Table 4-10. Studies of potential food sources and human consumption of 6PPD and 6PPD-q

Location	Information	Concentration	Method	Detection Limit
Beijing, China (Ji et al. 2022)	Fish and honey were purchased from a local supermarket and fish market. Samples were analyzed for 6PPD and 6PPD-q.	Fish: 6PPD Snakehead (0.669 µg/kg) Weever (0.481 µg/kg) 6PPD-q: Spanish mackerel (< LOQ) Honey: ND	Modified QuEChERS methods with (HPLC/MS-MS)	LOD: 6PPD: Fish: 0.00025 mg/kg Honey: 0.0003 mg/kg 6PPD-q: 0.0003 mg/kg LOQ: 6PPD: Fish: 0.00043 mg/kg Honey: 0.0001 mg/kg 6PPD-q: 0.001 mg/kg,
Laboratory in Hangzhou, China (Fang et al. 2023)	Zebrafish were analyzed after laboratory exposure to 6PPD.	After 7 days 6PPD in Zebrafish Larvae: TWA in Water 351 ng/g:1.35 ng/g BAF 265 2,685 ng/g:28.2 ng/g BAF 103	QuEChERS with (HPLC/MS-MS)	LOQ: 0.1 ng/mL
Laboratory in Wenzhou, China (Zhang et al. 2023)	Zebrafish were analyzed after laboratory exposure to 6PPD or 6PPD-q.	After 10 days, 6PPD and 6PPD-q in larvae was significantly higher at 0.2 and 0.8 mg/L exposure vs. control, but not at 0.025 mg/L exposure. Levels of 6PPD were higher than 6PPD-q. BAFs were not calculated.	Homogenization, poly filtration, and UPLC/MS-MS	Not specified
Laboratory in Norway (Hägg et al. 2023)	Lumpfish were exposed to seawater with fish feed mixed with crumb rubber in the lab for 7 days then fed uncontaminated feed for 14 days. Blood was analyzed for 6PPD and 6PPD-q (among other chemicals) at various timepoints throughout.	6PPD max on Day 9 of 1,206 pg/g 6PPD-q not detected in blood DTPD and TPPD also detected in blood	Blood was spiked with D5-6PPD-q, centrifuged, and run by GC-HRMS	Instrumental LOD 6PPD LOD: 0.1 pg 6PPD-q LOD: 0.5 pg
Laboratory in Germany (Grasse et al. 2023)	Zebrafish were analyzed after 24, 48, 72, and 96 hours of exposure in the lab.	Ratio of internal:external concentration 6PPD (exposure of 6.3 and 1.28 µg/L) Max of ~3,000 at 48 hours 6PPD-q (exposure of 20.0, 11.3, and 4.8 µg/L) Max of ~225 at 48 hours	HPLC/MS-MS Fish: FastPrep homogenizer, sonication, and centrifuging	LOD (ng/mL) 6PPD-q: 0.089 6PPD: 0.130 LOQ (ng/mL) (6PPD-q:6PPD) 0.439:0.638

Table 4-10. Studies of potential food sources and human consumption of 6PPD and 6PPD-q

Location	Information	Concentration	Method	Detection Limit
Laboratory in Austria (Castan et al. 2023)	Hydroponic solutions of lettuce were spiked with 1 mg/L of 6PPD-q or constantly leaching TWP over 14 days to analyze uptake and metabolism.	Spiked compounds max: 6PPD 0.78 µg/g 6PPD-q 2.19 µg/g Tire leachate max: 6PPD 0.4µg/g 6PPD-q 0.02 µg/g	Tissues extracted using acetonitrile, then run on LC-MS	Not specified
Laboratory in Toronto (Nair et al. 2023)	Rainbow trout were exposed to 6PPD-q (and other PPD-q) for 96 hours at 0.2, 0.8, 3, 12, and 50 µg/L, then the whole fish body was analyzed for 6PPD-q.	Dose-dependent increase of 6PPD-q concentration (n.34–432 ng/g) Whole-body BCFs of 6PPD-q were calculated as 2.9, 19, 25, and 17.2 293 L/kg at the water concentrations of 0.8, 3, 12, and 25 µg/L, respectively	Tissues extracted using acetonitrile, centrifuged, then run on LC-MS	Not specified
Laboratory in Japan (Hiki and Yamamoto 2022)	*S. leucomaenis pluvius*, *S. curilus*, and *O. masou masou* were exposed to up to 3.5–3.8 µg/L 6PPD-q for 24 hours, then brain and gill were analyzed for 6PPD-q.	6PPD-q max (brain/gill) (µg/kg-wet) *S. leucomaenis pluvius* ~50, *S. curilus* 25/70, *O. masou masou* 4.7/38 6PPD-q-OH (more in brain vs. gill) (µg/kg-wet) *S. leucomaenis pluvius* ~50, *S. curilus* ~50, *O. masou masou* ~25 ILC_{50} in *S. leucomaenis pluvius* of 4.0 µg/kg (brain) and 6.2 µg/kg (gill)	LC-MS/MS in the exposure solution. Tissue extracted using acetonitrile	Not specified
Canada (Wu et al. 2023)	Researchers measured levels of 6PPD-q-dG from tissue of frozen capelin from a Canadian supermarket. 6PPD-q-dG is the isomer of 3-hydroxy-1, N^2-6PPD-etheno-2′-deoxyguanosine.	Liver: median=6.69 (4.24–8.03) lesions/10^8 nucleosides Roe: median=10.9 (4.45–16.8) lesions/10^8 nucleosides Gill: median=11.2 (8.47–15.5) lesions/10^8 nucleosides	UPLC-ESI-MS/MS	LOD: 0.017 ng/mL LOQ: 0.056 ng/mL

Notes: µg/kg=micrograms per kilogram, µg/L=micrograms per liter, BAF=bioaccumulation factor, BCFs= bioconcentration factors, GC-HRMS=gas chromatography–high-resolution mass spectrometry, HPLC-MS/MS=high–performance liquid chromatography–tandem mass spectrometry, LC-MS=liquid chromatography / mass spectrometry, LC-MS/MS=liquid chromatography / tandem mass spectrometry, L/kg=liters per kilogram, LOD=limit of detection, LOQ=limit of quantitation, mg/kg=milligram per kilogram, mg/L=milligram per liter, ND=nondetect, ng/g=nanogram per gram, PPD-q=para-phenylenediamines-quinones, QuEChERS=A solid-phase extraction method: Quick, Easy, Cheap, Effective, Rugged, and Safe, TWA=time-weighted average, TWP=tire-wear particles, UPLC/MS-MS=ultra-performance liquid chromatography–tandem mass spectrometry, UPLC-ESI-MS/MS= ultra-performance liquid chromatography-electrospray ionization–tandem mass spectrometry

References

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.

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 (October):1219248. https://doi.org/10.3389/fenvs.2023.1219248.

Hiki, Kyoshiro, and Hiroshi Yamamoto. 2022. "The Tire-Derived Chemical 6PPD-Quinone Is Lethally Toxic to the White-Spotted Char *Salvelinus leucomaenis pluvius* but Not to Two Other Salmonid Species." *Environmental Science & Technology Letters* 9 (12): 1050–55. https://doi.org/10.1021/acs.estlett.2c00683.

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.

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.

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.

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.