACN, concentrated to near dryness with nitrogen, redissolved in ACN and filteredWater: HLB SPE Cartridge (60 mg, 3 mL), eluted with methanol−dichloromethane (1:9, v/v), concentrated to dryness with nitrogen, redissolved in ACN and 0.45 μm nylon filtered | UPLC-HRMS | Waters ACQUITY HSS T3 (1.8 μm, 2.1×100 mm) column with 0.1% formic acid in water and 0.1% formic acid in ACN mobile phase | ESI(+) mode full-scan and data-dependent acquisition mode | 269.2/212.1 | 269.2/93.1 | (Cao et al. 2022) |
| Recycled Tire Rubber Employed in Synthetic Football Fields | Suspect screening | Glass vial aluminum cap, stored in the dark at room temperature | Not available as of the publication date | In vitro simulation of digestion extraction, then solid-phase extraction or the bioaccessible fraction: 50 mg of Oasis HLB eluted with ethyl acetate.Ultrasound-assisted extraction for PAHs: crumb rubber in ethyl acetate, ultrasonic bath at 50 kHz for 20 min, PTFE filtered | GC/MS | Phenomenex Zebron ZB-Semivolatiles capillary column (30 mm×0.25 mm×0.25 μm film)  | SRM | Suspect screening | Not available as of the publication date | (Armada et al. 2023) |
| Solubilization of Organic Compounds from Tire Particles Using FishIn VitroDigestive Model | Digestate: LOD: 0.2 μg/LLOQ: 0.5 μg/LCryogenically milled tire tread: LOD: 0.3 μg/LLOQ: 0.9 μg/L |  Amber glass vessels | D5-6PPD-q, benzothiazole-d4, aniline-d5, and diphenylurea-d10, | FishIn VitroDigestive Model, then liquid/liquid extracted twice DCM, then concentrated for analysis | UHPLC-HRMS | Waters ACQUITY UPLC HSS T3 (100×2.1 mm, 1.8 μm) column with 0.1% formic acid in water and 0.1% formic acid in methanol mobile phase | ESI(+) modeMRM | 269.05/ 106.99 | 269.05/184.09 | (Masset et al. 2022) |
| Road Runoff | Not available as of the publication date | Water extracted within 48 hours of collection; glass bottles used for spikes | D5-6PPD-q | Filtered with 0.7-mm glass microfiber filters (Grade GF/F, cytiva), then SPE with Oasis 6 cc, 500 mg HLB cartridges, eluted with methanol | HPLC-MS/MS | Agilent InfinityLab Poroshell 120 EC-C8 LC (30 mm, 2.1 mm, 2.7 μm) column with 1 mM ammonium formate in water and methanol mobile phase | ESI(+) modeMRM | 269.3/184.2 | Not available as of the publication date | (Rodgers et al. 2023) |
| Urban River with Stormwater-Influenced Flows; Upstream, Downstream, and near WWTP Discharge | 0.0098 μg/L | PET bottle, stored frozen (−18°C)*protocol established prior to discovery of 6PPD‑q* | Not available as of the publication date | SPE with Waters HLB cartridges, 6 cc, 500 mg, eluted with methanol and concentrated | UPLC-HRMS | Kinetex 2.6 µm C18 column (50×4.6 mm). Solvent A, Milli-Q water (pH=7) with 0.1% of formic acid (A), and methanol with 0.1% of formic acid (B) mobile phase | Orbitrap HRMS, positive ionization mode with a HESI source (HESI-II probe). PRM for data acquisition | Targeted select ion monitoring: 269.20123 | Not available as of the publication date | (Johannessen, Helm, et al. 2022) |
| Surface Water at Five Urban Centers in Queensland, Australia; Surface Waters and Stormwater in Australian Urban Tributary | Not available as of the publication date | 600 mL polypropylene jars, frozen (−20°C) until analysis | Internal: d6-5-methylbenzotriazole and d5-atrazine Inject Internal: Caffeine-13C3 | Water: filtered through Whatman 47 mm, 1 μm, GFF/B, SPE with Waters Oasis 6 cm3 HLB cartridges, eluted with methanol concentrated with nitrogenParticles: Filter papers with particles dried in an incubator at 60°C for 3 hours and stored at 4°C for analysis; filters were cut into eight equal segments, and one segment was loaded into an 80 μL pyrolysis cup | Water: LC-MS/MSParticles: Pryo-GC/MS (not analyzed for 6PPD‑q) | LC: Phenomenex Kinetex biphenyl 100 Å analytical column (2.6 μm, 50×2.1 mm) column with 0.1% formic acid in water and 0.1% formic acid in methanol mobile phase pryo-GCMS: Particulates captured on the 1 μm filter analyzed for TRWPs and polymers by pryo-GC/MS | ESI(+) modeMRM | LC-MS/MS: 269/184Pyro-GC/MS: Full-scan mode over a mass range of 40 to 600 m/z | 269/107269/93 | (Rauert et al. 2022) |
| Exposure Concentrations during Acute Toxicity Studies | LOD: 0.5 μg /LLOQ: 1.8 μg /L | Glass tanks, beakers, and bottles | Not available as of the publication date | Direct-inject  | LC-MS/MS | Shim-pack VP ODS C18 (150×2.0 mm) column with 0.1% formic acid in water and 0.1% formic acid in methanol mobile phase | ESI(+) modeMRM | 269/185 | Not available as of the publication date | (Hiki et al. 2021) |
| Exposure Concentrations during Zebrafish Behavior and Neurotransmitter Studies | Not available as of the publication date | Not available as of the publication date | Not available as of the publication date | Extracted with ACN | HPLC-MS/MS | Athena C18-WP (2.1×50 mm, 3.0 μm) column with water and methanol mobile phase | ESI(+) modeMRM | 269.3/184.1 | 269.3/211.0 | (Ji, Huang, et al. 2022)  |
| Surface Water from Two Urbanized Watersheds | LOQ: 0.0065 μg/L | PE bottles, held for 72 hours refrigerated, and then frozen  | Atrazine-d5 and melamine-13C3 | To ensure efficient extraction of transformation products with unknown chemical structures, three different SPE methods were employed | UPLC-MS/MS | Kinetex 2.6 μm C18 column (50×4.6 mm), mobile phase A consisting of Milli-Q water (pH=7), and mobile phase B consisted of methanol | HESI source (HESI-II probe) operated in positive ionization mode. Data acquisition was achieved using PRM | 269.20123 | Not available as of the publication date | (Johannessen, Helm, and Metcalfe 2021)  |
| Surface Water, Groundwater, Stormwater, and Suspended Particles from Stormwater | MDL: 0.048 ng/LMQL: 0.160 ng/L | Stainless-steel bucket, 0.7 μm GFFs to collect suspended particles, water samples in HDPE bottles NaN3 (0.05%) to inhibit microbial activity, stored at 4°C  | D5-6PPD-q | Filtered through 0.7 μm GFFs (Grade GF/F; Whatman, UK), NaN3 (0.05%) was added into water samples to inhibit microbial activity. The water samples and suspended particles were stored at 4°C until they were extracted.SPE Water samples: adjusted to pH 2 and EDTA added, then Waters Oasis HLB cartridges (6 mL, 200 mg), eluted with methanol, taken to dryness with nitrogen and redissolved in 10% methanol and filtered with 0.22 μm nylon microfiltration membraneSuspended Particles: freeze-dried and sequential ultrasonicated with methanol, dryness with nitrogen and redissolved in 10% methanol and filtered with 0.22 μm nylon microfiltration membrane | UPLC-MS/MS | Waters XBridge BEH C18 column (2.1×100 mm, 2.5 μm) column with 0.05% formic acid in water and 0.05% formic acid in ACN mobile phase | ESI(+) modeMRM | 269.20/ 184.10 | 269.20/185.15 | (R. Zhang, Zhao, Liu, Tian, et al. 2023)  |
| Surface Water, Groundwater, and Stormwater, and Suspended Material | MDL: 0.048 ng/LMQL: 0.160 ng/L | Stainless-steel bucket, 0.7 μm GFFs to collect suspended particles, water samples in HDPE bottles NaN3 (0.05%) to inhibit microbial activity, stored at 4°C  | D5-6PPD-q | Water samples were adjusted to pH=2 using 3 mol/L HCl, SPE Oasis HLB cartridges, eluted with methanol, evaporated to almost dryness, redissolved in 10% methanol and nylon filtered | LC-MS/MS | Column: Poroshell HPH-C18 column (2.1×100 mm, 2.7 μm) with a C18 guard column (2.0 × 4 mm). Mobile Phase: water (0.1%formic acid) and methanol (0.1% formic acid) | ESI(+) modeMRM | 269.20/ 184.10 | 269.20/185.15 | (R. Zhang, Zhao, Liu, Tian, et al. 2023) |
| Mammalian Cells | LOQ: 0.11 ng/g | Not available as of the publication date | Internal: Coumaphos-d10 Surrogate: Benzophenone-d10 | Digestion mixtures were extracted by SPE with Waters Oasis HLB 1 cc 30 mg cartridges, eluted with 8:2 methanol: ACN, and concentrated by vacuum concentrator | UPLC-HRMS | Waters ACQUITY BEH C18 UPLC column (2.1×100 mm, 1.7 μm in particle size and 130 Å in pore size) with 0.1% formic acid in water and 0.1% formic acid in ACN mobile phase | Positive-ion modePRM mode | 269.0/184.1 | 269.0/212.4269.0/92.8 | (Wu et al. 2023) |
| Influent, Effluent, and Biosolids in Four WWTPs in Hong Kong | Influent: LOQ: 0.12 ng/L LOD: 0.037 ng/LOther: LOQ: 0.06 ng/LLOD: 0.018 ng/LBiosolids: LOQ: 0.31 ng/gLOD: 0.092 ng/g | Glass bottles, held on ice and transferred to lab within 2 hoursWastewater: glass microfiber filtered (1.2 μm, Whatman, Hillsboro, USA) to remove suspended particulate matter, added 5% (v/v) methanol to inhibit microbial growth, stored in the dark at 4°C until extraction. Biosolids and filtered suspended particulate matter: freeze-dried, homogenized, 60-mesh sieve, stored at−20°C until extraction | Surrogate: diphenylamine-d10, Internal: D5-6PPD-q | Glass bottles, held on ice and transferred to lab within 2 hoursWastewater: serial liquid/liquid dichloromethane extraction, purification with Envi-carbSPE cartridge and eluted with ethanol/dichloromethane(2:8, v/v), taken to near dryness with nitrogen, redissolved with ACN and nylon filteredBiosolids and filtered suspended particulate matter: serial ultrasonication with dichloromethane and ACN, purification with Envi-carbSPE cartridge and eluted with ethanol/dichloromethane(2:8, v/v), taken to near dryness with nitrogen, redissolved with ACN and nylon filtered | LC-MS/MS | Waters ACQUITY HSS T3 column (1.8μm, 2.1×100 mm), where the mobile phase consisted of 0.1% formic acid in deionized water (A) and 0.1% formic acid in ACN (B) | ESI(+) modeMRM | 269.2/93.1 | 269.2/ 184.1269.2/ 212.1 | (Cao et al. 2023) |
| Influent and Effluent from Municipal, Hospital, and Industrial WWTPs | LOD: 0.120 ng/L | Upon arrival at lab, hydrochloric acid added to a pH 2, stored at −20°C | D5-6PPD-q | 0.7 μm GFF then SPE Oasis HLB (6 mL, 200 mg) eluted with methanol, evaporated to almost dryness with nitrogen, redissolved with 10% methanol | LC-MS/'MS | Waters Xbridge BEH C18 column (2.1 mm ID, 100 mm, 2.5 μm), 0.05% formic acid in Milli-Q water (mobile phase A) and ACN (mobile phase B) | MRM | 269.2/184.10 | 269.2/185.15 | (R. Zhang, Zhao, Liu, Thomes, et al. 2023) |
| Urban Water System: Surface Water, Surface Rainfall Runoff (Hardened Pavement, Road, Farmland), Influents and Effluents WWTP, and Six Points Along Drinking Water Treatment Sections | LOD: 0.04 ng/LLOQ: 0.12 ng/L | Glass amber bottles, immediately adjusted to pH 3.0 with 4 M H2SO4, added 5% methanol (v/v) to inhibit microbial growth, transported in cold ice boxes, stored at (4°C) before processing and extracted within 48 hours | Not available as of the publication date | Filtered through 0.7 μm GFF membranes, filter membrane serial sonication extraction with methanol and 0.1% formic acid, added to filtered water. SPE Oasis HLB cartridges (500 mg, 6 mL), eluted methanol ethyl acetate, and dichloromethane. Taken to dryness with nitrogen and redissolved with methanol and PTFE filtered | UPLC-MS/MS | Column not listed; 0.1% formic acid; (A) and methanol (B) mobile phase | ESI(+) modeMRM | 269.05/92.7 | 269.05/ 185269.05/ 183.8 | (H.-Y. Zhang, Huang, Liu, Hu, et al. 2023) |
| TWP Solvent Extracts, TWP Aqueous Leachate, Roadway Runoff, Roadway-Impacted Creek Samples | CreekLOD: 3.3 ng/LLOQ: 4.9 ng/LRoadway RunoffLOD: 2.4 ng/LLOQ: 3.9 ng/LTWP LeachateLOD: 2.8 ng/LLOQ: 4 ng/LTWP Methanolic ExtractsLOD: 3.4 ug/gLOQ: 11 ug/g | Roadway Runoff: grab and ISCO sampler, stored at 4°C and extracted within 24 hours of sample collection | D5-6PPD-q | TWP samples methanol-extracted; TWP leachate, roadway runoff, and creek water samples were SPE extracted (Oasis HLB cartridges, eluted with methanol and concentrated to 1 mL) | LC-MS/MS | Agilent Poroshell HPH-C18 column (2.1×100 mm, 2.7 μm) preceded with a C18 guard column(2.0×4  mm). LC-MS grade water (A) and methanol (B), both with 0.1% formic acid as the mobile phase | ESI+ modedMRM | 269.2/184.0 | 269.2/107.1 | (Zhao et al. 2023)  |

Notes: μL=microliter, μm=micrometer, ACN=acetonitrile, ASE=accelerated solvent extractor, BEH=bridged ethyl-siloxane/silica hybrid, cc=cubic centimeter, DCM= dichloromethane, dMRM=dynamic multiple reaction monitoring mode, EDTA= ethylenediaminetetraacetic acid, ESI=electrospray ionization, g=gram, GAPS=Global Atmospheric Passive Sampling, GC=gas chromatography, GC/MS=gas chromatography / mass spectrometry, GFF=glass fiber filter, g/L=grams per liter, HDPE=high-density polyethylene, HESI=heated electrospray ionization, HLB=hydrophilic-lipophilic-balanced, HPLC=high-performance liquid chromatography, HPLC-HRMS=high-performance liquid chromatography–high-resolution mass spectrometry, HPLC-MS/MS=high-performance liquid chromatography– tandem mass spectrometry, HRGC/HRMS= high-resolution gas chromatography / high-resolution mass spectrometry , HRMS=high-resolution mass spectrometry, HSS T3= high-strength silica, trifunctionally bonded, ID=inner diameter, ISTD=internal standards, LC=liquid chromatography, LC-HRMS=liquid chromatography–high-resolution mass spectrometry, LC-MS/MS=liquid chromatography / tandem mass spectrometry, LOQ=limit of quantitation, MeOH=methanol, MDL=method detection limit mg=milligram, mg/kg=milligrams/kilogram, mL=milligrams per liter, mm=millimeter, mM=millimolar, mmol=millimol, mol/L=mol per liter, MQL=method quantification limit, MRL=method reporting limit, MRM=multiple reaction monitoring mode, MS2=tandem mass spectrometry, ng/L=nanograms per liter, PAH=polynuclear aromatic hydrocarbon, PE=polyethylene, pg/m3=picograms per cubic meter, PRM=Parallel reaction monitoring, PTFE=polytetrafluoroethylene, PUF=polyurethane foam, Pyro-GC/MS=pyrolysis gas chromatography / mass spectrometry, SPE=solid-phase extraction, SRM=selected reaction monitoring, SSTD=surrogate standard, TRC= Toronto Research Chemical, TRWP=tire- and road-wear particles, TWP=tire-wear particles, UPLC=ultra-performance liquid chromatography, UPLC-HRMS=ultra-performance liquid chromatography–high-resolution mass spectrometry, UPLC-MS/MS=ultra-performance liquid chromatography– tandem mass spectrometry, UPLC-TOF-MS=ultra-performance liquid chromatography–quadrupole time-of-flight–tandem mass spectrometry, v/v=volume per volume, WWTP=wastewater treatment plant.

**References**

Armada, Daniel, Antia Martinez-Fernandez, Maria Celeiro, Thierry Dagnac, and Maria Llompart. 2023. “Assessment of the Bioaccessibility of PAHs and Other Hazardous Compounds Present in Recycled Tire Rubber Employed in Synthetic Football Fields.” *Science of the Total Environment* 857 (January):159485. https://doi.org/10.1016/j.scitotenv.2022.159485.

Cao, Guodong, Wei Wang, Jing Zhang, Pengfei Wu, Han Qiao, Huankai Li, Gefei Huang, Zhu Yang, and Zongwei Cai. 2023. “Occurrence and Fate of Substituted P-Phenylenediamine-Derived Quinones in Hong Kong Wastewater Treatment Plants.” *Environmental Science & Technology*, October. https://doi.org/10.1021/acs.est.3c03758.

Cao, Guodong, Wei Wang, Jing Zhang, Pengfei Wu, Xingchen Zhao, Zhu Yang, Di Hu, and Zongwei Cai. 2022. “New Evidence of Rubber-Derived Quinones in Water, Air, and Soil.” *Environmental Science & Technology* 56 (7): 4142–50. https://doi.org/10.1021/acs.est.1c07376.

Castan, Stephanie, Anya Sherman, Ruoting Peng, Michael T. Zumstein, Wolfgang Wanek, Thorsten Hüffer, and Thilo Hofmann. 2023. “Uptake, Metabolism, and Accumulation of Tire Wear Particle–Derived Compounds in Lettuce.” *Environmental Science & Technology* 57 (1): 168–78. https://doi.org/10.1021/acs.est.2c05660.

Du, Bibai, Bowen Liang, Yi Li, Mingjie Shen, Liang-Ying Liu, and Lixi Zeng. 2022. “First Report on the Occurrence of *N*-(1,3-Dimethylbutyl)-*N′*-Phenyl-*p*-Phenylenediamine (6PPD) and 6PPD-Quinone as Pervasive Pollutants in Human Urine from South China.” *Environmental Science & Technology Letters*, November. https://doi.org/10.1021/acs.estlett.2c00821.

Fang, Chanlin, Liya Fang, Shanshan Di, Yundong Yu, Xinquan Wang, Caihong Wang, and Yuanxiang Jin. 2023. “Characterization of N-(1,3-Dimethylbutyl)-*N′*-Phenyl-*p*-Phenylenediamine (6PPD)-Induced Cardiotoxicity in Larval Zebrafish (*Danio Rerio*).” *Science of the Total Environment* 882 (July):163595. https://doi.org/10.1016/j.scitotenv.2023.163595.

Grasse, Nico, Bettina Seiwert, Riccardo Massei, Stefan Scholz, Qiuguo Fu, and Thorsten Reemtsma. 2023. “Uptake and Biotransformation of the Tire Rubber–Derived Contaminants 6-PPD and 6-PPD Quinone in the Zebrafish Embryo (*Danio rerio*).” *Environmental Science & Technology* 57 (41): 15598–607. https://doi.org/10.1021/acs.est.3c02819.

Hägg, Fanny, Dorte Herzke, Vladimir A. Nikiforov, Andy M. Booth, Kristine Hopland Sperre, Lisbet Sørensen, Mari Egeness Creese, and Claudia Halsband. 2023. “Ingestion of Car Tire Crumb Rubber and Uptake of Associated Chemicals by Lumpfish (*Cyclopterus Lumpus*).” *Frontiers in Environmental Science* 11. https://doi.org/10.3389/fenvs.2023.1219248.

Hiki, Kyoshiro, Kenta Asahina, Kota Kato, Takahiro Yamagishi, Ryo Omagari, Yuichi Iwasaki, Haruna Watanabe, and Hiroshi Yamamoto. 2021. “Acute Toxicity of a Tire Rubber–Derived Chemical, 6PPD Quinone, to Freshwater Fish and Crustacean Species.” *Environmental Science & Technology Letters* 8 (9): 779–84. https://doi.org/10.1021/acs.estlett.1c00453.

Huang, Wei, Yumeng Shi, Jialing Huang, Chengliang Deng, Shuqin Tang, Xiaotu Liu, and Da Chen. 2021. “Occurrence of Substituted *p*-Phenylenediamine Antioxidants in Dusts.” *Environmental Science & Technology Letters* 8 (5): 381–85. https://doi.org/10.1021/acs.estlett.1c00148.

Ji, Jiawen, Jinze Huang, Niannian Cao, Xianghong Hao, Yanhua Wu, Yongqiang Ma, Dong An, Sen Pang, and Xuefeng Li. 2022. “Multiview Behavior and Neurotransmitter Analysis of Zebrafish Dyskinesia Induced by 6PPD and Its Metabolites.” *Science of The Total Environment* 838 (September):156013. https://doi.org/10.1016/j.scitotenv.2022.156013.

Ji, Jiawen, Changsheng Li, Bingjie Zhang, Wenjuan Wu, Jianli Wang, Jianhui Zhu, Desheng Liu, et al. 2022. “Exploration of Emerging Environmental Pollutants 6PPD and 6PPDQ in Honey and Fish Samples.” *Food Chemistry* 396 (December):133640. https://doi.org/10.1016/j.foodchem.2022.133640.

Johannessen, Cassandra, Paul Helm, Brent Lashuk, Viviane Yargeau, and Chris D. Metcalfe. 2022. “The Tire Wear Compounds 6PPD-Quinone and 1,3-Diphenylguanidine in an Urban Watershed.” *Archives of Environmental Contamination and Toxicology* 82 (2): 171–79. https://doi.org/10.1007/s00244-021-00878-4.

Johannessen, Cassandra, Paul Helm, and Chris D. Metcalfe. 2021. “Detection of Selected Tire Wear Compounds in Urban Receiving Waters.” *Environmental Pollution* 287 (October):117659. https://doi.org/10.1016/j.envpol.2021.117659.

Johannessen, Cassandra, Amandeep Saini, Xianming Zhang, and Tom Harner. 2022. “Air Monitoring of Tire-Derived Chemicals in Global Megacities Using Passive Samplers.” *Environmental Pollution* 314 (December):120206. https://doi.org/10.1016/j.envpol.2022.120206.

Lane, Rachael Frances, Kelly L. Smalling, Paul M. Bradley, Justin B. Greer, Stephanie E. Gordon, John D. Hansen, Andrew R. Spanjer, Dana W. Kolpin, and Jason R. Masoner. 2024. “Tire-Derived Contaminants 6ppd and 6ppd-Q: Analysis, Sample Handling, and Reconnaissance of United States Stream Exposures.” https://doi.org/10.2139/ssrn.4824411.

Masset, Thibault, Benoit J. D. Ferrari, William Dudefoi, Kristin Schirmer, Alan Bergmann, Etienne Vermeirssen, Dominique Grandjean, Luke Christopher Harris, and Florian Breider. 2022. “Bioaccessibility of Organic Compounds Associated with Tire Particles Using a Fish In Vitro Digestive Model: Solubilization Kinetics and Effects of Food Coingestion.” *Environmental Science & Technology* 56 (22): 15607–16. https://doi.org/10.1021/acs.est.2c04291.

Maurer, Loïc, Eric Carmona, Oliver Machate, Tobias Schulze, Martin Krauss, and Werner Brack. 2023. “Contamination Pattern and Risk Assessment of Polar Compounds in Snow Melt: An Integrative Proxy of Road Runoffs.” *Environmental Science & Technology* 57 (10): 4143–52. https://doi.org/10.1021/acs.est.2c05784.

Nair, Pranav, Jianxian Sun, Linna Xie, Lisa Kennedy, Derek Kozakiewicz, Sonya Kleywegt, Chunyan Hao, et al. 2023. “In Process: Synthesis and Toxicity Evaluation of Tire Rubber–Derived Quinones.” Preprint. Chemistry. https://doi.org/10.26434/chemrxiv-2023-pmxvc.

Olubusoye, Boluwatife S., James V. Cizdziel, Matthew Bee, Matthew T. Moore, Marco Pineda, Viviane Yargeau, and Erin R. Bennett. 2023. “Toxic Tire Wear Compounds (6PPD-Q and 4-ADPA) Detected in Airborne Particulate Matter Along a Highway in Mississippi, USA.” *Bulletin of Environmental Contamination and Toxicology* 111 (6): 68. https://doi.org/10.1007/s00128-023-03820-7.

Rauert, Cassandra, Nathan Charlton, Elvis D. Okoffo, Ryan S. Stanton, Alon R. Agua, Michael C. Pirrung, and Kevin V. Thomas. 2022. “Concentrations of Tire Additive Chemicals and Tire Road Wear Particles in an Australian Urban Tributary.” *Environmental Science & Technology*, January. https://doi.org/10.1021/acs.est.1c07451.

Rodgers, Timothy F. M., Yanru Wang, Cassandra Humes, Matthew Jeronimo, Cassandra Johannessen, Sylvie Spraakman, Amanda Giang, and Rachel C. Scholes. 2023. “Bioretention Cells Provide a 10-Fold Reduction in 6PPD-Quinone Mass Loadings to Receiving Waters: Evidence from a Field Experiment and Modeling.” *Environmental Science & Technology Letters*, June. https://doi.org/10.1021/acs.estlett.3c00203.

Seiwert, Bettina, Maolida Nihemaiti, Mareva Troussier, Steffen Weyrauch, and Thorsten Reemtsma. 2022. “Abiotic Oxidative Transformation of 6-PPD and 6-PPD Quinone from Tires and Occurrence of Their Products in Snow from Urban Roads and in Municipal Wastewater.” *Water Research* 212 (April):118122. https://doi.org/10.1016/j.watres.2022.118122.

Silva, D. da, J. Gates, L. Harding, S. O’Neil, and I. Schultz. In preparation. “Analysis of Multiple Tire Rubber-Derived Chemicals (TRCs) in Fish Bile and Plasma: Analytical Method Field Assessment in Puget Sound, WA.”

Wang, Wei, Guodong Cao, Jing Zhang, Pengfei Wu, Yanyan Chen, Zhifeng Chen, Zenghua Qi, Ruijin Li, Chuan Dong, and Zongwei Cai. 2022. “Beyond Substituted *p*-Phenylenediamine Antioxidants: Prevalence of Their Quinone Derivatives in PM2.5.” *Environmental Science & Technology*, July, acs.est.2c02463. https://doi.org/10.1021/acs.est.2c02463.

Wu, Jiabin, Guodong Cao, Feng Zhang, and Zongwei Cai. 2023. “A New Toxicity Mechanism of *N-*(1,3-Dimethylbutyl)-*N′*-Phenyl-*p-*Phenylenediamine Quinone: Formation of DNA Adducts in Mammalian Cells and Aqueous Organisms.” *Science of the Total Environment* 866 (March):161373. https://doi.org/10.1016/j.scitotenv.2022.161373.

Zeng, Lixi, Yi Li, Yuxin Sun, Liang-Ying Liu, Mingjie Shen, and Bibai Du. 2023. “Widespread Occurrence and Transport of *p*-Phenylenediamines and Their Quinones in Sediments across Urban Rivers, Estuaries, Coasts, and Deep-Sea Regions.” *Environmental Science & Technology*, January, acs.est.2c07652. https://doi.org/10.1021/acs.est.2c07652.

Zhang, Hai-Yan, Zheng Huang, Yue-Hong Liu, Li-Xin Hu, Liang-Ying He, You-Sheng Liu, Jian-Liang Zhao, and Guang-Guo Ying. 2023. “Occurrence and Risks of 23 Tire Additives and Their Transformation Products in an Urban Water System.” *Environment International* 171 (January):107715. https://doi.org/10.1016/j.envint.2022.107715.

Zhang, Ruiling, Shizhen Zhao, Xin Liu, Margaret William Thomes, Chui Wei Bong, Dilanka N. D. Samaraweera, Tilak Priyadarshana, Guangcai Zhong, Jun Li, and Gan Zhang. 2023. “Fates of Benzotriazoles, Benzothiazoles, and *p*-Phenylenediamines in Wastewater Treatment Plants in Malaysia and Sri Lanka.” *ACS ES&T Water* 3 (6): 1630–40. https://doi.org/10.1021/acsestwater.2c00410.

Zhang, Ruiling, Shizhen Zhao, Xin Liu, Lele Tian, Yangzhi Mo, Xin Yi, Shiyang Liu, Jiaqi Liu, Jun Li, and Gan Zhang. 2023. “Aquatic Environmental Fates and Risks of Benzotriazoles, Benzothiazoles, and *p-*Phenylenediamines in a Catchment Providing Water to a Megacity of China.” *Environmental Research* 216 (January):114721. https://doi.org/10.1016/j.envres.2022.114721.

Zhang, Shu-Yun, Xiufeng Gan, Baoguo Shen, Jian Jiang, Huimin Shen, Yuhang Lei, Qiuju Liang, et al. 2023. “6PPD and Its Metabolite 6PPDQ Induce Different Developmental Toxicities and Phenotypes in Embryonic Zebrafish.” *Journal of Hazardous Materials* 455 (August):131601. https://doi.org/10.1016/j.jhazmat.2023.131601.

Zhang, Yanhao, Caihong Xu, Wenfen Zhang, Zenghua Qi, Yuanyuan Song, Lin Zhu, Chuan Dong, Jianmin Chen, and Zongwei Cai. 2022. “*P* -Phenylenediamine Antioxidants in PM 2.5 : The Underestimated Urban Air Pollutants.” *Environmental Science & Technology* 56 (11): 6914–21. https://doi.org/10.1021/acs.est.1c04500.

Zhao, Haoqi Nina, Ximin Hu, Zhenyu Tian, Melissa Gonzalez, Craig A. Rideout, Katherine T. Peter, Michael C. Dodd, and Edward P. Kolodziej. 2023. “Transformation Products of Tire Rubber Antioxidant 6PPD in Heterogeneous Gas-Phase Ozonation: Identification and Environmental Occurrence.” *Environmental Science & Technology* 57 (14): 5621–32. https://doi.org/10.1021/acs.est.2c08690.