2 Effects Characterization and Toxicity

ITRC has prepared an overview of the current understanding of 6PPD-q sources, exposure, fate, transport, toxicity, mitigation strategies, on-going research, and data needs. This section describes the following:

- the toxicity of 6PPD-q and its parent compound 6PPD
- the ecological and human health effects associated with exposure to these contaminants
- potential populations of concern that may come into contact with 6PPD-q and 6PPD
- current knowledge gaps regarding potential ecological and human health hazards

Section 1 (Introduction) and Section 4 (Occurrence, Fate, Transport, and Exposure Pathways) provide detailed discussions of sources, fate, and transport mechanisms and levels of 6PPD-q and 6PPD in environmental compartments and potential exposure pathways that are relevant to the effects discussed within this section.

2.1 Introduction

Human or ecological receptors may be exposed to 6PPD-q and its parent compound 6PPD via several different exposure pathways (see Figure 1-7).

Potential aquatic ecological receptors, which include freshwater and marine organisms (vertebrates, invertebrates, and plants), may be exposed to 6PPD-q and 6PPD through direct uptake of water through respiratory surfaces, ingestion, and absorption. The route of exposure may vary among species or life stages. Exposure in terrestrial organisms (vertebrates, invertebrates, and plants) is poorly characterized, but it is possible that terrestrial receptors may be exposed via ingestion, inhalation, or absorption.

Potential routes of exposures for humans include ingestion of soil, sediment, dust and water, dust inhalation, and dermal contact. Research supports the biological uptake of both 6PPD and 6PPD-q, which may potentially expose humans via food sources (see Section 4.5 and Table 4-10). At present, however, quantitative measurement of 6PPD-q, 6PPD, and their metabolic products in humans is limited. U.S.-based biomonitoring studies of 6PPD-q or 6PPD exposure are not currently available.

The effects of 6PPD-q and 6PPD on ecological and human health, as with most other chemicals, depends on the magnitude, frequency, and duration of exposure. Research on 6PPD-q toxicity is rapidly expanding and, while some data are presented herein for 6PPD-q, data gaps have been identified in our understanding of health effects. The knowledge base on human and ecological effects of both 6PPD and 6PPD-q is still evolving. 6PPD is the only known source of 6PPD-q in the environment, but the relationship between the toxicity of 6PPD and the toxicity of 6PPD-q is currently unknown. At this time, data gaps remain regarding the effects on human and ecological receptors of exposure to both 6PPD and 6PPD-q. Data on the effects of 6PPD are presented in case a read-across using 6PPD is deemed appropriate in future work as a method for predicting the effects of 6PPD-q. Currently, data are insufficient to conduct a read-across from 6PPD to 6PPD-q. In addition, regulatory authorities may need to address the impacts of 6PPD-q through regulation of the use of 6PPD in products. In doing so, regulatory agencies may have a need to evaluate potential effects of 6PPD relative to a proposed alternative.

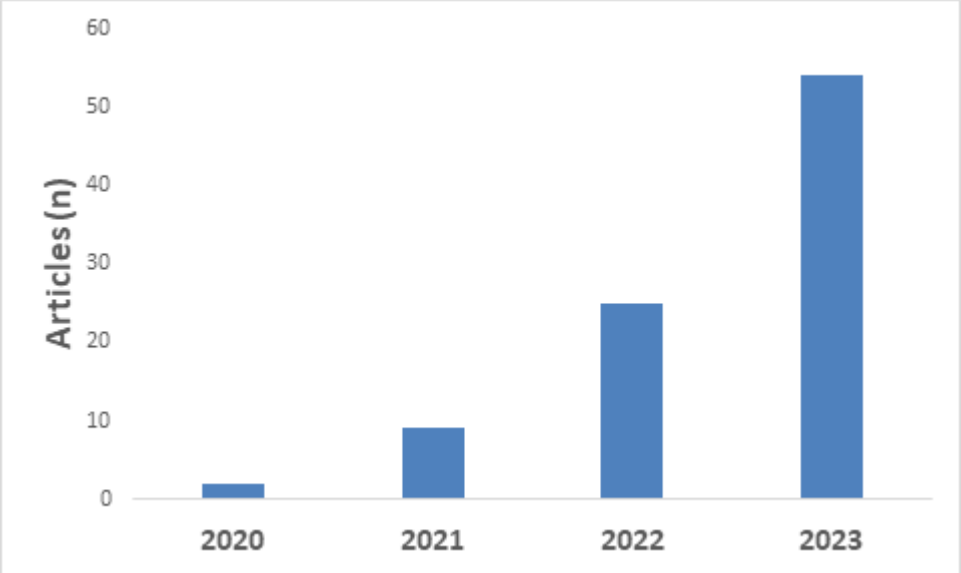

Figure 2-1. Number of articles mentioning 6PPD or 6PPD-q from 2020–2023.

Since the publication of work by Z. Tian et al. (Tian et al. 2021[X8BRFG3P] Tian, Zhenyu, Haoqi Zhao, Katherine T. Peter, Melissa Gonzalez, Jill Wetzel, Christopher Wu, Ximin Hu, et al. 2021. “A Ubiquitous Tire Rubber–Derived Chemical Induces Acute Mortality in Coho Salmon.” Science 371 (6525): 185–89. https://doi.org/10.1126/science.abd6951.), interest in 6PPD-q has grown substantially. A search of the PubMed database[3] shows that the number of articles has risen exponentially year-over-year (Figure 2-1). This section is not intended to represent a comprehensive review of 6PPD-q and 6PPD toxicity studies, and a formal study reliability evaluation was not performed for the studies discussed in this section. Given the active research on this topic, additional studies have been published since the completion of this document. While the intent of this section is to present the most salient and recently available information on the toxicological effects of 6PPD-q and 6PPD, interested readers are encouraged to search the scientific literature for newly available information.

2.2 Environmental Toxicology

This section reports the currently available toxicity information and knowledge gaps concerning the effects of 6PPD-q and 6PPD on freshwater, marine, and terrestrial organisms (vertebrates, invertebrates, and plants). Although 6PPD-q and 6PPD are similar in chemical structure, the toxicological relationship between the two chemicals is nuanced and depends highly on the species and endpoints under consideration as described in the subsections below. Given these considerations, each chemical will be discussed in its own subsection, and toxicity will be described on the basis of ecological taxa. The absence of a taxon in the following subsections represents a data gap at the time this document was published. Additional information is available from other previously published reports (DTSC 2022[2M3Z8Z4F] DTSC. 2022. “Product-Chemical Profile for Motor Vehicle Tires Containing N-(1,3-Dimethylbutyl)-N′-Phenyl-p-Phenylenediamine (6PPD) from the California Department of Toxic Substances Control (DTSC).” https://dtsc.ca.gov/wp-content/uploads/sites/31/2022/05/6PPD-in-Tires-Priority-Product-Profile_FINAL-VERSION_accessible.pdf .OECD 2004[FCJPCPVW] OECD. 2004. “SIDS Initial Assessment Report for N-(1,3-Dimethylbutyl)-N´-Phenyl-1,4-Phenylenediamine (6PPD), Organisation for Economic Co-Operation and Development (OECD).” https://hpvchemicals.oecd.org/UI/handler.axd?id=5e1a446c-5969-479c-9270-7ced8726952e.Washington State Department of Ecology 2024[A9RGZ5XI] Washington State Department of Ecology. 2024. “Safer Products for Washington 2024 Report.” Seattle, Washington. https://ecology.wa.gov/waste-toxics/reducing-toxic-chemicals/washingtons-toxics-in-products-laws/safer-products.ToxServices, LLC 2021[IDJRCJR2] ToxServices, LLC. 2021. “N-(1,3-Dimethylbutyl)-N’-Phenyl-p-Phenylenediamine (6PPD) (CAS #793-24-8) Greenscreen® for Safer Chemicals (Greenscreen®) Assessment.” GS-1204. Washington, D.C.: ToxServices Toxicology Risk Assessment Consulting. https://www.ezview.wa.gov/Portals/_1962/Documents/6ppd/GreenScreenExecutiveSummaryFor6PPD.pdf.OSPAR Commission 2006[SVMKJM7X] OSPAR Commission. 2006. “Hazardous Substances Series 4-(Dimethylbutylamino)Diphenylamine (6PPD) 2005 (2006 Update).” Publication Number: 271/2006. https://www.ospar.org/documents?v=7029.ECHA 2021[Y79Z3ZWV] ECHA. 2021.

“Substance Infocard: N-1,3-Dimethylbutyl-N’-Phenyl-p-Phenylenediamine. European Chemicals Agency (ECHA).” April 7, 2021. https://echa.europa.eu/substance-information/-/substanceinfo/100.011.222.).

2.2.1 Environmental Toxicity of 6PPD-q

As noted in Section 1: Introduction, 6PPD-q, a transformation product of the antiozonant 6PPD, was identified in stormwater samples in late 2020 as a causal toxicant in URMS among adult and juvenile coho salmon (*Oncorhynchus kisutch*) (Tian et al. 2021[X8BRFG3P] Tian, Zhenyu, Haoqi Zhao, Katherine T. Peter, Melissa Gonzalez, Jill Wetzel, Christopher Wu, Ximin Hu, et al. 2021. “A Ubiquitous Tire Rubber–Derived Chemical Induces Acute Mortality in Coho Salmon.” Science 371 (6525): 185–89. https://doi.org/10.1126/science.abd6951.). While a considerable amount of work has focused on characterizing aquatic toxicity in various fish species, there are still relatively few studies on invertebrates and algae and no data on aquatic-dependent amphibians. Research to date regarding the aquatic toxicity of 6PPD-q is discussed in the subsections below, and Tables 2-1 and Table 2-2 summarize the range of known effect levels observed in various species for acute and chronic toxicity, respectively. Compared to those of aquatic studies, data for terrestrial wildlife is generally limited. Currently, no data are available on the toxicity of 6PPD-q in birds, reptiles, and terrestrial amphibians, while information for other terrestrial receptors is scarce. The following subsections summarize key information on the toxicological effects of 6PPD-q for taxa with available data.

2.2.1.1 6PPD-q Toxicity in Fishes

6PPD-q’s Toxicity in Salmonids

- 6PPD-q is highly toxic to coho salmon.
- Of the fish species studied thus far, coho salmon are the most sensitive
- The effects of exposure to 6PPD-q in other aquatic species are varied, even in closely related species.
- Other sensitive fish species include rainbow trout (and likely steelhead), brook trout, lake trout, and coastal cutthroat trout.

In coho salmon, URMS begins with a common progression of behavioral symptoms, which include increased surface swimming, loss of equilibrium and buoyancy, gasping at the surface, and ultimately mortality (Chow et al. 2019[7RMZ3UNQ] Chow, Michelle I., Jessica I. Lundin, Chelsea J. Mitchell, Jay W. Davis, Graham Young, Nathaniel L. Scholz, and Jenifer K. McIntyre. 2019. “An Urban Stormwater Runoff Mortality Syndrome in Juvenile Coho Salmon.” Aquatic Toxicology 214 (September):105231. https://doi.org/10.1016/j.aquatox.2019.105231.Scholz et al. 2011[S8ASEIXU] Scholz, Nathaniel L., Mark S. Myers, Sarah G. McCarthy, Jana S. Labenia, Jenifer K. McIntyre, Gina M. Ylitalo, Linda D. Rhodes, et al. 2011. “Recurrent Die-Offs of Adult Coho Salmon Returning to Spawn in Puget Sound Lowland Urban Streams.” PLOS ONE 6 (12): e28013. https://doi.org/10.1371/journal.pone.0028013.Tian et al. 2022[BICQHLBC] Tian, Zhenyu, Melissa Gonzalez, Craig A. Rideout, Haoqi Nina Zhao, Ximin Hu, Jill Wetzel, Emma Mudrock, C. Andrew James, Jenifer K. McIntyre, and Edward P. Kolodziej. 2022. “6PPD-Quinone: Revised Toxicity Assessment and Quantification with a Commercial Standard.” Environmental Science & Technology Letters, January, acs.estlett.1c00910. https://doi.org/10.1021/acs.estlett.1c00910.). The behavioral characteristics of URMS are described in Section 2.2.1.1.2. After identification of this chemical in stormwater and surface water, follow-up laboratory toxicity tests using 6PPD-q confirmed that 6PPD-q is acutely toxic to coho salmon, with 24-hour median lethal concentration (LC_{50}) ranging from 0.041 µg/L in coho hatchlings (3 weeks post-swim up) to 0.095 µg/L in juveniles (1+ year old) (Lo et al. 2023[LA4CEWYX] Lo, Bonnie P., Vicki L. Marlatt, Xiangjun Liao, Sofya Reger, Carys Gallilee, Andrew R.S. Ross, and Tanya M. Brown. 2023. “Acute Toxicity of 6PPD-Quinone to Early Life Stage Juvenile Chinook (Oncorhynchus tshawytscha) and Coho (Oncorhynchus kisutch) Salmon.” Environmental Toxicology and Chemistry 42 (4): 815–22. https://doi.org/10.1002/etc.5568.Greer et al. 2023[P6RF5UFR] Greer, Justin B., Ellie M. Dalsky, Rachael F. Lane, and John D. Hansen. 2023. “Establishing an In Vitro Model to Assess the Toxicity of 6PPD-Quinone and Other Tire Wear Transformation Products.” Environmental Science & Technology Letters, May. https://doi.org/10.1021/acs.estlett.3c00196.Tian et al. 2022[BICQHLBC] Tian, Zhenyu, Melissa Gonzalez, Craig A. Rideout, Haoqi Nina Zhao, Ximin Hu, Jill Wetzel, Emma Mudrock, C. Andrew James, Jenifer K. McIntyre, and Edward P. Kolodziej. 2022. “6PPD-Quinone: Revised Toxicity Assessment and Quantification with a Commercial Standard.” Environmental Science & Technology Letters, January, acs.estlett.1c00910. https://doi.org/10.1021/acs.estlett.1c00910.). For comparison, Johannessen, Helm, et al. (Johannessen et al. 2022[E9K7U5U3] 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.) measured 6PPD-q at 2.85 µg/L in the Don River in Toronto,

Canada; this is the highest level we found in the literature for surface water.

Since the publication of Z. Tian et al. (Tian et al. 2021[X8BRFG3P] Tian, Zhenyu, Haoqi Zhao, Katherine T. Peter, Melissa Gonzalez, Jill Wetzel, Christopher Wu, Ximin Hu, et al. 2021. “A Ubiquitous Tire Rubber–Derived Chemical Induces Acute Mortality in Coho Salmon.” Science 371 (6525): 185–89. https://doi.org/10.1126/science.abd6951.), acute mortality has been evaluated in more than a dozen species of fish (see Tables 2-1 and Table 2-2). Of the fish species studied thus far, coho salmon are the most sensitive. Lethal concentrations vary widely among fish (24-hour LC_{50} of 0.041 µg/L in coho salmon to a 96-hour LC_{50} greater than 500 µg/L in the Chinese rare minnow (*Gobiocypris rarus*), highlighting the likelihood of a unique species-specific physiological mechanism for URMS (Lo et al. 2023[LA4CEWYX] Lo, Bonnie P., Vicki L. Marlatt, Xiangjun Liao, Sofya Reger, Carys Gallilee, Andrew R.S. Ross, and Tanya M. Brown. 2023. “Acute Toxicity of 6PPD-Quinone to Early Life Stage Juvenile Chinook (Oncorhynchus tshawytscha) and Coho (Oncorhynchus kisutch) Salmon.” Environmental Toxicology and Chemistry 42 (4): 815–22. https://doi.org/10.1002/etc.5568.Di et al. 2022[BLEFEP7S] Di, Shanshan, Zhenzhen Liu, Huiyu Zhao, Ying Li, Peipei Qi, Zhiwei Wang, Hao Xu, Yuanxiang Jin, and Xinquan Wang. 2022. “Chiral Perspective Evaluations: Enantioselective Hydrolysis of 6PPD and 6PPD-Quinone in Water and Enantioselective Toxicity to Gobiocypris Rarus and Oncorhynchus Mykiss.” Environment International 166 (August):107374. https://doi.org/10.1016/j.envint.2022.107374.). To date, no known adverse outcome pathway can explain the difference in sensitivities among species; however, hypothesized mechanisms of action are discussed in Section 2.2.1.1.2.

Toxicological responses to 6PPD-q vary among species and within species. 6PPD-q appears to exhibit age-dependent toxicity in coho salmon, with Lo et al. (Lo et al. 2023[LA4CEWYX] Lo, Bonnie P., Vicki L. Marlatt, Xiangjun Liao, Sofya Reger, Carys Gallilee, Andrew R.S. Ross, and Tanya M. Brown. 2023. “Acute Toxicity of 6PPD-Quinone to Early Life Stage Juvenile Chinook (Oncorhynchus tshawytscha) and Coho (Oncorhynchus kisutch) Salmon.” Environmental Toxicology and Chemistry 42 (4): 815–22. https://doi.org/10.1002/etc.5568.) reporting a 24-hour LC_{50} of 0.041 µg/L in newly feeding (approximately 3 weeks post-swim up) fish, Greer et al. (Greer et al. 2023[P6RF5UFR] Greer, Justin B., Ellie M. Dalsky, Rachael F. Lane, and John D. Hansen. 2023. “Establishing an In Vitro Model to Assess the Toxicity of 6PPD-Quinone and Other Tire Wear Transformation Products.” Environmental Science & Technology Letters, May. https://doi.org/10.1021/acs.estlett.3c00196.) reporting a 12-hour LC_{50} of 0.080 µg/L in fish with a mean age of 189 days, and Z. Tian et al. (Tian et al. 2022[BICQHLBC] Tian, Zhenyu, Melissa Gonzalez, Craig A. Rideout, Haoqi Nina Zhao, Ximin Hu, Jill Wetzel, Emma Mudrock, C. Andrew James, Jenifer K. McIntyre, and Edward P. Kolodziej. 2022. “6PPD-Quinone: Revised Toxicity Assessment and Quantification with a Commercial Standard.” Environmental Science & Technology Letters, January, acs.estlett.1c00910. https://doi.org/10.1021/acs.estlett.1c00910.) reporting a 24-hour LC_{50} of 0.095 µg/L in fish greater than 1 year old. Greer et al. (Greer et al. 2023[PSLHUQ22] Greer, Justin B., Ellie M. Dalsky, Rachael F. Lane, and John D. Hansen. 2023. “Tire-Derived Transformation Product 6PPD-Quinone Induces Mortality and Transcriptionally Disrupts Vascular Permeability Pathways in Developing Coho Salmon.” Environmental Science & Technology, July. https://doi.org/10.1021/acs.est.3c01040.) exposed unhatched coho embryos (starting at the stage when their eyes became apparent) to a 24-hour pulse of 6PPD-q (0.10, 0.90, or 7.22 µg/L) twice weekly, which simulated intermittent rain events under laboratory conditions. Exposures occurred until most of the embryos had hatched during the final (fourth) exposure pulse. No significant embryo mortality occurred in the 0.10 µg/L treatment, even though this exposure is about the same as the 24-hour LC_{50} for juveniles. Significant mortality occurred in the 7.22 µg/L treatment by the second exposure pulse with only 21% of embryos surviving to hatching and egg yolk absorption. These data suggest that the chorion, a protective membrane enveloping embryos, potentially mitigates uptake of some waterborne toxicants like 6PPD-q to the embryo (see Table 2-2).

Sensitivity to 6PPD-q does not seem to be correlated with genetic relationships. Although effect concentrations of 6PPD-q have been reported for several salmon species, none are as sensitive as the coho. Figure 2-2 shows several LC_{50} values for species that have been tested in laboratory studies and highlights that toxicity to 6PPD-q does not follow a phylogenetic relationship among fish. Additional data regarding the toxicity of 6PPD-q for other species is described in Tables 2-1 and Table 2-2.

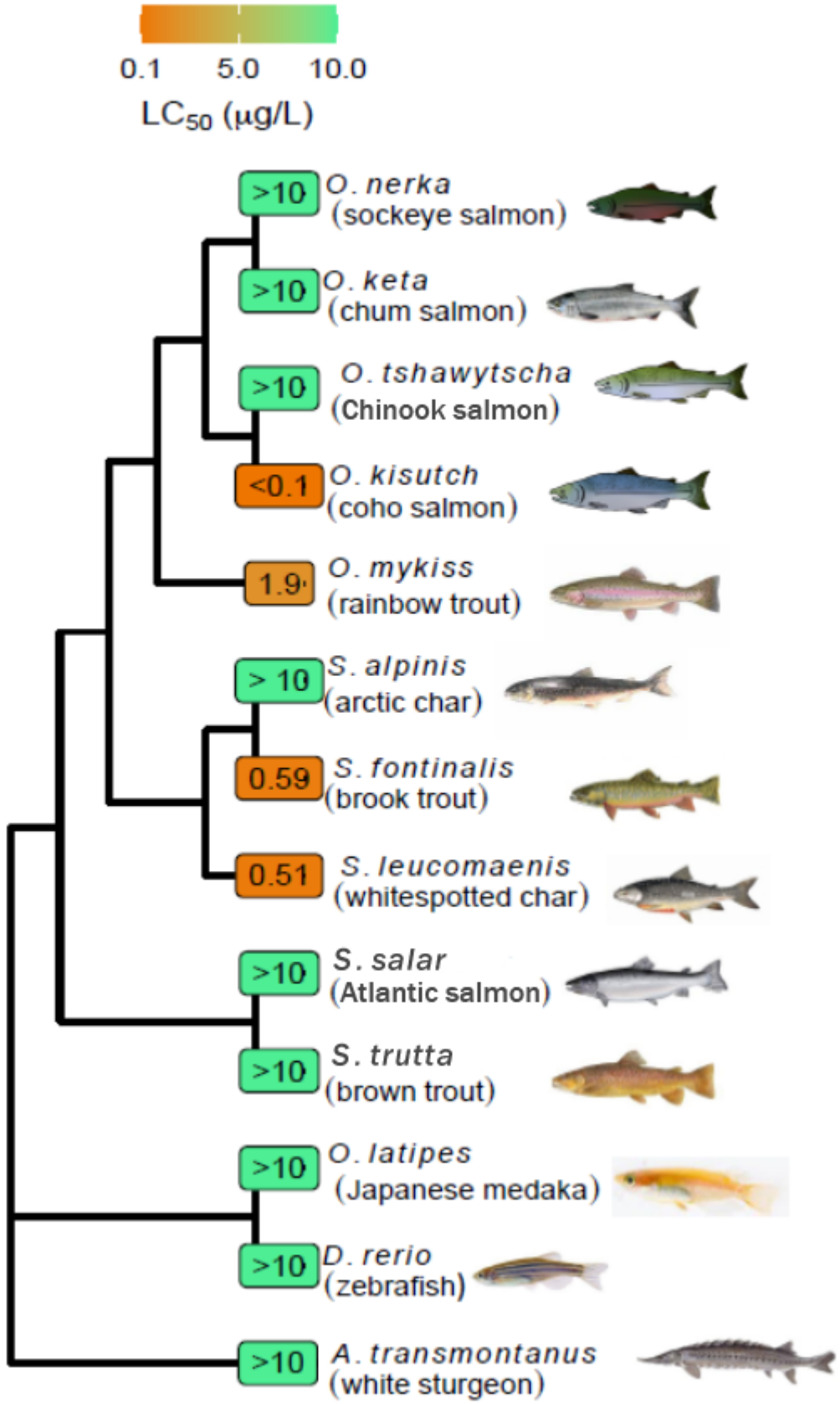

Figure 2-2. Toxicity of 6PPD-q does not follow a phylogenetic relationship. The diagram shows the phylogeny of fish that have been tested for acute toxicity to 6PPD-q as of mid-2023. For each fish, the associated LC_{50} in µg/L (based on an individual study) is represented by the color-gradient scale and numeric value. Genus abbreviations: *A.* (*Acipenser*), *D.* (*Danio*), *O.* (*Oncorhynchus*), *S.* (*Salmo* or *Salvelinus*). Several species are not included in the figure because the results were released late in the production of this document. Pink salmon

(*O. gorbuscha*) have an LC_{50} greater than 10 (Foldvik et al. 2024[NFMULGUG] Foldvik, Anders, Fedor Kryuchkov, Eva Ulvan, Roar Sandodden, and Elii Kvingedal. 2024. "Acute Toxicity Testing of Pink Salmon (Oncorhynchus gorbuscha) with the Tire Rubber–Derived Chemical 6PPD-Quinone." Environmental Toxicology and Chemistry. https://doi.org/https://doi.org/10.1002/etc.5875.). Lake trout (*Salvelinus namaycush*), 24-hour LC_{50} is 0.51 µg/L (Roberts et al. 2024[FMG8VP7Y] Roberts, Catherine, Junyi Lin, Evan Kohlman, Niteesh Jain, Mawuli Amekor, Alper James Alcaraz, Natacha Hogan, Markus Hecker, and Markus Brinkmann. 2024. "Acute and Sub-Chronic Toxicity of 6PPD-Quinone to Early-Life Stage Lake Trout (Salvelinus namaycush)." bioRxiv. https://doi.org/10.1101/2024.03.26.586843.). These results were publicly released prior to peer review. The analysis of the LC_{50} of coastal cutthroat trout (*Oncorhynchus clarkii clarkii*) has not been released (Shankar et al. 2024[FBNQNIWI] Shankar, Prarthana, Ellie M. Dalsky, Joanne E Salzer, Justin B Greer, Rachael F. Lane, William N Batts, Jacob Gregg, Gael Kurath, Paul K Hershberger, and John D Hansen. 2024. "Evaluation of Lethal and Sublethal Effects of 6PPD-Q on Coastal Cutthroat Trout (Oncorhynchus Clarkii Clarkii)." Csv,xml. U.S. Geological Survey. https://doi.org/10.5066/P16SMKIJ.). The highest-known levels 6PPD-q in surface water, 2.85 µg/L, were measured by Johannessen, Helm, et al. (Johannessen et al. 2022[E9K7U5U3] 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.) in the Don River in Toronto, Canada.

Source: Justin Greer (reproduced with permission of the U.S. Geological Survey).

2.2.1.1.1 Sublethal or Chronic Toxicity in Salmonids

Greer et al. (Greer et al. 2023[PSLHUQ22] Greer, Justin B., Ellie M. Dalsky, Rachael F. Lane, and John D. Hansen. 2023. "Tire-Derived Transformation Product 6PPD-Quinone Induces Mortality and Transcriptionally Disrupts Vascular Permeability Pathways in Developing Coho Salmon." Environmental Science & Technology, July. https://doi.org/10.1021/acs.est.3c01040.) identified sublethal impacts to coho embryos and alevin (hatchlings) but at doses equal to or higher than the LC_{50} of later life stages. See the description of the study in Section 2.2.1.1. In a preprint publication (not yet peer-reviewed), Roberts et al. (Roberts et al. 2024[FMG8VP7Y] Roberts, Catherine, Junyi Lin, Evan Kohlman, Niteesh Jain, Mawuli Amekor, Alper James Alcaraz, Natacha Hogan, Markus Hecker, and Markus Brinkmann. 2024. "Acute and Sub-Chronic Toxicity of 6PPD-Quinone to Early-Life Stage Lake Trout (Salvelinus namaycush)." bioRxiv. https://doi.org/10.1101/2024.03.26.586843.) observed developmental abnormalities, including pooling blood, yolk sac edema, and spinal curvature, in chronic toxicity experiments on lake trout (*Salvelinus namaycush*) alevin at environmentally relevant concentrations. These abnormalities became evident after 72 hours and continued to manifest until day 28 of exposure; exposure lasted for 45 days. Philibert et al. (Philibert et al. 2024[2M74ZR52] Philibert, Danielle, Ryan S. Stanton, Christine Tang, Naomi L. Stock, Tillmann Benfey, Michael Pirrung, and Benjamin de Jourdan. 2024. "The Lethal and Sublethal Impacts of Two Tire Rubber–Derived Chemicals on Brook Trout (Salvelinus fontinalis) Fry and Fingerlings." Chemosphere, May. https://doi.org/10.1016/j.chemosphere.2024.142319.) demonstrated that blood chemistry parameters and gill morphology were altered in brook trout fingerlings that survived a 24-hour exposure to 0.5 µg/L 6PPD-q. Further research is needed to characterize how long these alterations persist and how they might impact long-term fitness.

2.2.1.1.2 Unknown 6PPD-q Mechanism of Action in Fish

The specific mechanism, or mechanisms, that underlie the acute mortality associated with 6PPD-q are not fully understood. While observable effects may provide some insight into potential mechanisms of toxicity, tracing them back to a discrete molecular initiating event remains a work in progress.

The Toxicological Mechanism of 6PPD-q Remains Unknown

- Behavioral symptoms of URMS were categorized into six successive stages:

1. Asymptomatic with discrete surfacing events (less than 0.25 seconds)
2. Short episodes of surfacing (0.25–2 seconds)
3. Sustained episodes of surface swimming
4. Spiraling and loss of equilibrium
5. Loss of buoyancy

6. Moribund and unresponsive to touch

- Physiological and transcriptional profiling have implicated dysfunction of the vasculature, including the blood-brain barrier.
- Studies have implicated mitochondrial uncoupling and biotransformation in the toxicity of 6PPD-q.

Prior to the discovery of 6PPD-q, URMS was investigated in laboratory studies by exposing coho salmon to urban runoff.

Chow et al. (Chow et al. 2019[7RMZ3UNQ] Chow, Michelle I., Jessica I. Lundin, Chelsea J. Mitchell, Jay W. Davis, Graham Young, Nathaniel L. Scholz, and Jenifer K. McIntyre. 2019. “An Urban Stormwater Runoff Mortality Syndrome in Juvenile Coho Salmon.” Aquatic Toxicology 214 (September):105231. https://doi.org/10.1016/j.aquatox.2019.105231.) found that the symptomology of URMS in juvenile coho salmon was consistent even within and between storm runoff events. Because of this, the authors were able to divide the behavioral characteristics of URMS into six stages. In Stage 1, fish were generally asymptomatic but had discrete surfacing events shorter than 0.25 seconds. These events included swimming within 1 cm of the exposure tank’s surface. Stage 2 was characterized by short surface-swimming episodes between 0.25 and 2 seconds. In Stage 3, fish exhibited sustained (more than 2 seconds) surface swimming, which included both linear and circular swimming paths. By Stage 4, fish appeared to lose equilibrium and exhibited swimming patterns characterized by side swimming and spiraling. In Stage 5, fish lost their buoyancy and settled at the bottom of the exposure tank. Finally, fish in Stage 6 were considered moribund and exhibited changes in ventilation rate, gaping, spasms, and no response to touch.

Greater than 96% of fish were in Stage 6 of URMS within 7 hours of exposure to runoff. Chow et al. (Chow et al. 2019[7RMZ3UNQ] Chow, Michelle I., Jessica I. Lundin, Chelsea J. Mitchell, Jay W. Davis, Graham Young, Nathaniel L. Scholz, and Jenifer K. McIntyre. 2019. “An Urban Stormwater Runoff Mortality Syndrome in Juvenile Coho Salmon.” Aquatic Toxicology 214 (September):105231. https://doi.org/10.1016/j.aquatox.2019.105231.) additionally showed that symptomatic coho salmon were moribund and did not recover if transferred to a clean (runoff-free) exposure medium.

Studies evaluating the blood chemistry of runoff-exposed coho salmon noted decreased blood ion content and increased blood glucose, lactate, and hematocrit, along with progressively increased blood acidosis (McIntyre et al. 2018[G7QW7PSD] McIntyre, Jenifer, Jessica Lundin, James Cameron, Michelle Chow, Jay Davis, John Incardona, and Nathaniel Scholz. 2018. “Interspecies Variation in the Susceptibility of Adult Pacific Salmon to Toxic Urban Stormwater Runoff.” Environmental Pollution 238:196–203. https://doi.org/https://doi.org/10.1016/j.envpol.2018.03.012.Chow et al. 2019[7RMZ3UNQ] Chow, Michelle I., Jessica I. Lundin, Chelsea J. Mitchell, Jay W. Davis, Graham Young, Nathaniel L. Scholz, and Jenifer K. McIntyre. 2019. “An Urban Stormwater Runoff Mortality Syndrome in Juvenile Coho Salmon.” Aquatic Toxicology 214 (September):105231. https://doi.org/10.1016/j.aquatox.2019.105231.). These physiological changes, which were indicative of hypoxia and acute stress, were consistent with the behaviors observed in URMS. A subsequent study showed that these changes in blood chemistry may have been attributable to the loss of blood plasma due to vascular dysfunction. Blair et al. (Blair, Barlow, and McIntyre 2021[B647MUXL] Blair, Stephanie I., Clyde H. Barlow, and Jenifer K. McIntyre. 2021. “Acute Cerebrovascular Effects in Juvenile Coho Salmon Exposed to Roadway Runoff.” Canadian Journal of Fisheries and Aquatic Sciences, February. https://doi.org/10.1139/cjfas-2020-0240.) used dye tracing to demonstrate that plasma accumulated in the brain and olfactory rosettes of coho salmon following runoff exposure. Additionally, when a high concentration of dye was used, blood plasma visibly leaked through the gills of exposed fish. The authors suggested that disruption of blood vasculature, particularly the blood–brain barrier and possibly the blood vessels of the gills, underlies the physiological and behavioral effects of URMS.

By the time the report by Blair et al. (Blair, Barlow, and McIntyre 2021[B647MUXL] Blair, Stephanie I., Clyde H. Barlow, and Jenifer K. McIntyre. 2021. “Acute Cerebrovascular Effects in Juvenile Coho Salmon Exposed to Roadway Runoff.” Canadian Journal of Fisheries and Aquatic Sciences, February. https://doi.org/10.1139/cjfas-2020-0240.) was published, 6PPD-q was discovered as the causal agent of URMS and could be studied more directly. To characterize the molecular effects of 6PPD-q exposure, Greer et al. (Greer et al. 2023[PSLHUQ22] Greer, Justin B., Ellie M. Dalsky, Rachael F. Lane, and John D. Hansen. 2023. “Tire-Derived Transformation Product 6PPD-Quinone Induces Mortality and Transcriptionally Disrupts Vascular Permeability Pathways in Developing Coho Salmon.” Environmental Science & Technology, July. https://doi.org/10.1021/acs.est.3c01040.) profiled gene expression changes in coho salmon hatchlings exposed to a chemical standard of 6PPD-q. Using whole-transcriptome ribonucleic acid (RNA) sequencing, the authors observed dose-dependent effects on the expression of genes that regulate endothelial permeability and cell–cell contacts. They also noted that some of the behavioral and physiological characteristics of URMS parallel a disease state known in humans as capillary leak syndrome, a condition in which blood plasma diffuses into surrounding tissue and can cause swelling around the brain. This further implicated blood vasculature,

including the blood–brain barrier, as a target tissue of 6PPD-q toxicity.

It is still unclear why some species are susceptible to 6PPD-q while others are tolerant. Current evidence suggests that the ability to biotransform 6PPD-q into less-toxic metabolites may play a role.

Mahoney et al. (Mahoney et al. 2022[V5HSELRG] Mahoney, Hannah, Francisco C. da Silva Junior, Catherine Roberts, Matthew Schultz, Xiaowen Ji, Alper James Alcaraz, David Montgomery, et al. 2022. “Exposure to the Tire Rubber-Derived Contaminant 6PPD-Quinone Causes Mitochondrial Dysfunction In Vitro.” Environmental Science & Technology Letters 9 (9): 765–71. https://doi.org/10.1021/acs.estlett.2c00431.) reported that rainbow trout liver cells were not sensitive to 6PPD-q, although cell viability was decreased and oxygen consumption rate was increased by a proposed uncoupling of the mitochondrial electron transport chain in gill cells. Monohydroxy metabolites of 6PPD-q were detected in the liver but not gill tissues, suggesting that the chemical was more effectively metabolized in the liver (Mahoney et al. 2022[V5HSELRG] Mahoney, Hannah, Francisco C. da Silva Junior, Catherine Roberts, Matthew Schultz, Xiaowen Ji, Alper James Alcaraz, David Montgomery, et al. 2022. “Exposure to the Tire Rubber-Derived Contaminant 6PPD-Quinone Causes Mitochondrial Dysfunction In Vitro.” Environmental Science & Technology Letters 9 (9): 765–71. https://doi.org/10.1021/acs.estlett.2c00431.). In another recent study, biliary O-glucuronide metabolites of 6PPD-q were found to be present at higher levels in tolerant species than in sensitive species, such as coho salmon. The authors suggest that species-specific differences in expression of biotransformation enzymes may be a key factor in the variable toxicity of 6PPD-q (Montgomery et al. 2023[X3FANIWH] Montgomery, David, Xiaowen Ji, Jenna Cantin, Danielle Philibert, Garrett Foster, Summer Selinger, Niteesh Jain, et al. 2023. “Not Yet Peer Reviewed: Toxicokinetic Characterization of the Inter-Species Differences in 6PPD-Quinone Toxicity Across Seven Fish Species: Metabolite Identification and Semi-Quantification.” bioRxiv. https://doi.org/10.1101/2023.08.18.553920.). In a study of zebrafish embryos exposed to 6PPD-q, Grasse et al. (zotpress items=”{4911552:WJHX578U}” style=”chicago-author-date”) used mass spectrometry (MS) to semi-quantify transformation products based on measured peak area. Within 96 hours of exposure, more than 95% of 6PPD-q was biotransformed with “6PPD-q + O + glucuronide” accounting for more than 80% of the total peak area. These results suggested that zebrafish embryos can tolerate 6PPD-q exposure by rapidly detoxifying it through metabolism. In a separate study (preprint, not yet peer-reviewed), Nair et al. (Nair et al. 2023[9V5ES4MI] 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.) exposed juvenile rainbow trout to 6PPD and seven different PPD-quinones (including 6PPD-q) for 96 hours and subsequently analyzed whole-body homogenates for nontargeted identification of metabolites. The LC_{50} for 6PPD-q was 0.64 micrograms per liter (µg/L); however, no toxicity occurred for any of the other PPD-quinones up to 50 µg/L. Tissue concentrations of 6PPD-q were an order of magnitude higher than the six other PPD-quinones. Fish that survived 6PPD-q exposure also exhibited lower concentrations of 6PPD-q, again suggesting that survival may depend on an organism’s ability to biotransform PPD-quinones to less-toxic hydroxy-metabolites. It is not fully understood why 6PPD-q was uniquely toxic among the tested PPD-quinones. Nair et al. (Nair et al. 2023[9V5ES4MI] 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.) reported that two hydroxylated 6PPD-q isomers were detected in fish exposed to 6PPD-q. One isomer exhibited hydroxylation on the alkyl side chain, and one exhibited it on the aromatic ring. In contrast, the other six less-toxic PPD-quinones only appeared to undergo aromatic ring hydroxylation. It is unknown whether this alkyl side chain isomer may elicit toxicity through interaction with an unknown protein target (Nair et al. 2023[9V5ES4MI] 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.).

Some data indicate potential enantioselective toxicity of 6PPD-q. In a study by Di et al. (Di et al. 2022[BLEFEP7S] Di, Shanshan, Zhenzhen Liu, Huiyu Zhao, Ying Li, Peipei Qi, Zhiwei Wang, Hao Xu, Yuanxiang Jin, and Xinquan Wang. 2022. “Chiral Perspective Evaluations: Enantioselective Hydrolysis of 6PPD and 6PPD-Quinone in Water and Enantioselective Toxicity to Gobiocypris Rarus and Oncorhynchus Mykiss.” Environment International 166 (August):107374. https://doi.org/10.1016/j.envint.2022.107374.), rainbow trout and the rare minnow (*G. rarus*) were exposed to *R*-enantiomer, *S*-enantiomer, or a racemate of both 6PPD and 6PPD-q (Figure 2-3) for 96 hours. In *G. rarus*, LC_{50} values were identical for both *R*-6PPD and *S*-6PPD (201 µg/L), while the 6PPD racemate was slightly lower (162 µg/L). No mortality occurred up to 500 µg/L *R*-6PPD-q, *S*-6PPD-q, or 6PPD-q racemate. In contrast, rainbow trout were not sensitive to 6PPD, and no mortality occurred at concentrations up to 400 µg/L. Rainbow trout responded differently to *R*-6PPD-q, *S*-6PPD-q, or 6PPD-q racemate,

with LC_{50} values of 4.31, 1.66, and 2.26 µg/L, respectively. As rainbow trout are known to be sensitive to 6PPD-q, it is feasible, as the authors propose, that 6PPD-q enantiomers may bind a presently unknown molecular target with varying affinity.

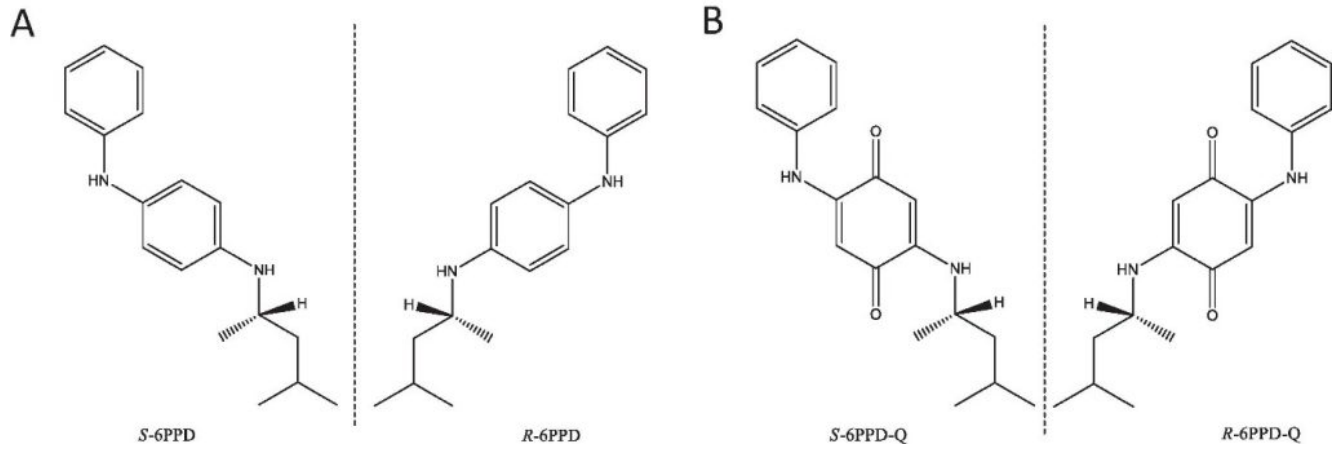

Figure 2-3. Molecular structures of (A) 6PPD and (B) 6PPD-q enantiomers.

Source: Reproduced from Di et al. (Di et al. 2022[BLEFEP7S] Di, Shanshan, Zhenzhen Liu, Huiyu Zhao, Ying Li, Peipei Qi, Zhiwei Wang, Hao Xu, Yuanxiang Jin, and Xinquan Wang. 2022. "Chiral Perspective Evaluations: Enantioselective Hydrolysis of 6PPD and 6PPD-Quinone in Water and Enantioselective Toxicity to Gobiocypris Rarus and Oncorhynchus Mykiss." Environment International 166 (August):107374. https://doi.org/10.1016/j.envint.2022.107374.) under the Creative Commons Public License (https://creativecommons.org/licenses/by-nc-nd/4.0/).

2.2.1.2 6PPD-q Toxicity in Aquatic and Benthic Invertebrates

Acute toxicity tests using invertebrate species thus far have shown that, among the species tested, aquatic and benthic invertebrates are not acutely sensitive to 6PPD-q. No significant mortality was observed in the following freshwater species: amphipods (*Hyalella azteca*), water flea (*Daphnia magna*), rotifer (*Brachionus calyciflorus*), file ramshorn snail (*Planorbella pilsbryi*), washboard mussel (*Megalonaias nervosa*), or mayfly (*Hexagenia* spp.) (see Table 2-1) (Klauschies and Isanta-Navarro 2022[B9C96GRR] Klauschies, Toni, and Jana Isanta-Navarro. 2022. "The Joint Effects of Salt and 6PPD Contamination on a Freshwater Herbivore." Science of the Total Environment 829:154675. https://doi.org/10.1016/j.scitotenv.2022.154675.Hiki et al. 2021[WZF69GXC] 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.Prosser, Salole, and Hang 2023[X2FC6LV6] Prosser, R. S., J. Salole, and S. Hang. 2023. "Toxicity of 6PPD-Quinone to Four Freshwater Invertebrate Species." Environmental Pollution, September, 122512. https://doi.org/10.1016/j.envpol.2023.122512.). Klauschies and Isanta-Navarro (Klauschies and Isanta-Navarro 2022[B9C96GRR] Klauschies, Toni, and Jana Isanta-Navarro. 2022. "The Joint Effects of Salt and 6PPD Contamination on a Freshwater Herbivore." Science of the Total Environment 829:154675. https://doi.org/10.1016/j.scitotenv.2022.154675.) also reported no mortality in *B. calyciflorus* after 12 days of exposure to 6PPD-q. Sublethal endpoints such as fecundity and oxidative stress were also not affected in *B. calyciflorus* at concentrations up to 1,000 µg/L 6PPD-q. Additionally, the only two studies currently available that assessed acute toxicity of 6PPD-q to marine invertebrates also found no significant mortality in the amphipod *Parhyale hawaiensis* and the rotifer *Brachionus koreanus*, although 6PPD-q was found to be mutagenic to *P. hawaiensis* (Maji et al. 2023[D8QNSW4K] Maji, Usha Jyoti, Kyuhyeong Kim, In-Cheol Yeo, Kyu-Young Shim, and Chang-Bum Jeong. 2023. "Toxicological Effects of Tire Rubber–Derived 6PPD-Quinone, a Species-Specific Toxicant, and Dithiobisbenzanilide (DTBBA) in the Marine Rotifer Brachionus koreanus." Marine Pollution Bulletin 192 (July):115002. https://doi.org/10.1016/j.marpolbul.2023.115002.Botelho et al. 2023[WZC7F928] Botelho, Marina Tenório, Gabriely Groto Militão, Markus Brinkmann, and Gisela de Aragão Umbuzeiro. 2023. "Toxicity and Mutagenicity Studies of 6PPD-Quinone in a Marine Invertebrate Species and Bacteria." Environmental and Molecular Mutagenesis 64 (6): 335–41. https://doi.org/10.1002/em.22560.) . Therefore, it appears that aquatic and benthic invertebrates are not acutely sensitive to 6PPD-q, while sublethal and chronic effects represent a data gap.

2.2.1.3 6PPD-q Toxicity in Algae and Aquatic Plants

To date, one study has specifically evaluated the toxicity to 6PPD-q in algae (Wu et al. 2023[PYQQU7AG] 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 866:161373. https://doi.org/10.1016/j.scitotenv.2022.161373.). Cultures of the single-celled green alga *Chlamydomonas reinhardtii* were exposed to 6PPD-q for 72 hours at concentrations of 250 and 1,000 µg/L, and cell viability was evaluated by calculating absorbance ratios between exposed and unexposed groups. The study observed a significant treatment effect with an increase in algal cell death between the exposed and unexposed groups, but there was no major difference in viability between the 250 and 1,000 µg/L treatments (Wu et al. 2023[PYQQU7AG] 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 866:161373. https://doi.org/10.1016/j.scitotenv.2022.161373.). Although the exposure concentrations exceeded environmental relevance, the study provided evidence of a possible genotoxicity mechanism (Wu et al. 2023[PYQQU7AG] 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 866:161373. https://doi.org/10.1016/j.scitotenv.2022.161373.).

2.2.1.4 6PPD-q Toxicity in Mammals

Some data are available that may be used to evaluate the sublethal effects of 6PPD-q on mammalian wildlife (Table 2-3). One study found that oral exposure of laboratory mice to 10 and 100 milligrams per kilograms (mg/kg) 6PPD-q in corn oil for 6 weeks resulted in fatty liver syndrome, an outcome that was shared with the parent compound 6PPD; however, the authors noted that these relatively high concentrations were selected for hazard assessment and were not intended to represent environmentally relevant exposure concentrations (Fang et al. 2023[B66X64SI] Fang, Liya, Chanlin Fang, Shanshan Di, Yundong Yu, Caihong Wang, Xinquan Wang, and Yuanxiang Jin. 2023. “Oral Exposure to Tire Rubber–Derived Contaminant 6PPD and 6PPD-Quinone Induce Hepatotoxicity in Mice.” Science of the Total Environment 869 (April):161836. https://doi.org/10.1016/j.scitotenv.2023.161836.). The authors determined that 6PPD tended to affect gene expression related to fatty acid metabolism; in comparison, 6PPD-q affected inflammatory pathways. Mice were not monitored for apical outcomes. Although steatosis is an adverse outcome and indicator of liver damage in human health evaluations, no studies in wild mammals are available that would define steatosis as an adverse apical outcome in nonhuman organisms. Another study (He, Gu, and Wang 2023[6MPWVZGE] He, Wenmiao, Aihua Gu, and Dayong Wang. 2023. “Four-Week Repeated Exposure to Tire-Derived 6-PPD Quinone Causes Multiple Organ Injury in Male BALB/c Mice.” Science of the Total Environment 894 (October):164842. https://doi.org/10.1016/j.scitotenv.2023.164842.) evaluated toxicity to multiple organs in rodents and found that 28 days of 6PPD-q injection (intraperitoneal) exposure damaged the liver and other organs. Concentrations in the livers in this study were approximately 20 nanograms per gram (ng/g), which was similar to what was found in their oral exposure study. Additionally, H. N. Zhao, Thomas, et al. (Zhao et al. 2023[GZZX9DMJ] Zhao, Haoqi Nina, Sydney P. Thomas, Mark J. Zylka, Pieter C. Dorrestein, and Wenxin Hu. 2023. “Urine Excretion, Organ Distribution, and Placental Transfer of 6PPD and 6PPD-Quinone in Mice and Potential Developmental Toxicity through Nuclear Receptor Pathways.” Environmental Science & Technology 57 (36): 13429–38. https://doi.org/10.1021/acs.est.3c05026.) found that oral gavage exposure to 4 mg/kg 6PPD-q in pregnant mice resulted in chemical transfer to pups, resulting in concentrations in livers (mean 200 ng/g) that were greater than in the studies cited above. Although the route of exposure is not environmentally relevant, these results may be of use for ecological risk assessment based on tissue residue values if an apical outcome was associated with organ damage as shown in the study.

2.2.1.5 6PPD-q Toxicity in Soil Invertebrates

A limited number of studies examine the effects of 6PPD-q on terrestrial invertebrates and can be directly used for ecological risk assessments (see Table 2-2). The most relevant study was conducted in springtails (*Folsomia candida*), a commonly tested soil-dwelling organism with a global distribution (Xu et al. 2023[WDVQCW7M] Xu, Qiao, Wei Wu, Zufei Xiao, Xin Sun, Jun Ma, Jing Ding, Zhe Zhu, and Gang Li. 2023. “Responses of Soil and Collembolan (Folsomia candida) Gut Microbiomes to 6PPD-Q Pollution.” Science of the Total Environment 900 (November):165810. https://doi.org/10.1016/j.scitotenv.2023.165810.). The authors estimated a nominal 28-day LC_{50} of 16.31 micrograms of 6PPD per kilogram (µg/kg) of soil for adult springtails. Reproduction was not significantly affected by 6PPD-q exposure at nominal soil concentrations up to 5,000 µg/kg. Notably, genomic analysis showed a difference in effect on microbes from soil compared to those from the guts of springtails.

Exposure to 5,000 µg/kg was associated with an increase in nitrogen cycling genes in soil microbes and a decrease in springtail gut microbes. Changes to microbial community structure and function in response to 6PPD-q exposure may be an important endpoint to examine with respect to the provisioning of terrestrial ecosystem services.

Further work with invertebrate taxa has been conducted in *C. elegans* and may provide information about effects in soil invertebrates (Hua et al. 2023[RJ86H9T3] Hua, Xin, Xiao Feng, Geyu Liang, Jie Chao, and Dayong Wang. 2023. “Long-Term Exposure to 6-PPD Quinone Reduces Reproductive Capacity by Enhancing Germline Apoptosis Associated with Activation of Both DNA Damage and Cell Corpse Engulfment in Caenorhabditis elegans.” Journal of Hazardous Materials 454 (July):131495. https://doi.org/10.1016/j.jhazmat.2023.131495.Wang, Hua, and Wang 2023[PMPW53J5] Wang, Yuxing, Xin Hua, and Dayong Wang. 2023. “Exposure to 6-PPD Quinone Enhances Lipid Accumulation through Activating Metabolic Sensors of SBP-1 and MDT-15 in Caenorhabditis elegans.” Environmental Pollution 333 (September):121937. https://doi.org/10.1016/j.envpol.2023.121937.Hua et al. 2023[LJTX7EWE] Hua, Xin, Xiao Feng, Geyu Liang, Jie Chao, and Dayong Wang. 2023. “Exposure to 6-PPD Quinone at Environmentally Relevant Concentrations Causes Abnormal Locomotion Behaviors and Neurodegeneration in Caenorhabditis elegans.” Environmental Science & Technology, March. https://doi.org/10.1021/acs.est.2c08644.) (see Table 2-2). Because this work involved aqueous exposures (0.1, 1, and 10 µg/L 6PPD-q), extrapolation to exposure concentrations in the terrestrial environment is uncertain. Nevertheless, it is worth summarizing effects because *C. elegans* is a soil-dwelling organism. Hua et al. (Hua et al. 2023[RJ86H9T3] Hua, Xin, Xiao Feng, Geyu Liang, Jie Chao, and Dayong Wang. 2023. “Long-Term Exposure to 6-PPD Quinone Reduces Reproductive Capacity by Enhancing Germline Apoptosis Associated with Activation of Both DNA Damage and Cell Corpse Engulfment in Caenorhabditis elegans.” Journal of Hazardous Materials 454 (July):131495. https://doi.org/10.1016/j.jhazmat.2023.131495.) found that reproductive capacity declined with increasing aqueous 6PPD-q concentration, potentially as related to gonadal development. 6PPD-q exposure at concentrations as low as 1 µg/L reduced locomotion in *C. elegans*. It also led to the development of neurodegeneration, with the former occurring at lower concentrations than the latter (Hua et al. 2023[LJTX7EWE] Hua, Xin, Xiao Feng, Geyu Liang, Jie Chao, and Dayong Wang. 2023. “Exposure to 6-PPD Quinone at Environmentally Relevant Concentrations Causes Abnormal Locomotion Behaviors and Neurodegeneration in Caenorhabditis elegans.” Environmental Science & Technology, March. https://doi.org/10.1021/acs.est.2c08644.), indicating a potential for neurobehavioral and thus apical effects in this species. The same exposure conditions also led to an accumulation of lipids in vivo, suggesting that 6PPD-q has the potential to alter lipid metabolism in invertebrates such as *C. elegans* (Zhang et al. 2024[BZQEGEXI] Zhang, Jing, Guodong Cao, Wei Wang, Han Qiao, Yi Chen, Xiaoxiao Wang, Fuyue Wang, Wenlan Liu, and Zongwei Cai. 2024. “Stable Isotope-Assisted Mass Spectrometry Reveals in Vivo Distribution, Metabolism, and Excretion of Tire Rubber-Derived 6PPD-Quinone in Mice.” Science of the Total Environment 912 (February):169291. https://doi.org/10.1016/j.scitotenv.2023.169291.).

2.2.1.6 6PPD-q Toxicity in Terrestrial Plants

Although it has been shown that 6PPD-q can be taken up in plant tissues (Castan et al. 2023[3RBDETGD] 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.), no published plant toxicity tests could be found as of the writing of this summary.

2.2.1.7 6PPD-q Toxicity in Terrestrial Microorganisms

There are limited ecologically relevant studies examining the effect of 6PPD-q on terrestrial microorganisms. One study, as described above, assessed the impact of 6PPD-q in soil exposure on microbial diversity and taxonomic abundance, with potential ramifications for nitrogen cycling (Xu et al. 2023[WDVQCW7M] Xu, Qiao, Wei Wu, Zufei Xiao, Xin Sun, Jun Ma, Jing Ding, Zhe Zhu, and Gang Li. 2023. “Responses of Soil and Collembolan (Folsomia candida) Gut Microbiomes to 6PPD-Q Pollution.” Science of the Total Environment 900 (November):165810. https://doi.org/10.1016/j.scitotenv.2023.165810.). Although these findings suggest the potential for 6PPD-q to impact ecosystem services through its effects on terrestrial microorganisms, additional research is needed in this area.

2.2.2 Environmental Toxicity of 6PPD

2.2.2.1 6PPD Toxicity in Fishes

Coho salmon (*O. kisutch)* are less sensitive to 6PPD compared to 6PPD-q, with a reported nominal 24-hour LC_{50} of 251 µg/L

for 6PPD (Tian et al. 2021[X8BRFG3P] Tian, Zhenyu, Haoqi Zhao, Katherine T. Peter, Melissa Gonzalez, Jill Wetzel, Christopher Wu, Ximin Hu, et al. 2021. “A Ubiquitous Tire Rubber–Derived Chemical Induces Acute Mortality in Coho Salmon.” Science 371 (6525): 185–89. https://doi.org/10.1126/science.abd6951.) (see Table 2-1). This study notes a lack of confidence in the measured concentrations of 6PPD in water due to its poor solubility, high instability, and formation of transformation products during exposure. As such, only nominal values were used to estimate the LC_{50} value. In another study using the common laboratory test species, zebrafish (*Danio rerio),* the 96-hour LC_{50} for 6PPD was 442.62 µg/L (Varshney et al. 2022[APRMZJBS] Varshney, Shubham, Adnan H. Gora, Prabhugouda Siriyappagouder, Viswanath Kiron, and Pål A. Olsvik. 2022. “Toxicological Effects of 6PPD and 6PPD Quinone in Zebrafish Larvae.” Journal of Hazardous Materials 424 (February):127623. https://doi.org/10.1016/j.jhazmat.2021.127623.). Similarly, this study noted that the LC_{50} values for 6PPD should be used with caution due to the chemical’s low water solubility and stability, high Kow, and formation of transformation products. Another study, conducted under good laboratory practice, reported a 96-hour LC_{50} for 6PPD equal to 28 µg/L (measured) for Japanese medaka (*O. latipes)* (ECHA 2021[Y79Z3ZWV] ECHA. 2021. “Substance Infocard: N-1,3-Dimethylbutyl-N’-Phenyl-p-Phenylenediamine. European Chemicals Agency (ECHA).” April 7, 2021. https://echa.europa.eu/substance-information/-/substanceinfo/100.011.222.). A similar study using Japanese medaka corroborated these results and reported 80% mortality in medaka after a 96-hour exposure to a time-weighted average concentration of 107 µg/L 6PPD (Hiki et al. 2021[WZF69GXC] 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.), indicating that the LC_{50} was lower than 107 µg/L. Two long-term studies tested the toxicity of 6PPD in fathead minnow (*P. promelas*), and the LC_{50} values ranged from 35 µg/L (21-day exposure) to 150 µg/L (28-day exposure) (Prosser et al. 2017[G5HZ4XYX] Prosser, Ryan S., Joanne L. Parrott, Melissa Galicia, Kallie Shires, Cheryl Sullivan, John Toito, Adrienne J. Bartlett, Danielle Milani, Patty L. Gillis, and Vimal K. Balakrishnan. 2017. “Toxicity of Sediment-Associated Substituted Phenylamine Antioxidants on the Early Life Stages of Pimephales promelas and a Characterization of Effects on Freshwater Organisms.” Environmental Toxicology and Chemistry 36 (10): 2730–38. https://doi.org/10.1002/etc.3828.Monsanto Company 1979[QBCWTBKB] Monsanto Company. 1979. “Dynamic Toxicity of Santoflex 13 to Fatheads Minnows (Pimephales promelas). Curated Toxicity Data Were Retrieved from the ECOTOXicology Knowledgebase, U.S. Environmental Protection Agency. Http:/Www.Epa.Gov/Ecotox/ (November 10, 2023).” 21850-A/AB-780121B. St. Louis, Missouri.). 6PPD was not acutely toxic to rainbow trout (*O. mykiss)* exposed to a concentration of 50 µg/L for 96 hours (Nair et al. 2023[9V5ES4MI] 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.). Overall, the reported LC_{50} values for 6PPD acute toxicity in fish species are much higher than the maximum measured concentrations of 6PPD in river water (0.00129 µg/L) and road runoff (0.00752 µg/L) (Zhang et al. 2023[JWRBWTKN] 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.).

Several studies using zebrafish (*D. rerio*) reported sublethal effects after exposure to 6PPD. For example, endpoints such as development, locomotor behavior, respiration and heart rate, or oxidative damage were affected after exposure to 6PPD at various concentrations tested in the literature (see Table 2-2) (Ji et al. 2022[QJ23CAKR] 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 838:156013. https://doi.org/10.1016/j.scitotenv.2022.156013.Fang et al. 2023[FFFKR3MY] 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.Hiki et al. 2021[WZF69GXC] 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.Varshney et al. 2022[APRMZJBS] Varshney, Shubham, Adnan H. Gora, Prabhugouda Siriyappagouder, Viswanath Kiron, and Pål A. Olsvik. 2022. “Toxicological Effects of 6PPD and 6PPD Quinone in Zebrafish Larvae.” Journal of Hazardous Materials 424 (February):127623.

https://doi.org/10.1016/j.jhazmat.2021.127623.Zhang et al. 2023[3FCHDXBN] 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.).

2.2.2.2 6PPD Toxicity in Aquatic and Benthic Invertebrates

Data are limited on the acute toxicity of 6PPD to aquatic and benthic invertebrates (see Table 2-1). In water flea (*D. magna),* a 48-hour LC_{50} value equal to 230 µg/L was reported from a good laboratory practice study based on measured concentrations (ECHA 2021[Y79Z3ZWV] ECHA. 2021. “Substance Infocard: N-1,3-Dimethylbutyl-N’-Phenyl-p-Phenylenediamine. European Chemicals Agency (ECHA).” April 7, 2021. https://echa.europa.eu/substance-information/-/substanceinfo/100.011.222.). A more recent study observed 100% mortality of *D. magna* after 48-hour exposure to 6PPD at a time-weighted average concentration of 138 µg/L, indicating that the 48-hour LC_{50} is less than 138 µg/L (Hiki et al. 2021[WZF69GXC] 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.). It is unclear why there are inconsistencies between the studies that tested *D. magna*. Physicochemical properties of 6PPD such as low water solubility and stability, high Kow, and formation of transformation products may have resulted in uncertainties regarding the derived LC_{50} values.

In the epibenthic amphipod *H. azteca*, the 96-hour LC_{50} based on measured concentrations was 250 µg/L (Prosser et al. 2017[N22D9LHY] Prosser, R. S., A. J. Bartlett, D. Milani, E. A. M. Holman, H. Ikert, D. Schissler, J. Toito, J. L. Parrott, P. L. Gillis, and V. K. Balakrishnan. 2017. “Variation in the Toxicity of Sediment-Associated Substituted Phenylamine Antioxidants to an Epibenthic (Hyalella azteca) and Endobenthic (Tubifex tubifex) Invertebrate.” Chemosphere 181 (August):250–58. https://doi.org/10.1016/j.chemosphere.2017.04.066.). Chronic toxicity (28-day exposure) tests were also performed as part of this study in both water and sediment, and the 28-day LC_{50} was equal to 13 µg/L for water exposures and 135 µg per gram (µg/g) sediment dry weight for sediment exposures (see Table 2-2). The results from the chronic water and sediment toxicity tests indicated that 6PPD is more bioavailable in water versus sediment, and the authors suggested this may be due to sorption of 6PPD to organic matter in sediment. Additionally, the concentration of 6PPD in sediment and water declined over the study duration, and the authors noted that bioavailability of 6PPD to aquatic and sediment-dwelling organisms in freshwater ecosystems should be specifically investigated due to the chemical’s apparent degradation. Prosser, Bartlett, et al. (Prosser et al. 2017[N22D9LHY] Prosser, R. S., A. J. Bartlett, D. Milani, E. A. M. Holman, H. Ikert, D. Schissler, J. Toito, J. L. Parrott, P. L. Gillis, and V. K. Balakrishnan. 2017. “Variation in the Toxicity of Sediment-Associated Substituted Phenylamine Antioxidants to an Epibenthic (Hyalella azteca) and Endobenthic (Tubifex tubifex) Invertebrate.” Chemosphere 181 (August):250–58. https://doi.org/10.1016/j.chemosphere.2017.04.066.) also exposed aquatic worms (*Tubifex* sp.) in a chronic (28-day exposure) sediment toxicity test. Their results indicated that mortality was the least sensitive endpoint (28-day LC_{50} of 67 µg/g dry weight), while reproduction was a more sensitive endpoint. Exposed worms produced fewer total juveniles and fewer large juveniles (those greater than 500 micrometers (µm) in length). The EC_{10} for both these outcomes was 3 µg/g, and the concentration at which an effect was observed in 50 percent of test subjects (EC_{50}) was 4 µg/g for dry weight sediment (EC_{10}, the concentration at which an effect was observed in 10 percent of test subjects). Compared to *H. azteca* in sediment, their results suggested that *Tubifex* may be more exposed to 6PPD due to species-specific differences in behavior (for example, *Tubifex* remains buried in sediment to feed on organic matter and is likely exposed to sediment-bound 6PPD and 6PPD in pore water consumed by the worms).

In a study that investigated the effects of 6PPD on different life stages of freshwater mussels, the viability of glochidia (larvae) of two mussel species (the fatmucket mussel, *Lampsilis siliquoidea,* and the wavy-rayed lampmussel *Lampsilis fasciola*) was assessed after a 48-hour exposure to 6PPD in water (Prosser et al. 2017[L96F85XM] Prosser, R. S., P.L. Gillis, E.A.M. Holman, D. Schissler, H. Ikert, J. Toito, E. Gilroy, et al. 2017. “Effect of Substituted Phenylamine Antioxidants on Three Life Stages of the Freshwater Mussel Lampsilis siliquoidea.” Environmental Pollution 229:281–89. https://doi.org/10.1016/j.envpol.2017.05.086.). The 48-hour median effect concentration (EC_{50}) values for larval viability were 439 µg/L 6PPD for *L. siliquoidea* and 137 µg/L for *L. fasciola* (see Table 2-1). For the juvenile stage of fatmucket mussels (*L. siliquoidea),* the same study exposed test organisms to 6PPD for 28 days in sediment and water. The reported 28-day LC_{50} measurements were 62 µg/g dry weight sediment and 17 µg/L water. Adult mussels exposed for 28 days did not exhibit

significant sublethal impacts, including a lack of oxidative stress in any tissue examined (gill, gonad, and digestive), no adverse effect on viability of hemocytes, and no deoxyribonucleic acid (DNA) damage in hemocytes. The authors concluded that genotoxicity was not observed in mussel hemocytes at concentrations up to 115.9 µg/g dry weight sediment (Prosser et al. 2017[L96F85XM] Prosser, R. S., P.L. Gillis, E.A.M. Holman, D. Schissler, H. Ikert, J. Toito, E. Gilroy, et al. 2017. “Effect of Substituted Phenylamine Antioxidants on Three Life Stages of the Freshwater Mussel Lampsilis siliquoidea.” Environmental Pollution 229:281–89. https://doi.org/10.1016/j.envpol.2017.05.086.).

2.2.2.3 6PPD Toxicity in Algae and Aquatic Plants

There are few studies on the toxicity of 6PPD to algae and aquatic plants. An algal study presented in the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) dossier for 6PPD reported a 96-hour EC_{50} equal to 600 µg/L and a 96-hour no-observed-effect concentration for growth rate equal to 200 µg/L in the green algae species *Pseudokirchneriella subcapitata* (previously *Selenastrum capricornutum*) (ECHA 2021[Y79Z3ZWV] ECHA. 2021. “Substance Infocard: N-1,3-Dimethylbutyl-N’-Phenyl-p-Phenylenediamine. European Chemicals Agency (ECHA).” April 7, 2021. https://echa.europa.eu/substance-information/-/substanceinfo/100.011.222.). This study was categorized as not reliable (Klimisch score of 3) in the REACH dossier due to “significant methodological deficiencies” including the lack of exponential growth of the test organism during the incubation period, which is required in most standardized algal toxicity tests. Therefore, the reported effect levels cannot reliably be interpreted as a result of exposure to 6PPD.

2.2.2.4 6PPD Toxicity in Mammals

Some studies on the toxicity of 6PPD to mammals were conducted in parallel with 6PPD-q; therefore, the available information is similar to that provided in Section 2.2.1.4 and Table 2-3.

One study found that oral exposure at 10 and 100 mg/kg 6PPD to mice for 6 weeks resulted in fatty liver syndrome (Fang et al. 2023[B66X64SI] Fang, Liya, Chanlin Fang, Shanshan Di, Yundong Yu, Caihong Wang, Xinquan Wang, and Yuanxiang Jin. 2023. “Oral Exposure to Tire Rubber–Derived Contaminant 6PPD and 6PPD-Quinone Induce Hepatotoxicity in Mice.” Science of the Total Environment 869 (April):161836. https://doi.org/10.1016/j.scitotenv.2023.161836.), similar to 6PPD-q. Unlike 6PPD-q, which affected inflammatory pathways, the authors determined that 6PPD tended to affect gene expression related to fatty acid metabolism. The mice were not monitored for apical outcomes. Although steatosis is an adverse outcome and indicator of liver damage in human health evaluations, no studies in wild mammals are available that would define steatosis as an adverse apical outcome in nonhuman organisms. H. N. Zhao, Thomas, et al. (Zhao et al. 2023[GZZX9DMJ] Zhao, Haoqi Nina, Sydney P. Thomas, Mark J. Zylka, Pieter C. Dorrestein, and Wenxin Hu. 2023. “Urine Excretion, Organ Distribution, and Placental Transfer of 6PPD and 6PPD-Quinone in Mice and Potential Developmental Toxicity through Nuclear Receptor Pathways.” Environmental Science & Technology 57 (36): 13429–38. https://doi.org/10.1021/acs.est.3c05026.) found that oral gavage exposure to 4 mg/kg 6PPD in pregnant mice resulted in chemical transfer to pups, resulting in concentrations in livers (mean 200 ng/g) that were greater than in the studies cited above. These results may be of use for ecological risk assessment based on tissue residue values if an apical outcome was associated with organ damage as shown in the study.

2.3 Potential for Bioaccumulation and Adduct Formation

Additional data are required to thoroughly evaluate the bioaccumulation potential of 6PPD-q and 6PPD in aquatic organisms and potential toxic effects, such as adduct formation, that result from uptake of the chemicals. See Section 3.5: Biological Uptake for further discussion of biological uptake and bioaccumulation. In addition, Table 4-10 summarizes concentrations of 6PPD-q measured in studies of aquatic organisms.

2.4 Human Health and Toxicology

This section highlights 6PPD-q toxicity studies relevant to the evaluation of human health effects. Data summarizing the human health effects of 6PPD are presented below and have been included in case a read-across is conducted in the future when data on 6PPD might be used to infer toxicologic endpoints where data are currently lacking for 6PPD-q. At this point in time, data are currently insufficient to conduct such a read-across.

6PPD-quinone Toxicity Relevant to Humans

- Mouse studies indicate that 6PPD-q accumulates primarily in the liver and lung, as well as adipose tissue, kidneys, the brain, and other organs.

- 6PPD-q can cross both the placenta and blood–brain barrier.
- Oral doses of 6PPD-q, at levels much higher than found in the environment, caused liver toxicity in mice.
- 6PPD-q can be excreted in urine and fecal matter.
- Some early studies have identified neurotoxicity, reproductive toxicity, and genotoxicity in various assays. More studies are needed to replicate these findings.

A summary of toxicology data relevant to the assessment of human health for 6PPD-q and its parent 6PPD is provided in Table 2-3.

2.4.1 Toxicity of 6PPD-q

2.4.1.1 Toxicokinetics of 6PPD-q

Toxicokinetic studies provide insights into how substances are absorbed, distributed, metabolized, and excreted in living organisms. These studies can provide insights into potential toxic effects.

Repeat dose intraperitoneal administration, which allows for rapid and efficient absorption and increased bioavailability of 6PPD-q (Al Shoyaib, Archie, and Karamyan 2019[E4MBH586] Al Shoyaib, Abdullah, Sabrina Rahman Archie, and Vardan T. Karamyan. 2019. “Intraperitoneal Route of Drug Administration: Should It Be Used in Experimental Animal Studies?” Pharmaceutical Research 37 (1): 12. https://doi.org/10.1007/s11095-019-2745-x.), indicated accumulation in the liver and lung of mice (He, Gu, and Wang 2023[6MPWVZGE] He, Wenmiao, Aihua Gu, and Dayong Wang. 2023. “Four-Week Repeated Exposure to Tire-Derived 6-PPD Quinone Causes Multiple Organ Injury in Male BALB/c Mice.” Science of the Total Environment 894 (October):164842. https://doi.org/10.1016/j.scitotenv.2023.164842.). In mice fed a single dose of deuterated 6PPD-q_s, 6PPD-q was primarily distributed in the adipose tissue followed by the kidney, lung, testis, liver, spleen, heart, and muscle (Zhang et al. 2024[BZQEGEXI] Zhang, Jing, Guodong Cao, Wei Wang, Han Qiao, Yi Chen, Xiaoxiao Wang, Fuyue Wang, Wenlan Liu, and Zongwei Cai. 2024. “Stable Isotope-Assisted Mass Spectrometry Reveals in Vivo Distribution, Metabolism, and Excretion of Tire Rubber-Derived 6PPD-Quinone in Mice.” Science of the Total Environment 912 (February):169291. https://doi.org/10.1016/j.scitotenv.2023.169291.). Following oral gavage of pregnant mice, 6PPD-q was distributed to the brain and liver of dams and fetal body and fetal brain (Zhao et al. 2023[GZZX9DMJ] Zhao, Haoqi Nina, Sydney P. Thomas, Mark J. Zylka, Pieter C. Dorrestein, and Wenxin Hu. 2023. “Urine Excretion, Organ Distribution, and Placental Transfer of 6PPD and 6PPD-Quinone in Mice and Potential Developmental Toxicity through Nuclear Receptor Pathways.” Environmental Science & Technology 57 (36): 13429–38. https://doi.org/10.1021/acs.est.3c05026.). Lower concentrations of 6PPD-q were measured in the fetal brain as compared with the dam brain, which indicates the partial protection effect of the placenta (Zhao et al. 2023[GZZX9DMJ] Zhao, Haoqi Nina, Sydney P. Thomas, Mark J. Zylka, Pieter C. Dorrestein, and Wenxin Hu. 2023. “Urine Excretion, Organ Distribution, and Placental Transfer of 6PPD and 6PPD-Quinone in Mice and Potential Developmental Toxicity through Nuclear Receptor Pathways.” Environmental Science & Technology 57 (36): 13429–38. https://doi.org/10.1021/acs.est.3c05026.). (The 6PPD results from H. N. Zhao, Thomas, et al. (Zhao et al. 2023[GZZX9DMJ] Zhao, Haoqi Nina, Sydney P. Thomas, Mark J. Zylka, Pieter C. Dorrestein, and Wenxin Hu. 2023. “Urine Excretion, Organ Distribution, and Placental Transfer of 6PPD and 6PPD-Quinone in Mice and Potential Developmental Toxicity through Nuclear Receptor Pathways.” Environmental Science & Technology 57 (36): 13429–38. https://doi.org/10.1021/acs.est.3c05026.) are discussed in Section 2.4.2.1). A study conducted by J. Zhang et al. (Zhang et al. 2024[BZQEGEXI] Zhang, Jing, Guodong Cao, Wei Wang, Han Qiao, Yi Chen, Xiaoxiao Wang, Fuyue Wang, Wenlan Liu, and Zongwei Cai. 2024. “Stable Isotope-Assisted Mass Spectrometry Reveals in Vivo Distribution, Metabolism, and Excretion of Tire Rubber-Derived 6PPD-Quinone in Mice.” Science of the Total Environment 912 (February):169291. https://doi.org/10.1016/j.scitotenv.2023.169291.) demonstrated that 6PPD-q penetrates the blood–brain barrier of mice.

Urine concentrations measured in male and female mice dosed with 6PPD-q or 6PPD indicated lower excretion of 6PPD-q than 6PPD (Zhao et al. 2023[GZZX9DMJ] Zhao, Haoqi Nina, Sydney P. Thomas, Mark J. Zylka, Pieter C. Dorrestein, and Wenxin Hu. 2023. “Urine Excretion, Organ Distribution, and Placental Transfer of 6PPD and 6PPD-Quinone in Mice and Potential Developmental Toxicity through Nuclear Receptor Pathways.” Environmental Science & Technology 57 (36): 13429–38. https://doi.org/10.1021/acs.est.3c05026.). J. Zhang et al. (Zhang et al. 2024[BZQEGEXI] Zhang, Jing, Guodong Cao, Wei Wang, Han Qiao, Yi Chen, Xiaoxiao Wang, Fuyue Wang, Wenlan Liu, and Zongwei Cai. 2024. “Stable Isotope-Assisted Mass Spectrometry Reveals in Vivo Distribution, Metabolism, and Excretion of Tire Rubber-Derived 6PPD-Quinone in Mice.” Science of the Total Environment 912 (February):169291. https://doi.org/10.1016/j.scitotenv.2023.169291.) identified fecal excretion

rather than urine excretion as the main excretory pathway for 6PPD-q and two newly identified novel hydroxylated metabolites of 6PPD-q (D5-6PPD-q-OH and D5-6PPD-q-2OH).

Human biomonitoring conducted in China (see Section 2.5) has measured 6PPD-q and 6PPD in urine with 6PPD-q concentrations significantly higher than 6PPD and higher concentrations of both chemicals observed in pregnant woman than in in adults or children. Daily excretion rates of 6PPD-q in urine of adults, children, and pregnant women were 11.3 ng per kg of body weight (ng/kg-bw)/day, 2.18 ng/kg-bw/day, and 90.9 ng/kg-bw/day, respectively (Du et al. 2022[DWFYR89F] 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.).

6PPD-q was detected in CSF from a small sample of Parkinson’s disease (PD) patients and a control group (Fang et al. 2024[2L4QI2CG] Fang, Jiacheng, Xiaoxiao Wang, Guodong Cao, Fuyue Wang, Yi Ru, Bolun Wang, Yanhao Zhang, et al. 2024. “6PPD-Quinone Exposure Induces Neuronal Mitochondrial Dysfunction to Exacerbate Lewy Neurites Formation Induced by α-Synuclein Preformed Fibrils Seeding.” Journal of Hazardous Materials 465 (March):133312. https://doi.org/10.1016/j.jhazmat.2023.133312.).

2.4.1.2 Acute Toxicity of 6PPD-q

No acute toxicity data are available for 6PPD-q.

2.4.1.3 Irritation/Sensitization of 6PPD-q

No irritation or sensitization data are available for 6PPD-q.

2.4.1.4 Chronic Toxicity and Systemic Effects of 6PPD-q

In a subchronic toxicity study by L. Fang et al. (Fang et al. 2023[B66X64SI] Fang, Liya, Chanlin Fang, Shanshan Di, Yundong Yu, Caihong Wang, Xinquan Wang, and Yuanxiang Jin. 2023. “Oral Exposure to Tire Rubber–Derived Contaminant 6PPD and 6PPD-Quinone Induce Hepatotoxicity in Mice.” Science of the Total Environment 869 (April):161836. https://doi.org/10.1016/j.scitotenv.2023.161836.), 6PPD-q increased lipid accumulation in the livers of mice that were given oral doses of 10 mg/kg-bw/day for 6 weeks. In addition, 6PPD-q increased liver triglycerides at all doses tested (10, 30, and 100 mg/kg-bw/day), and an altered expression of liver enzymes and inflammatory markers in the liver were also reported (Fang et al. 2023[B66X64SI] Fang, Liya, Chanlin Fang, Shanshan Di, Yundong Yu, Caihong Wang, Xinquan Wang, and Yuanxiang Jin. 2023. “Oral Exposure to Tire Rubber–Derived Contaminant 6PPD and 6PPD-Quinone Induce Hepatotoxicity in Mice.” Science of the Total Environment 869 (April):161836. https://doi.org/10.1016/j.scitotenv.2023.161836.).

In a subchronic toxicity study by He, Gu, and Wang (Zhao et al. 2023[GZZX9DMJ] Zhao, Haoqi Nina, Sydney P. Thomas, Mark J. Zylka, Pieter C. Dorrestein, and Wenxin Hu. 2023. “Urine Excretion, Organ Distribution, and Placental Transfer of 6PPD and 6PPD-Quinone in Mice and Potential Developmental Toxicity through Nuclear Receptor Pathways.” Environmental Science & Technology 57 (36): 13429–38. https://doi.org/10.1021/acs.est.3c05026.), “…significant pathological changes were formed in liver, kidney, lung, spleen, testis, and brain…” in mice repeatedly administered 0.4 and 4 mg/kg 6PPD-q via IP administration. According to He, Gu, and Wang (Zhao et al. 2023[GZZX9DMJ] Zhao, Haoqi Nina, Sydney P. Thomas, Mark J. Zylka, Pieter C. Dorrestein, and Wenxin Hu. 2023. “Urine Excretion, Organ Distribution, and Placental Transfer of 6PPD and 6PPD-Quinone in Mice and Potential Developmental Toxicity through Nuclear Receptor Pathways.” Environmental Science & Technology 57 (36): 13429–38. https://doi.org/10.1021/acs.est.3c05026.), “…[b]iochemical parameters of liver [metabolism] (alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP)) and kidney [function] (urea and creatinine) were all significantly upregulated by repeated injection with 0.4 and 4 mg/kg 6PPD-q…”, indicating potential injury to the liver and kidney. But IP studies have significant caveats because it is not a relevant exposure pathway.

2.4.1.5 Carcinogenicity and Genotoxicity of 6PPD-q

6PPD-q was mutagenic to *Parhyale hawaiensis*, a marine invertebrate, and weakly mutagenic to bacteria (Botelho et al. 2023[WZC7F928] Botelho, Marina Tenório, Gabriely Groto Militão, Markus Brinkmann, and Gisela de Aragão Umbuzeiro. 2023. “Toxicity and Mutagenicity Studies of 6PPD-Quinone in a Marine Invertebrate Species and Bacteria.” Environmental and Molecular Mutagenesis 64 (6): 335–41. https://doi.org/10.1002/em.22560.). *Caenorhabditis elegans*, a round worm frequently used in the lab, exposed to 1 µg/L and 10 µg/L 6PPD-q experienced increased DNA damage and increased expression of DNA

damage-related genes (Hua et al. 2023[RJ86H9T3] Hua, Xin, Xiao Feng, Geyu Liang, Jie Chao, and Dayong Wang. 2023. “Long-Term Exposure to 6-PPD Quinone Reduces Reproductive Capacity by Enhancing Germline Apoptosis Associated with Activation of Both DNA Damage and Cell Corpse Engulfment in Caenorhabditis elegans.” Journal of Hazardous Materials 454 (July):131495. https://doi.org/10.1016/j.jhazmat.2023.131495.). DNA adducts were detected in 6PPD-q-treated mammalian cells; removal of 6PPD-q led to decreased levels of the adduct, which suggested potential repair pathways (Wu et al. 2023[PYQQU7AG] 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 866:161373. https://doi.org/10.1016/j.scitotenv.2022.161373.). No carcinogenicity data are available.

2.4.1.6 Reproductive/Developmental Toxicity of 6PPD-q

In a reproductive toxicity study by Hua et al. (Hua et al. 2023[RJ86H9T3] Hua, Xin, Xiao Feng, Geyu Liang, Jie Chao, and Dayong Wang. 2023. “Long-Term Exposure to 6-PPD Quinone Reduces Reproductive Capacity by Enhancing Germline Apoptosis Associated with Activation of Both DNA Damage and Cell Corpse Engulfment in Caenorhabditis elegans.” Journal of Hazardous Materials 454 (July):131495. https://doi.org/10.1016/j.jhazmat.2023.131495.), exposure to 1 µg/L and 10 µg/L 6PPD-q reduced reproductive capacity and negatively affected gonad development in *C. elegans*. The relevance to human health of an effect in *C. elegans* worms is not clear.

In a prenatal developmental toxicity study, pregnant female mice were treated with 4 mg/kg 6PPD-q or 6PPD from embryonic day 11.5 to 15.5. 6PPD-q was found to cross the placenta and was detected in fetal whole-body tissue and fetal brain samples at higher concentrations than 6PPD (Zhao et al. 2023[GZZX9DMJ] Zhao, Haoqi Nina, Sydney P. Thomas, Mark J. Zylka, Pieter C. Dorrestein, and Wenxin Hu. 2023. “Urine Excretion, Organ Distribution, and Placental Transfer of 6PPD and 6PPD-Quinone in Mice and Potential Developmental Toxicity through Nuclear Receptor Pathways.” Environmental Science & Technology 57 (36): 13429–38. https://doi.org/10.1021/acs.est.3c05026.) . This study did not measure fetotoxicity, just distribution to the fetus. In addition, the authors found that 6PPD-q can activate both the human retinoic acid receptor α (RARα) and retinoid X receptor α (RXRα) in human embryonic kidney cells and indicates the potential for developmental effects (Zhao et al. 2023[GZZX9DMJ] Zhao, Haoqi Nina, Sydney P. Thomas, Mark J. Zylka, Pieter C. Dorrestein, and Wenxin Hu. 2023. “Urine Excretion, Organ Distribution, and Placental Transfer of 6PPD and 6PPD-Quinone in Mice and Potential Developmental Toxicity through Nuclear Receptor Pathways.” Environmental Science & Technology 57 (36): 13429–38. https://doi.org/10.1021/acs.est.3c05026.).

2.4.1.7 Neurotoxicity of 6PPD-q

6PPD Toxicity Relevant to Humans

- 6PPD is a dermal sensitizer.
- Chronic toxicity studies in mice indicate the liver and blood are the target organs
- ECHA classifies 6PPD as a category 1B reproductive toxicant.
- 6PPD can cross both the placenta and blood–brain barrier.
- 6PPD can be excreted in urine; in vitro studies indicate that 6PPD-q is not a predominant metabolite of 6PPD.

PD may cause leakage in the blood–brain barrier, creating the possibility that chemicals, such as 6PPD-q, can enter CSF and the brain. In this study, 6PPD-q was detected in the CSF of all the PD patients but only 64% of controls, and the levels were twice as high (11.18 nanograms per milliliter [ng/mL]) in PD patients compared to controls (5.07 ng/mL) (Fang et al. 2024[2L4QI2CG] Fang, Jiacheng, Xiaoxiao Wang, Guodong Cao, Fuyue Wang, Yi Ru, Bolun Wang, Yanhao Zhang, et al. 2024. “6PPD-Quinone Exposure Induces Neuronal Mitochondrial Dysfunction to Exacerbate Lewy Neurites Formation Induced by α-Synuclein Preformed Fibrils Seeding.” Journal of Hazardous Materials 465 (March):133312. https://doi.org/10.1016/j.jhazmat.2023.133312.). The sample sizes of both groups were small, so establishing the population-level significance of the results requires additional investigation. Further, J. Fang et al. (Fang et al. 2024[2L4QI2CG] Fang, Jiacheng, Xiaoxiao Wang, Guodong Cao, Fuyue Wang, Yi Ru, Bolun Wang, Yanhao Zhang, et al. 2024. “6PPD-Quinone Exposure Induces Neuronal Mitochondrial Dysfunction to Exacerbate Lewy Neurites Formation Induced by α-Synuclein Preformed Fibrils Seeding.” Journal of Hazardous Materials 465 (March):133312. https://doi.org/10.1016/j.jhazmat.2023.133312.) investigated the potential for 6PPD-q to impact the pathophysiology of PD by studying 6PPD-q’s effects on mouse primary dopaminergic neurons, a cell type impacted by PD. The researchers found some additive effects with 6PPD-q in mitochondria and in outgrowths (neurites) when the neurons were pre-seeded with

insoluble protein alpha-synuclein preformed fibrils (α-syn PFF), that can cause Parkinsonian deposits. The results were not compared to other molecules that may also induce oxidative stress. More research is needed to determine whether 6PPD-q can act as a human neurotoxin and whether it can cause mitochondrial dysfunction.

2.4.2 Toxicity of 6PPD

2.4.2.1 Toxicokinetics of 6PPD

No in vivo absorption studies of 6PPD are available. Nonetheless, toxicity studies at acute and repeated doses allow for the derivation of certain conclusions. The primary bioavailability of 6PPD through oral and dermal exposure is demonstrated by the emergence of systemic toxicity following exposure (OECD 2004[FCJPCPVW] OECD. 2004. “SIDS Initial Assessment Report for N-(1,3-Dimethylbutyl)-N´-Phenyl-1,4-Phenylenediamine (6PPD), Organisation for Economic Co-Operation and Development (OECD).” https://hpvchemicals.oecd.org/UI/handler.axd?id=5e1a446c-5969-479c-9270-7ced8726952e.ECHA 2022[Z9UAAF4F] ECHA. 2022. “6PPD: 1,4-Benzenediamine, N1-(1,3-Dimethylbutyl)-N4-Phenyl- Registration Dossier - European Chemicals Agency (ECHA).” https://echa.europa.eu/registration-dossier/-/registered-dossier/15367/1/2.). Following oral gavage of pregnant mice, 6PPD was shown to distribute to the brain and liver of dams and body and brain of fetuses (Zhao et al. 2023[GZZX9DMJ] Zhao, Haoqi Nina, Sydney P. Thomas, Mark J. Zylka, Pieter C. Dorrestein, and Wenxin Hu. 2023. “Urine Excretion, Organ Distribution, and Placental Transfer of 6PPD and 6PPD-Quinone in Mice and Potential Developmental Toxicity through Nuclear Receptor Pathways.” Environmental Science & Technology 57 (36): 13429–38. https://doi.org/10.1021/acs.est.3c05026.). Lower concentrations of 6PPD were measured in the fetal brain as compared with the dam brain, indicating a partial protective effect of the placenta (Zhao et al. 2023[GZZX9DMJ] Zhao, Haoqi Nina, Sydney P. Thomas, Mark J. Zylka, Pieter C. Dorrestein, and Wenxin Hu. 2023. “Urine Excretion, Organ Distribution, and Placental Transfer of 6PPD and 6PPD-Quinone in Mice and Potential Developmental Toxicity through Nuclear Receptor Pathways.” Environmental Science & Technology 57 (36): 13429–38. https://doi.org/10.1021/acs.est.3c05026.). (Note: The 6PPD-q results from H. N. Zhao, Thomas, et al. (Zhao et al. 2023[GZZX9DMJ] Zhao, Haoqi Nina, Sydney P. Thomas, Mark J. Zylka, Pieter C. Dorrestein, and Wenxin Hu. 2023. “Urine Excretion, Organ Distribution, and Placental Transfer of 6PPD and 6PPD-Quinone in Mice and Potential Developmental Toxicity through Nuclear Receptor Pathways.” Environmental Science & Technology 57 (36): 13429–38. https://doi.org/10.1021/acs.est.3c05026.) are discussed in Section 2.4.1.1).

In an in vitro metabolism study, in the presence of simulated gastric juice, 6PPD had a half-life of 36.9 hours. The major observed hydrolysis product was aniline with trace amounts of benzoquinone imine-N-phenyl; N-1,3 dimethyl-butylamine p-phenol; quinone; and 2-amino-4-methylpentane (ToxServices, LLC 2021[IDJRCJR2] ToxServices, LLC. 2021. “N-(1,3-Dimethylbutyl)-N’-Phenyl-p-Phenylenediamine (6PPD) (CAS #793-24-8) Greenscreen® for Safer Chemicals (Greenscreen®) Assessment.” GS-1204. Washington, D.C.: ToxServices Toxicology Risk Assessment Consulting. https://www.ezview.wa.gov/Portals/_1962/Documents/6ppd/GreenScreenExecutiveSummaryFor6PPD.pdf.).

In another study (Du et al. 2022[DWFYR89F] 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.), 6PPD was metabolized via human liver microsomes. After 3 hours of incubation with liver microsomes under in vitro conditions, 65% of 6PPD was depleted. Only 2% of the 6PPD was metabolized into 6PPD-q; its production rates were measured at corresponding time intervals, indicating 6PPD may predominantly metabolize into other metabolites.

In mice, 6PPD has been shown to rapidly excrete in urine (Zhao et al. 2023[GZZX9DMJ] Zhao, Haoqi Nina, Sydney P. Thomas, Mark J. Zylka, Pieter C. Dorrestein, and Wenxin Hu. 2023. “Urine Excretion, Organ Distribution, and Placental Transfer of 6PPD and 6PPD-Quinone in Mice and Potential Developmental Toxicity through Nuclear Receptor Pathways.” Environmental Science & Technology 57 (36): 13429–38. https://doi.org/10.1021/acs.est.3c05026.). Human biomonitoring studies have detected 6PPD in urine (Mao et al. 2024[MJI3WKBH] Mao, Weili, Hangbiao Jin, Ruyue Guo, Ping Chen, Songyang Zhong, and Xilin Wu. 2024. “Occurrence of p-Phenylenediamine Antioxidants in Human Urine.” Science of the Total Environment 914 (March):170045. https://doi.org/10.1016/j.scitotenv.2024.170045.Du et al. 2022[DWFYR89F] 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.ECHA 2022[Z9UAAF4F] ECHA. 2022. “6PPD:

1,4-Benzenediamine, N1-(1,3-Dimethylbutyl)-N4-Phenyl- Registration Dossier - European Chemicals Agency (ECHA)."

https://echa.europa.eu/registration-dossier/-/registered-dossier/15367/1/2.). Du et al. (Du et al. 2022[DWFYR89F] 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.) reported that pregnant women had higher daily excretion rates of 6PPD (median value of 2.13 ng/kg-bw/day) compared with adults (0.51 ng/kg-bw/day) and children (0.43 ng/kg-bw/day). Daily excretion rates of 6PPD measured by Mao et al. (Mao et al.

2024[MJI3WKBH] Mao, Weili, Hangbiao Jin, Ruyue Guo, Ping Chen, Songyang Zhong, and Xilin Wu. 2024. "Occurrence of p-Phenylenediamine Antioxidants in Human Urine." Science of the Total Environment 914 (March):170045. https://doi.org/10.1016/j.scitotenv.2024.170045.) in adults were 34 ng/kg-bw/day (mean) and 30 ng/kg-bw/day (median). The differences in the results indicate that more studies are necessary.

2.4.2.2 Acute Toxicity of 6PPD

Acute toxicity of 6PPD is moderate via the oral route of exposure and low via the dermal route of exposure, based on GHS thresholds. Oral median lethal dose (LD_{50}) values measured in rats range between 893 mg/kg and 5,000 mg/kg (ECHA

2022[Z9UAAF4F] ECHA. 2022. "6PPD: 1,4-Benzenediamine, N1-(1,3-Dimethylbutyl)-N4-Phenyl- Registration Dossier - European Chemicals Agency (ECHA)." https://echa.europa.eu/registration-dossier/-/registered-dossier/15367/1/2.). Substance preparation (dosing vehicle) influences the bioavailability after oral application, which may explain the broad range of oral

LD_{50} observed (OECD 2004[FCJPCPVW] OECD. 2004. "SIDS Initial Assessment Report for N-(1,3-Dimethylbutyl)-N´-Phenyl-1,4-Phenylenediamine (6PPD), Organisation for Economic Co-Operation and Development (OECD)." https://hpvchemicals.oecd.org/UI/handler.axd?id=5e1a446c-5969-479c-9270-7ced8726952e.).

Currently, dermal exposure studies of acute toxicity following guidelines from the Organisation for Economic Co-Operation and Development (OECD) guideline have not been completed. However, two older studies (ECHA 2022[Z9UAAF4F] ECHA. 2022. "6PPD: 1,4-Benzenediamine, N1-(1,3-Dimethylbutyl)-N4-Phenyl- Registration Dossier - European Chemicals Agency (ECHA)." https://echa.europa.eu/registration-dossier/-/registered-dossier/15367/1/2.) are available to evaluate dermal acute toxicity. In one study, a dermal LD_{50} value greater than 7,940 mg/kg-bw, the highest dose tested, was measured in rabbits. In a second study, a lethal dose in the range between 3,160 and 5,010 mg/kg-bw was established. According to OECD (2004), symptoms of toxicity included reduced food consumption, hypoactivity, and lethargy. Reliable data were not available to evaluate acute toxicity of 6PPD through an inhalation route of exposure (ECHA 2022[Z9UAAF4F] ECHA. 2022. "6PPD: 1,4-Benzenediamine, N1-(1,3-Dimethylbutyl)-N4-Phenyl- Registration Dossier - European Chemicals Agency (ECHA)." https://echa.europa.eu/registration-dossier/-/registered-dossier/15367/1/2.).

2.4.2.3 Irritation/Sensitization of 6PPD

6PPD is considered to be a skin sensitizer based on multiple animal studies (ECHA 2022[Z9UAAF4F] ECHA. 2022. "6PPD: 1,4-Benzenediamine, N1-(1,3-Dimethylbutyl)-N4-Phenyl- Registration Dossier - European Chemicals Agency (ECHA)." https://echa.europa.eu/registration-dossier/-/registered-dossier/15367/1/2.). Patch tests in human populations resulted in similar observations. Those who had previously been sensitized to rubber samples showed a higher rate of sensitization to 6PPD, whereas healthy volunteers who had not previously been exposed to test rubber formulations showed no or very little sensitization (OECD 2004[FCJPCPVW] OECD. 2004. "SIDS Initial Assessment Report for N-(1,3-Dimethylbutyl)-N´-Phenyl-1,4-Phenylenediamine (6PPD), Organisation for Economic Co-Operation and Development (OECD)."

https://hpvchemicals.oecd.org/UI/handler.axd?id=5e1a446c-5969-479c-9270-7ced8726952e.ECHA 2022[Z9UAAF4F] ECHA. 2022. "6PPD: 1,4-Benzenediamine, N1-(1,3-Dimethylbutyl)-N4-Phenyl- Registration Dossier - European Chemicals Agency (ECHA)." https://echa.europa.eu/registration-dossier/-/registered-dossier/15367/1/2.). Based on these animal and human test results (see Table 2-3), The European Chemicals Agency (ECHA) (2022) classifies 6PPD as a category 1 skin sensitizer without subcategorization.

Studies of rabbits determined that 6PPD was not irritating to skin but slightly irritated eyes (ECHA 2022[Z9UAAF4F] ECHA. 2022. "6PPD: 1,4-Benzenediamine, N1-(1,3-Dimethylbutyl)-N4-Phenyl- Registration Dossier - European Chemicals Agency (ECHA)." https://echa.europa.eu/registration-dossier/-/registered-dossier/15367/1/2.).

2.4.2.4 Chronic Toxicity and Systemic Effects of 6PPD

In a subchronic toxicity study by L. Fang et al. (Fang et al. 2023[B66X64SI] Fang, Liya, Chanlin Fang, Shanshan Di, Yundong Yu, Caihong Wang, Xinquan Wang, and Yuanxiang Jin. 2023. “Oral Exposure to Tire Rubber–Derived Contaminant 6PPD and 6PPD-Quinone Induce Hepatotoxicity in Mice.” Science of the Total Environment 869 (April):161836. https://doi.org/10.1016/j.scitotenv.2023.161836.), 6PPD increased lipid accumulation in the livers of mice that were given oral doses of 10 mg/kg-bw/day for 6 weeks. Similarly, ECHA (ECHA 2022[Z9UAAF4F] ECHA. 2022. “6PPD: 1,4-Benzenediamine, N1-(1,3-Dimethylbutyl)-N4-Phenyl- Registration Dossier - European Chemicals Agency (ECHA).” https://echa.europa.eu/registration-dossier/-/registered-dossier/15367/1/2.) identified the liver and blood cells as targets of toxicity in a 28-day oral exposure rat study. In the rat study, effects on the liver for both sexes were reversible at 20 mg/kg-bw/day (the established no adverse effect level or NOAEL), and both sexes showed fat deposition in the liver and anemia at 100 mg/kg-bw/day (the established lowest observed adverse effect level) (ECHA 2022[Z9UAAF4F] ECHA. 2022. “6PPD: 1,4-Benzenediamine, N1-(1,3-Dimethylbutyl)-N4-Phenyl- Registration Dossier - European Chemicals Agency (ECHA).” https://echa.europa.eu/registration-dossier/-/registered-dossier/15367/1/2.). A sex-specific sensitivity was also observed in the study data where a no-observed-effect level (NOEL) of 4 mg/kg-bw/day was established for female rats only. Mild effects (reversible periportal fatty change of the liver without an increase of liver weight; increased total serum protein) were observed in female rats at the lowest observed effects level (LOEL) of 20 mg/kg-bw/day, which formed the basis of the NOAEL (OECD 2004[FCJPCPVW] OECD. 2004. “SIDS Initial Assessment Report for N-(1,3-Dimethylbutyl)-N´-Phenyl-1,4-Phenylenediamine (6PPD), Organisation for Economic Co-Operation and Development (OECD).” https://hpvchemicals.oecd.org/UI/handler.axd?id=5e1a446c-5969-479c-9270-7ced8726952e.).

2.4.2.5 Carcinogenicity and Genotoxicity of 6PPD

6PPD is not likely to be mutagenic or genotoxic based on negative mutagenicity data in vitro in bacterial or mammalian cells and negative clastogenicity data in vivo (OECD 2004[FCJPCPVW] OECD. 2004. “SIDS Initial Assessment Report for N-(1,3-Dimethylbutyl)-N´-Phenyl-1,4-Phenylenediamine (6PPD), Organisation for Economic Co-Operation and Development (OECD).” https://hpvchemicals.oecd.org/UI/handler.axd?id=5e1a446c-5969-479c-9270-7ced8726952e.ECHA 2022[Z9UAAF4F] ECHA. 2022. “6PPD: 1,4-Benzenediamine, N1-(1,3-Dimethylbutyl)-N4-Phenyl- Registration Dossier - European Chemicals Agency (ECHA).” https://echa.europa.eu/registration-dossier/-/registered-dossier/15367/1/2.). 6PPD has low potential for carcinogenicity as indicated in data collected from two-year chronic feeding studies in rats and negative results in an in vitro cell transformation assay with BALB/3T3 cells (ECHA 2022[Z9UAAF4F] ECHA. 2022. “6PPD: 1,4-Benzenediamine, N1-(1,3-Dimethylbutyl)-N4-Phenyl- Registration Dossier - European Chemicals Agency (ECHA).” https://echa.europa.eu/registration-dossier/-/registered-dossier/15367/1/2.).

2.4.2.6 Reproductive/Developmental Toxicity of 6PPD

6PPD is listed as a category 1B reproductive toxicant by ECHA (ECHA 2022[Z9UAAF4F] ECHA. 2022. “6PPD: 1,4-Benzenediamine, N1-(1,3-Dimethylbutyl)-N4-Phenyl- Registration Dossier - European Chemicals Agency (ECHA).” https://echa.europa.eu/registration-dossier/-/registered-dossier/15367/1/2.). Dose-dependent dystocia (difficult birth) was found in multiple treatment groups in rats. A NOAEL of 7 mg/kg-bw/day was established for female reproductive toxicity (ECHA 2022[Z9UAAF4F] ECHA. 2022. “6PPD: 1,4-Benzenediamine, N1-(1,3-Dimethylbutyl)-N4-Phenyl- Registration Dossier - European Chemicals Agency (ECHA).” https://echa.europa.eu/registration-dossier/-/registered-dossier/15367/1/2.). Other studies did not identify reproductive effects in either rats or rabbits (OECD 2004[FCJPCPVW] OECD. 2004. “SIDS Initial Assessment Report for N-(1,3-Dimethylbutyl)-N´-Phenyl-1,4-Phenylenediamine (6PPD), Organisation for Economic Co-Operation and Development (OECD).” https://hpvchemicals.oecd.org/UI/handler.axd?id=5e1a446c-5969-479c-9270-7ced8726952e.OSPAR Commission 2006[SVMKJM7X] OSPAR Commission. 2006. “Hazardous Substances Series 4-(Dimethylbutylamino)Diphenylamine (6PPD) 2005 (2006 Update).” Publication Number: 271/2006. https://www.ospar.org/documents?v=7029.).

Developmental toxicity tests on 6PPD have not been conclusive. No indications for teratogenic or developmental effects were observed in rats up to oral doses of 250 mg/kg-bw/day (the highest dose tested) (OECD 2004[FCJPCPVW] OECD. 2004. “SIDS Initial Assessment Report for N-(1,3-Dimethylbutyl)-N´-Phenyl-1,4-Phenylenediamine (6PPD), Organisation for Economic Co-Operation and Development (OECD).” https://hpvchemicals.oecd.org/UI/handler.axd?id=5e1a446c-5969-479c-9270-7ced8726952e.ECHA 2022[Z9UAAF4F] ECHA. 2022.

“6PPD: 1,4-Benzenediamine, N1-(1,3-Dimethylbutyl)-N4-Phenyl- Registration Dossier - European Chemicals Agency (ECHA).” https://echa.europa.eu/registration-dossier/-/registered-dossier/15367/1/2.). In a prenatal developmental toxicity study, no conclusion could be drawn regarding whether the developmental effects (lower mean fetal body weights) observed in rabbits were due to decreased maternal feeding or specific effects of 6PPD on the fetus. A dosage level of 25 mg/kg-bw/day was established as the NOAEL for embryo/fetal development (ECHA 2022[Z9UAAF4F] ECHA. 2022. “6PPD: 1,4-Benzenediamine, N1-(1,3-Dimethylbutyl)-N4-Phenyl- Registration Dossier - European Chemicals Agency (ECHA).” https://echa.europa.eu/registration-dossier/-/registered-dossier/15367/1/2.). This NOAEL is supported by other studies where “…[e]xposure during the gestation period demonstrated the absence of a fetotoxic or teratogenic potential and of maternal toxicity in rabbits with doses up to 30 mg/kg-bw/day (highest dose tested)” (OECD 2004[FCJPCPVW] OECD. 2004. “SIDS Initial Assessment Report for N-(1,3-Dimethylbutyl)-N´-Phenyl-1,4-Phenylenediamine (6PPD), Organisation for Economic Co-Operation and Development (OECD).” https://hpvchemicals.oecd.org/UI/handler.axd?id=5e1a446c-5969-479c-9270-7ced8726952e.).

In a prenatal developmental toxicity study, pregnant female mice were treated with 4 mg/kg 6PPD from embryonic day 11.5 to 15.5. 6PPD was found to cross the placenta and was detected in fetal whole-body tissue and fetal brain samples (Zhao et al. 2023[GZZX9DMJ] Zhao, Haoqi Nina, Sydney P. Thomas, Mark J. Zylka, Pieter C. Dorrestein, and Wenxin Hu. 2023. “Urine Excretion, Organ Distribution, and Placental Transfer of 6PPD and 6PPD-Quinone in Mice and Potential Developmental Toxicity through Nuclear Receptor Pathways.” Environmental Science & Technology 57 (36): 13429–38. https://doi.org/10.1021/acs.est.3c05026.). Zhao et al. also found that 6PPD acted as an agonist on RXRα in human embryonic kidney cells. Collectively, these results suggest the potential for developmental effects. More research is needed to understand whether these results translate into observable outcomes in the embryos (apical endpoints).

2.5 Biomonitoring for 6PPD and 6PPD-q

At the time this document was prepared, there was limited information about biological measures of exposure to 6PPD and 6PPD-q in people. The kinds of samples used to characterize human exposure may vary with the chemical contaminant of concern but can include blood, hair, and urine, among other biological materials. Detection of 6PPD and 6PPD-q in urine, serum, and CSF is summarized below.

6PPD and 6PPD-q were detected in the urine of adults, children, and pregnant individuals living in South China (Du et al. 2022[DWFYR89F] 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.). 6PPD had an overall detection frequency of 68%, and 6PPD-q had a 97% detection frequency. Pregnant women’s urine had the highest levels of 6PPD and 6PPD-q out of all the demographic groups in the study, with 6PPD-q concentrations greater than 6PPD concentrations by two orders of magnitude. Higher levels of 6PPD and 6PPD-q were detected in the urine of adult women than that of adult men, but this difference was not present in children. Additionally, 6PPD-q urine concentrations increased with age in children, with levels higher in children aged 7–13 years and 4–6 years compared to 1–3 years. Du et al. (Du et al. 2022[DWFYR89F] 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.) hypothesized this age difference is due to a difference in toxicokinetics between younger and older children, as well as the change in diet from breast milk/formula to solid foods. Du et al. (Du et al. 2022[DWFYR89F] 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.) only analyzed for 6PPD and 6PPD-q, but there may be other metabolic transformation products of 6PPD and 6PPD-q in urine that are not yet accounted for. For example, J. Zhang et al. (Zhang et al. 2024[BZQEGEXI] Zhang, Jing, Guodong Cao, Wei Wang, Han Qiao, Yi Chen, Xiaoxiao Wang, Fuyue Wang, Wenlan Liu, and Zongwei Cai. 2024. “Stable Isotope-Assisted Mass Spectrometry Reveals in Vivo Distribution, Metabolism, and Excretion of Tire Rubber-Derived 6PPD-Quinone in Mice.” Science of the Total Environment 912 (February):169291. https://doi.org/10.1016/j.scitotenv.2023.169291.) identified two novel hydroxylated metabolites of 6PPD-q (D5-6PPD-q-OH and D5-6PPD-q-2OH) that were not included in the pioneering human urine biomonitoring study by Du et al. (Du et al.

2022[DWFYR89F] 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.).

Mao et al. (Mao et al. 2024[MJI3WKBH] Mao, Weili, Hangbiao Jin, Ruyue Guo, Ping Chen, Songyang Zhong, and Xilin Wu. 2024. “Occurrence of p-Phenylenediamine Antioxidants in Human Urine.” Science of the Total Environment 914 (March):170045. https://doi.org/10.1016/j.scitotenv.2024.170045.) analyzed urine sampled from 151 adults for 6PPD but not 6PPD-q in China. 6PPD had an 82% detection frequency. Higher levels of 6PPD were detected in the urine from adult women than from adult men, which supports the findings of Du et al. (Du et al. 2022[DWFYR89F] 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.). A general trend of decreasing 6PPD concentration with increasing age was also noted, with significance achieved for the less than 20 years versus 50+ years comparison.

J. Zhang et al. (Zhang et al. 2024[BZQEGEXI] Zhang, Jing, Guodong Cao, Wei Wang, Han Qiao, Yi Chen, Xiaoxiao Wang, Fuyue Wang, Wenlan Liu, and Zongwei Cai. 2024. “Stable Isotope-Assisted Mass Spectrometry Reveals in Vivo Distribution, Metabolism, and Excretion of Tire Rubber-Derived 6PPD-Quinone in Mice.” Science of the Total Environment 912 (February):169291. https://doi.org/10.1016/j.scitotenv.2023.169291.) collected serum from 30 volunteers and analyzed for 6PPD-q to identify environmentally relevant exposure concentrations for a laboratory study in mice. The detection frequency of 6PPD-q in these serum samples was 100%, and the median concentration was 0.24 ng/mL. The quality of the data is not confirmed because the paper provides limited methodological information for the serum analysis. Further studies designed to characterize exposure and toxicokinetic properties of 6PPD and 6PPD-q in people are needed.

6PPD-q was detected in CSF from PD patients (n=13; 100% detection frequency) and a control group (n=11; 64% detection frequency) (Fang et al. 2024[2L4QI2CG] Fang, Jiacheng, Xiaoxiao Wang, Guodong Cao, Fuyue Wang, Yi Ru, Bolun Wang, Yanhao Zhang, et al. 2024. “6PPD-Quinone Exposure Induces Neuronal Mitochondrial Dysfunction to Exacerbate Lewy Neurites Formation Induced by α-Synuclein Preformed Fibrils Seeding.” Journal of Hazardous Materials 465 (March):133312. https://doi.org/10.1016/j.jhazmat.2023.133312.). Differences were noted between the populations, but could be caused by different exposure or different distribution to CSF in the disease state.

A single study on human breastmilk analyzed 120 samples from healthy lactating women in South China for several antioxidant chemicals. 6PPD was detected in 52% of samples with a median concentration of 4.1 picograms per milliliter (pg/mL). 6PPD-q was below the method quantitation limit in all samples (Liang et al. 2024[BL32NURZ] Liang, Bowen, Jiali Ge, Qing Deng, Yi Li, Bibai Du, Ying Guo, and Lixi Zeng. 2024. “Occurrence of Multiple Classes of Emerging Synthetic Antioxidants, Including p -Phenylenediamines, Diphenylamines, Naphthylamines, Macromolecular Hindered Phenols, and Organophosphites, in Human Milk: Implications for Infant Exposure.” Environmental Science & Technology Letters 11 (3): 259–65. https://doi.org/10.1021/acs.estlett.4c00010.).

Older studies of occupational exposure to 6PPD detected levels in the urine of rubber industry workers. 6PPD-q was not recognized at the time and was not measured. In all, 15% of urine samples of 21 Italian rubber industry workers who were exposed by both inhalation and dermal contact contained 6PPD (the maximum was 1.3 µg/L 6PPD in urine). 6PPD was detected in air samples taken in the work area at a concentration range of 0.01–1 micrograms per cubic meter (µg/m^3) (Carlucci et al. 1984[4TK2MX2U] Carlucci, Giovanni, Luisa Airoldi, Roberto Fanelli, and Biagio Laguzzi. 1984. “Quantitative Analysis of Aromatic Amines in Human Urine by Gas Chromatography—Mass Spectrometry—Selected-Ion Monitoring.” Journal of Chromatography B: Biomedical Sciences and Applications 311:141–47. https://www.sciencedirect.com/science/article/abs/pii/S0378434700847003.). A peak value of 580 µg/g 6PPD was detected in another biomonitoring study of 341 Italian rubber industry workers (1982–1987). The urine concentration of 6PPD of workers was found to correlate with the level of 6PPD detected in the air, which reached a maximum concentration of 6.6 mg/m^3 (Rimatori and Castellino 1989, as cited in OECD 2004[FCJPCPVW] OECD. 2004. “SIDS Initial Assessment Report for N-(1,3-Dimethylbutyl)-N´-Phenyl-1,4-Phenylenediamine (6PPD), Organisation for Economic Co-Operation and Development (OECD).” https://hpvchemicals.oecd.org/UI/handler.axd?id=5e1a446c-5969-479c-9270-7ced8726952e.).

2.6 Potential Populations of Concern

Potential Populations of Concern

- Certain populations may be differentially exposed to 6PPD-q, such as those with occupational exposure.
- There may be environmental justice concerns related to 6PPD-q due to the potential for disproportionate exposures to vulnerable communities.
- Additional research is needed to understand the potential for bioaccumulation in fish tissue and to inform the potential for human exposure in populations that rely on subsistence fishing.

People may be more likely to experience health effects from a chemical if they are biologically susceptible, have other health stressors, or are more highly exposed due to either behavioral exposure factors or increased contact with contaminated environmental media. For example, some people may have higher exposures to 6PPD-q and 6PPD than others because of their occupations or where they live. This section identifies some possible populations of concern based on potential exposure patterns, possible vulnerabilities, and populations that engage in subsistence fishing for coho salmon or other impacted species. Relationships between these potential exposures and health outcomes is a data gap.

Some workplaces in the United States contain tires and tire debris. As described by the California Department of Toxic Substances Control (DTSC) (DTSC 2022[2M3Z8Z4F] DTSC. 2022. “Product-Chemical Profile for Motor Vehicle Tires Containing N-(1,3-Dimethylbutyl)-N′-Phenyl-p-Phenylenediamine (6PPD) from the California Department of Toxic Substances Control (DTSC).” https://dtsc.ca.gov/wp-content/uploads/sites/31/2022/05/6PPD-in-Tires-Priority-Product-Profile_FINAL-VERSION_accessible.pdf.), “…[s]ome of these occupations include tire manufacturers, mechanics, highway workers,… street sweepers, car washers, and parking attendants.” Workers involved in handling, shredding or otherwise processing waste tires and other products may be at higher risk of exposure due to their direct contact with rubber materials containing 6PPD. These workers may have dermal exposure to the rubber as well as dust and particulate matter inhalation exposures. In addition, workers with prior skin sensitization to 6PPD or other phenylenediamine compounds may be more highly susceptible to allergic skin responses upon exposure to 6PPD. Children who live, play, attend school, and play sports near roadways or in environments that use crumb rubber could be at a heightened risk of exposure to 6PPD and 6PPD-q in TRWPs and road dusts. Children who play on fields amended with crumb rubber or with playground structures made from recycled tires may also be at elevated risk for exposure to 6PPD and 6PPD-q released from these materials, although exposure levels that result from these sources is not yet documented. Children’s lower body weight, greater dust ingestion and inhalation rates, and dust to skin adherence factor result in a higher dose from exposure compared to adults, which might make them more vulnerable to any potential adverse effects (Jin et al. 2023[P9WXQJUR] Jin, Ruihe, Yan Wu, Qun He, Pei Sun, Qiqing Chen, Chunjie Xia, Ye Huang, Jing Yang, and Min Liu. 2023. “Ubiquity of Amino Accelerators and Antioxidants in Road Dust from Multiple Land Types: Targeted and Nontargeted Analysis.” Environmental Science & Technology 57 (28): 10361–72. https://doi.org/10.1021/acs.est.3c01448.). Additionally, older children may be at higher risk of exposure compared to younger children as indicated by higher urine concentrations of 6PPD-q in 4–13 year olds compared to 1–3 year olds (Du et al. 2022[DWFYR89F] 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.).

Those who spend more time near roadways may have increased exposure to 6PPD and 6PPD-q. Likewise, air transport of directly emitted and resuspended tire and road particles containing 6PPD and 6PPD-q could lead to higher local concentrations of these compounds in ambient air and potentially contaminate air and dust in near-road residences, schools, and workplaces, impacting the health of individuals in these communities.

In a biomonitoring study (Du et al. 2022[DWFYR89F] 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.) (Section 2.5), 6PPD and 6PPD-q were detected in the urine of pregnant women, children, and general adult populations at varying concentrations in each subpopulation. Environmental justice concerns arise when vulnerable communities, often including low-income and minority populations, are disproportionately exposed to environmental hazards. This can result in unequal health impacts and socioeconomic disparities. Populations living near roadways in the United States are disproportionately nonwhite and low-income (Rowangould 2013[9KKD6N4U] Rowangould, G.M.

2013. “A Census of the US Near-Roadway Population: Public Health and Environmental Justice Considerations.” Transportation Research, Part D: Transport and Environment 2013 (25): 59–67. https://doi.org/10.1016/j.trd.2013.08.003.) and may be at higher risk for exposure and health effects of 6PPD and 6PPD-q.

Tribal members, recreational fishers, and communities that harvest salmon for subsistence in urbanized areas may experience higher exposure if it is found that these chemicals accumulate in edible fish tissue. See Section 1.3.3: Tribal Nations and Section 1.3.4: Potential Economic and Community Health Concerns for further discussion of environmental justice.