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Global Occurrence and Ecological Risk Assessment of 6PPD and 6PPD-Quinone: Derivation of PNECs Using Species Sensitivity Distribution

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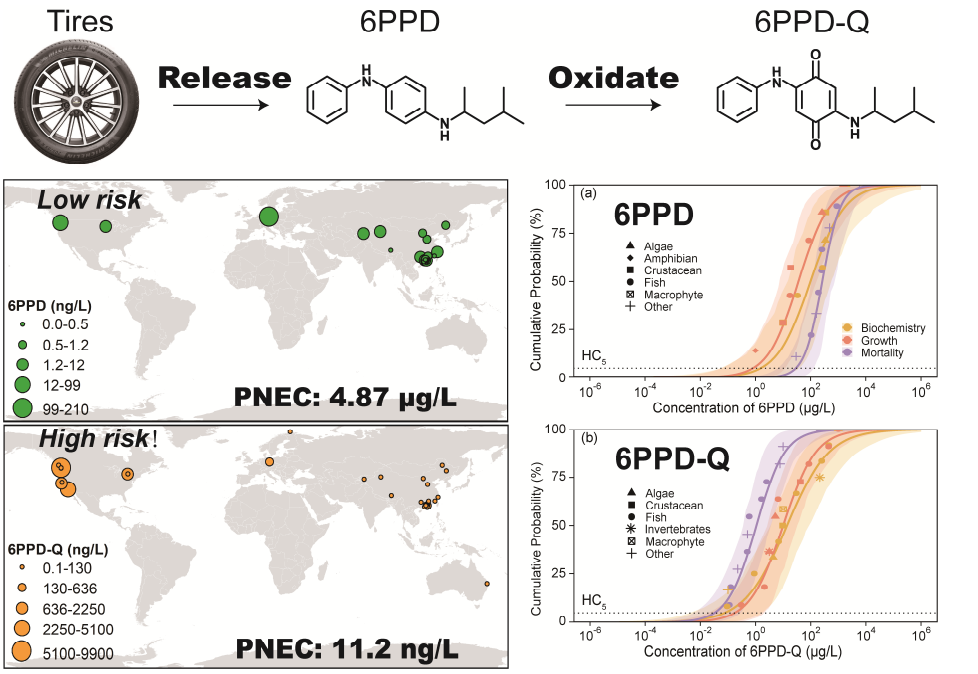
Highlights

- Global SSD-based PNECs were derived for 6PPD and 6PPD-Q
- 6PPD-Q showed much higher toxicity than 6PPD, with extreme sensitivity in salmonids
- 6PPD-Q exhibited elevated probabilistic ecological risk in surface waters

Abstract: Tire antioxidant *N*-(1,3-dimethylbutyl)-*N*′-phenyl-p-phenylenediamine (6PPD) and its transformation product 6PPD-quinone (6PPD-Q) have recently been recognized as emerging contaminants of global concern due to their widespread occurrence and high toxicity to aquatic organisms. Although the rapidly accumulating monitoring and toxicological data suggesting substantial ecological risks, a quantitative and cross-species assessment framework remains lacking. Here, we compiled 77 reported concentration records of 6PPD and 6PPD-Q from 21 studies worldwide and assembled 991 toxicity endpoints across 34 species to derive Predicted No-Effect Concentrations (PNECs) by species sensitivity distributions (SSDs) and probabilistic ecological risk estimates. Both compounds were ubiquitously detected in surface waters, with 6PPD-Q frequently exceeding the concentration of its parent compound. Salmonids exhibited exceptional sensitivity to 6PPD-Q, with lethal thresholds in the nanogram-per-liter range, whereas tolerance varied markedly among non-salmonid taxa. Model-averaged SSDs yielded mortality-based hazardous concentrations for 5% of species (HC_5) of 24.3 μg/L for 6PPD and 0.0559 μg/L for 6PPD-Q, corresponding to PNECs of 4.87 and 0.0112 μg/L, respectively. Probabilistic risk characterization indicated negligible global risk for 6PPD, whereas 6PPD-Q exhibited elevated risk potential, with mortality-based overall risk probabilities reaching 11.4%. Risk levels followed the pattern of surface runoff > river waters > wastewater effluent, and were higher in North America and Europe than in Asia. Regional differences in species sensitivity and environmental exposure contributed to substantial uncertainties, underscoring the need for localized PNEC derivation and expanded toxicity datasets, particularly for transformation products. This study provides the first integrated global SSD-based benchmarks for 6PPD and 6PPD-Q, offering a quantitative foundation for monitoring, regulation, and ecological protection of tire-derived contaminants.



1. Introduction

Tire-derived contaminants, particularly *N*-(1,3-dimethylbutyl)-*N*′-phenyl-*p*-phenylenediamine (6PPD) and its transformation product 6PPD-quinone (6PPD-Q), have emerged as significant pollutants in urban aquatic ecosystems [1,2]. 6PPD is extensively used as an antioxidant in tire manufacturing to prevent ozone-induced degradation [3,4]. In China, annual production of 6PPD reaches approximately 200,000 tons, accounting for about 54% of total rubber antioxidant output [5]. Upon reaction with ozone, 6PPD is converted into 6PPD-Q, a transformation product recently identified as highly toxic to aquatic organisms, such as coho salmon (*Oncorhynchus kisutch*), with a 24-h LC_{50} as low as 0.095 μg/L [6,7]. The discovery of 6PPD-Q as the causative agent of the "urban runoff mortality syndrome" has drawn global attention to its environmental occurrence and ecotoxicological implications.

Beyond the acute lethality, 6PPD and 6PPD-Q elicit a broad spectrum of toxic effects across aquatic organisms, indicating substantial sublethal and chronic impacts. Both compounds reduce photosynthetic performance, induce oxidative damage, and limit nutrient uptake in the floating macrophyte *Eichhornia crassipes* [8]. In corals, 6PPD causes acute toxicity and impairs the photosynthetic function of their symbiotic algae, leading to reduced growth and whole-organism respiration in *Pocillopora damicornis* [9]. Animal studies reveal further vulnerabilities: 6PPD induces developmental abnormalities in zebrafish (*Danio rerio*; 96-h LC_{50} = 2200 μg/L) [10], and environmentally relevant 6PPD-Q levels compromise intestinal barrier integrity in *Caenorhabditis elegans* [11]. Exposure to approximately 10^2 μg/L 6PPD further provokes oxidative stress and apoptosis in zebrafish larvae, alters cardiotoxicity-related gene expression, and perturbs the growth hormone/insulin-like growth factor (GH/IGF) and hypothalamic–pituitary–thyroid (HPT) endocrine axes, leading to growth inhibition and developmental defects [10,12]. At the molecular level, 6PPD-Q reacts with DNA deoxyguanosine to form 6PPD-Q–dG adducts that accumulate in fish eggs and gills, suggesting genotoxic potential and tissue-specific bioaccumulation [13]. In mammals, repeated exposure induces pulmonary inflammation and fibrosis, indicating potential human health concerns [14]. In addition, population-level assays revealed that 6PPD poses greater reproductive risk than 6PPD-Q to freshwater rotifers (*Brachionus calyciflorus*) at equivalent concentrations [15]. Collectively, these findings underscore the broad biological reactivity and ecological relevance of these compounds.

Environmental monitoring has confirmed the widespread presence of 6PPD and 6PPD-Q across multiple environmental compartments, including wastewater effluents [16], surface waters [17,18], stormwater runoff [1,19], sediments [5], and aquatic biota [20]. Concentrations in surface waters often reach several hundred nanograms per liter, with urban runoff containing up to 120 ng/L of 6PPD-Q [1]. Furthermore, several PPDs have demonstrated bioaccumulation and trophic transfer potential in estuarine food webs [20]. Despite this growing body of evidence, toxicity data remain fragmented across species and endpoints, and no quantitative framework currently exists to integrate exposure and sensitivity information for comprehensive ecological risk characterization.

To address this limitation, the Species Sensitivity Distribution (SSD) approach provides a probabilistic method to quantify interspecies variation in chemical

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sensitivity and to derive key ecological protection thresholds, such as the hazardous concentration affecting 5% of species (HC_5) and the Predicted No-Effect Concentration (PNEC). The SSD represents the cumulative distribution of species-specific toxicity thresholds and assumes that the tested organisms reflect the sensitivity spectrum of an ecosystem [21]. This approach, endorsed by OECD and ECHA guidance, enables probabilistic estimation of ecological protection levels and has been widely applied in deriving scientifically defensible PNECs [22,23]. Statistical models commonly used for SSD fitting include the "log-logistic", "log-normal", "Weibull", and "gamma" distributions [24]. However, SSD-based HC_5 and PNEC values for 6PPD and 6PPD-Q have not yet been established at a global scale, limiting our ability to assess their ecosystem-level risks quantitatively.

Therefore, this study aims to: (1) review and compile global occurrence data of 6PPD and 6PPD-Q in environmental surface runoff, wastewater treatment plants, and river surface water; (2) develop SSD-based PNECs for both compounds by integrating toxicity data across various taxa; and (3) quantify the ecological risks at regional and global scales. The derived PNECs and risk assessment results of 6PPD and 6PPD-Q will provide a solid scientific foundation for future monitoring and management of tire-derived contaminants.

2. Materials and Methods

2.1. Sampling Data Collection

A systematic literature survey was conducted to compile reported concentrations of 6PPD and 6PPD-Q in various aquatic matrices, including surface runoff, wastewater treatment plants (WWTPs), and river surface waters. Searches were performed across multiple bibliographic databases (Web of Science, ACS Publications, and Google Scholar) using combinations of the following keywords: "6PPD", "6PPD-quinone", "6PPD-Q", "stormwater", "surface water", "runoff", "river", "wastewater treatment plant", and "concentration". Additional references were identified through backward citation tracking (snowballing). Studies were included if they (i) reported quantitative concentrations of 6PPD or 6PPD-Q in environmental samples and (ii) provided sufficient metadata to identify sampling locations and matrices. In total, 21 studies published between 2022 and 2025 were included, yielding 77 valid data points (Tables S1–S3). All concentrations were converted to ng/L for consistency. When multiple values were reported, mean concentrations were preferentially used; if unavailable, median values were selected; and when neither was provided, the average of the maximum and minimum values was adopted. For WWTPs, effluent concentrations were used for spatial visualization. When pre- and post-rainfall data were available, post-rainfall concentrations were selected to represent storm-event exposure. Data were organized by site (Table S4) and continent for content analysis (Table S5), and spatial distributions were mapped using ArcGIS (version 10.2).

2.2. Toxicity Data Collection and SSD Construction

SSDs were developed to derive hazardous concentration (HC) values and estimate predicted PNECs for 6PPD and 6PPD-Q. A total of 991 aquatic toxicity records spanning 34 species were compiled from peer-reviewed literature and the U.S. EPA ECOTOX database with duplicate entries removed (Table S6). The retrieval of toxicity data from peer-reviewed literature followed the same approach as for sampling concentrations, using keywords including "6PPD", "6PPD-quinone", "6PPD-Q", "LC_{50}", "EC_{50}", "No Observed Effect Concentration (NOEC)", "Lowest Observed Effect Concentration (LOEC)", and "toxicity". Notably, all toxicity concentrations were standardized to μg/L to ensure comparability among data from different sources, and all concentration values cited in this study are reported with a precision that remains consistent with the original references, ensuring data fidelity and clarity. SSDs were constructed only when data were available for at least seven taxonomically diverse species representing a minimum of two trophic levels (e.g., algae, invertebrates, and fish) to ensure statistical robustness. All datasets were quality-checked for completeness and normality (Kolmogorov–Smirnov test, $p > 0.05$). Three toxicity endpoint categories, including mortality, growth, and biochemical effects were analyzed to capture both acute and sublethal responses. Mortality-based SSDs were derived from acute LC_{50} or EC_{50} data, whereas growth- and biochemical-based SSDs were constructed using chronic No Observed Effect Concentration (NOEC) or Lowest Observed Effect Concentration (LOEC) values. Geometric means were applied to minimize the influence of outliers, consistent with the assumption of log-normal toxicity distributions [25].

To minimize model-specific bias, SSDs were generated using a model-averaging approach integrating four commonly applied distributions: log-logistic (llogis), log-normal (lnorm), Weibull, and Gamma. The best-fitting models were identified using the Akaike Information Criterion (AIC), and the corrected AIC (AIC_c) was used for small sample sizes (sample size/parameter number < 40) [26]. This model-averaged SSD framework provides a more reliable estimate of species sensitivity and ecological protection thresholds than any single-model approach [27]. The AIC_c values were calculated as follows:

$$AIC_c = 2p - 2\ln(\hat{L}) + \frac{2p(p+1)}{n-p-1} \quad (1)$$

Here, p denotes the number of parameters in the model, and $\hat{L}$ represents the maximum value of the model's likelihood function.

The weight of model m among a total of k models was calculated using the following formula:

$$weight_m = e^{-\frac{1}{2}(\mathrm{AIC_{c,m}}-\mathrm{AIC_{c,min}})} / \sum_{i=1}^{k} e^{-\frac{1}{2}(\mathrm{AIC_{c,}i}-\mathrm{AIC_{c,min}})} \quad (2)$$

Here, $\mathrm{AIC_{c,min}}$ denotes the minimum $\mathrm{AIC_c}$ value among the set of k models.

The model-averaged SSD was calculated by incurporating the weights of the individual models as follows:

$$\widehat{HC_x} = \sum_{m=1}^{k} weight_m HC_{xm} \quad (3)$$

Here, HC_x represents the concentration affecting x% of species in the ecosystem. The predicted no-effect concentration (PNEC) was calculated by dividing the derived HC_5 by an assessment factor (AF) of 5. In SSD-based assessments, the AF typically ranges from 1 to 5 [23,28]. The selection of AF is both a scientific and a policy decision [28]. When uncertainty is high, higher AFs are generally warranted to ensure protective risk estimates [29]. Therefore, AF selection should be based on known sources of uncertainty and variability in the available toxicity data [30]. Considering the substantial variability in toxicity data and the elevated level of concern associated with these two compounds, we conservatively selected an AF of 5, consistent with a previous SSD-based study [23].

2.3. Probabilistic Ecological Risk Assessment of 6PPD and 6PPD-Q

We evaluated the ecological risks of 6PPD and 6PPD-Q using a tiered probabilistic framework comprising three sequential levels: Level 1 (hazard quotient, HQ), Level 2 (overall risk probability, ORP), and Level 3 (distribution-based quotient, DBQ). HQ was calculated as the measured or predicted environmental concentration divided by the PNEC, with risk categories defined as <0.1 (negligible), 0.1–1 (potential), and >1 (significant) [31]. Environmental concentrations were statistically characterized by fitting observed or modeled data to appropriate probability distributions, and the probabilities of exceeding HC_5 and PNEC were derived from cumulative distributions. A joint probability curve (JPC) integrating exposure and sensitivity distributions was constructed, and ORP was obtained as the area under the JPC curve to quantify the probability of adverse effects across the aquatic community, with ORP values of 0.1–1 indicating potential risk and >1 indicating clear risk [23]. To account for uncertainties and variability in exposure and toxicity data, 20,000 Monte Carlo simulations were performed. In each simulation, environmental concentrations and toxicological endpoints were randomly sampled to calculate the distribution-based quotient (DBQ):

$$\mathrm{DBQ} = \frac{C_{\mathrm{exposure}}}{\mathrm{PNEC_{sampled}}} \quad (4)$$

The resulting DBQ probability distribution was used to estimate the probability of exceeding critical thresholds, representing ecological risk. All analyses were conducted in R (v4.4.2), with SSD fitting, simulations, and visualization performed using the "fitdistrplus", "ssdtools", and "ggplot2" packages. The key R scripts are provided in Supplementary Information Text S1, ensuring full reproducibility of the results.

3. Results and Discussion

3.1. Global Concentration Profiles Regarding 6PPD and 6PPD-Q

Both 6PPD and its transformation product 6PPD-Q have been ubiquitously detected in aquatic environments worldwide, with maximum reported concentrations reaching 783 and 2850 ng/L, respectively (Table S1). Of the 24 studies that concurrently quantified both compounds in surface waters, 20 reported higher mean concentrations of 6PPD-quinone than of 6PPD (Tables S1–S4), suggesting that 6PPD undergoes rapid ozonation after entering surface waters [32,33]. Concentration data from these paired samples also revealed a significant positive correlation between 6PPD and 6PPD-Q ($r = 0.641$, $p < 0.01$), suggesting that the formation of 6PPD-Q is directly linked to the availability of 6PPD. These observations are consistent with 6PPD's role as an antiozonant in rubber, where oxidation at the polymer surface depletes local 6PPD and promotes migration of internal 6PPD toward the exterior, thereby sustaining protection against oxidative stress [34]. Compared with surface runoff and riverine waters, 6PPD (0.1–5.3 ng/L) and 6PPD-Q (<MDL–21 ng/L) levels in wastewater treatment plant (WWTP) effluents are relatively low (Table S5), indicating the limited contribution of WWTPs to the environmental occurrence of these compounds [35]. The similar concentration ranges of targeted compounds in surface runoff and rivers further imply that surface runoff is the predominant pathway through which these contaminants are introduced into fluvial systems [2].

For surface runoff and river samples, 6PPD-Q concentrations in North America and Europe were generally higher than those observed in Asia (Figure 1 and Table S5). Notably, 6PPD-Q concentrations reached up to 5100 ng/L in roadway runoff from Los Angeles [7] and 428 ng/L in road surface snow melt from Germany [36]. Such elevated levels may be associated with the high traffic density in certain urban areas and regional differences in the use of 6PPD in tire formulations. Coho salmon (*Oncorhynchus kisutch*), which is highly sensitive to 6PPD-Q toxicity, is native to the Pacific coasts of the United States and Canada. In these regions, reported 6PPD-Q concentrations in rivers (12–2020 ng/L) are generally close to or even exceed the 96-h LC_{50} for coho salmon (95 ng/L). In contrast, concentrations detected in Asian surface rivers were lower (up to 47 ng/L), and Asia is not a primary habitat of this species. These observations indicate that the ecological implications of 6PPD-Q contamination may vary regionally. To date, most studies on

6PPD and 6PPD-Q in surface waters have been conducted in Asia, North America, and Europe, whereas data from South America, Africa, and Oceania remain scarce [37].

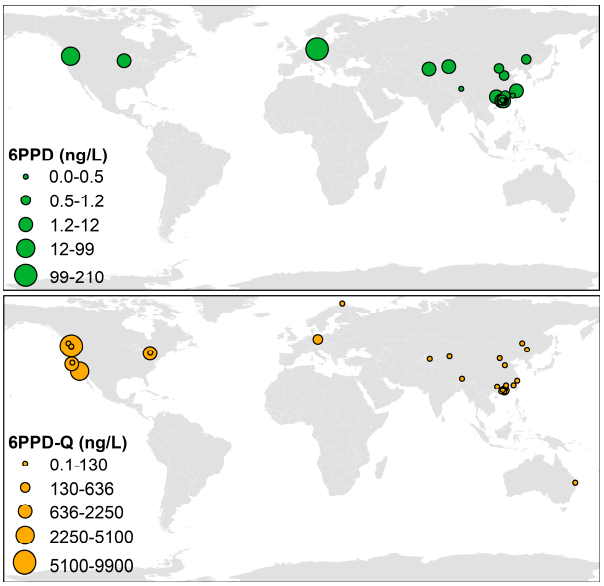

Figure 1. Global distribution of 6PPD and 6PPD-Q in water.

3.2. Toxicity Data of 6PPD Based on Various Endpoints

The raw toxicity data of 6PPD and 6PPD-Q based on various endpoints are exhibited in Table S6. Toxicity information for 6PPD was relatively comprehensive, comprising 60 mortality endpoints, 76 growth-related endpoints, and 301 biochemical endpoints. The corresponding concentration ranges were 5–14,740 μg/L (geometric mean: 360 μg/L), 1–1922.64 μg/L (91.45 μg/L), and 1–1922.64 μg/L (43.15 μg/L), respectively. The geometric mean of mortality data for 6PPD was approximately four times higher than that of growth endpoints and eight times higher than that of biochemical endpoints.

Among the mortality endpoints, *Hyalella azteca*, *Oryzias latipes*, and *Oncorhynchus mykiss* were the most sensitive species, with geometric mean LC_{50} values of 30, 105, and 192 μg/L, respectively, whereas *Pocillopora damicornis* was markedly less sensitive, exhibiting a geometric mean LC_{50} of 5886 μg/L. For growth-related endpoints, *Pelophylax nigromaculatus* and *Daphnia pulex* were identified as the most sensitive species, with geometric mean NOEC/LOEC values of 1 and 10 μg/L, respectively (Table S6). In contrast, *Chlorella pyrenoidosa* showed substantially lower sensitivity, with a geometric mean NOEC/LOEC value of 1552.5 μg/L (Table S6). This represents an inter-species sensitivity spread of over three orders of magnitude, highlighting pronounced variability in toxicological responses across taxa.

For biochemical endpoints, *Pelophylax nigromaculatus* and *Eichhornia crassipes* exhibited the highest sensitivity, with geometric mean NOEC/LOEC values of 1 and 10 μg/L, respectively (Table S6). Similar to the pattern observed for growth, *Chlorella pyrenoidosa* was among the least sensitive species, with a geometric mean NOEC/LOEC of 1922.64 μg/L (Table S6). This taxonomic pattern suggests that primary producers generally exhibit reduced susceptibility to 6PPD compared to higher-trophic organisms. Collectively, these results demonstrate substantial variation in species-specific sensitivity to 6PPD across trophic levels and biological endpoints. Such variability underscores the necessity of incorporating diverse taxa and sub-lethal indicators into ecological risk assessments to avoid underestimating potential impacts on sensitive species and early-warning biological processes.

3.3. Toxicity Data of 6PPD-Q Based on Various Endpoints

For 6PPD-Q, 26 mortality records, 75 growth records, and 173 biochemical records were available, with corresponding concentration ranges of 0.0396 μg/L–308.67 μg/L (geometric mean: 0.98 μg/L), 0.05–7500 μg/L (28.66 μg/L), and 0.01–2000 μg/L (18.01 μg/L), respectively. The geometric mean value for mortality endpoints was approximately three orders of magnitude lower for 6PPD-Q compared to 6PPD, while the geometric means for growth and biochemical endpoints were each roughly three-fold lower. For example, compared with 6PPD, 6PPD-Q induced stronger oxidative stress in *Chlorella vulgaris*, disrupting cell membrane permeability and mitochondrial membrane potential [38], and exerted greater effects on lipid metabolism in male black-spotted frogs [39]. The higher intrinsic toxicity of 6PPD-Q is likely attributable to its quinone moiety, which enhances electrophilicity and enables covalent binding with biological nucleophiles, including DNA, potentially resulting in DNA adducts and mutagenic effects [32]. Additionally, its higher aqueous solubility (log K_{ow} 3.98 vs. 4.47) and greater environmental persistence, 13.5–14.2 d versus 0.7–1.5 d in aerobic soils [40] and 12.8–16.3 d versus 4.83–64.1 h in aqueous solution [41], likely increase bioavailability across multiple taxa, thereby lowering the concentrations at which toxic effects occur. Together, these findings suggest that transformation products such as 6PPD-Q may pose equal or even greater ecological risks than their parent compounds, highlighting the importance of including degradation intermediates in ecological risk assessments.

Regarding mortality, the toxicity of 6PPD-Q varied widely among different fish species upon individual exposure. For example, the relatively tolerant species *Danio rerio* exhibited a geometric mean LC_{50} of 199.4 μg/L, which is approximately three orders of magnitude higher than the most sensitive species, such as *Oncorhynchus kisutch* (0.125 μg/L), *Salvelinus fontinalis* (0.59 μg/L), *Salvelinus leucomaenis pluvius* (0.51 μg/L), *Oncorhynchus clarkii clarkii* (0.112 μg/L), and *Salvelinus namaycush* (0.5 μg/L) (Table S6). Previous studies have reported that, following 96 h of exposure, zebrafish embryos biotransform approximately 50% of 6PPD and 95% of

6PPD-Q. Such a high metabolic capacity may partially account for the pronounced species-specific tolerance of zebrafish embryos toward 6PPD-Q [42]. Among the tested species, the growth and biochemical toxicity data indicated that *Sparus aurata* (geometric mean of 1000 μg/L) and *Sciaenops ocellatus* (449.85 μg/L) were the most tolerant to 6PPD-Q exposure (Table S6). Similarly, *Brachionus koreanus* also exhibited marked tolerance at biochemical endpoints, with a NOEC of 1000 μg/L. In contrast, the salmonids *Oncorhynchus kisutch* (0.3 μg/L) and *Salvelinus namaycush* (2.1 μg/L) were the most sensitive species based on growth-related toxicity endpoints. For biochemical endpoints, *Salvelinus fontinalis* (0.898 μg/L), *Oncorhynchus clarkii clarkii* (0.093 μg/L), and *Ceratophyllum demersum L.* (0.1 μg/L) exhibited the highest sensitivity among the tested species (Table S6). Notably, the SSD curve for 6PPD-Q based on mortality endpoints is shifted to the left relative to those derived from growth and biochemical endpoints (Figure 2). This pattern likely arises from two factors. First, 6PPD-Q can induce rapid lethal effects in salmonids, including respiratory or cardiovascular failure and acute metabolic disruption, resulting in mortality that may occur before detectable changes in chronic endpoints such as growth, reproduction, or enzyme activities [43]. Second, the inclusion of highly sensitive taxa, such as cold-water salmonids, statistically lowers the left tail of the mortality-based SSD, resulting in a reduced HC_5.

Cold-water salmonids such as *Oncorhynchus kisutch*, *Salvelinus namaycush*, *Salvelinus fontinalis*, and *Oncorhynchus clarkii clarkii* responded to 6PPD-Q at sub-microgram concentrations, highlighting the potential of salmonids as emerging model species for monitoring the impacts of environmental changes on aquatic ecosystems [44]. Molecular investigations in *Oncorhynchus kisutch* embryos revealed that 6PPD-Q disrupts vascular integrity by dysregulating pathways involved in endothelial permeability, suggesting blood–brain barrier and cardiovascular system impairment as plausible modes of action [45]. In addition, both 6PPD and 6PPD-Q contain chiral carbon atoms and exist as a pair of enantiomers. Studies have shown that the *S*-enantiomer of 6PPD-Q is 2.6 times more toxic to rainbow trout than the *R*-enantiomer [41]. Therefore, enantioselective metabolism studies in salmonids suggest that the toxicity and biotransformation of 6PPD-Q may differ substantially between its stereoisomers, reflecting protein-binding specificity and toxicokinetic factors that contribute to species sensitivity [46]. Collectively, species-specific sensitivity to 6PPD-Q is likely shaped by both toxicodynamic factors, which determine the chemical's interactions with biological targets, and toxicokinetic processes, which govern its absorption, distribution, metabolism, and excretion in the organism [47]. Further studies are needed to elucidate the mechanisms driving these species-specific toxic responses [6,48].

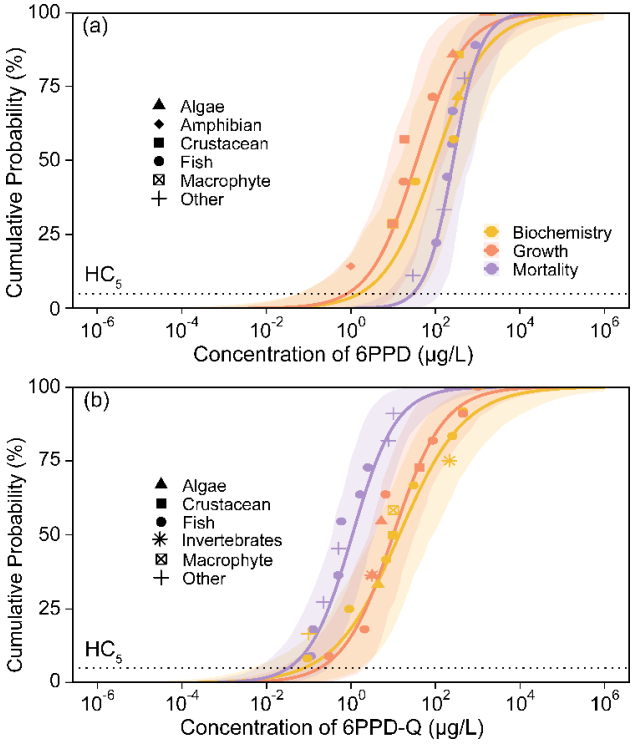

Figure 2. Species sensitivity distribution of 6PPD (**a**) and 6PPD-Q (**b**) based on different endpoints.

3.4. SSD Modeling Based on the Model-Averaging

Table 1 presents various statistics for the SSDs constructed for 6PPD and 6PPD-Q. Model fit was assessed using AICc and ΔAICc, with smaller values indicating better fit [49]. Based on these criteria, the "llogis" distribution provided the best fit to the mortality data for both 6PPD and 6PPD-Q, followed by the "lnorm" distribution. The "gamma" and "weibull" distributions yielded relatively low HC_5 values, likely because smaller shape parameters produce a steeper initial ascent of the SSD curves, which may result in an overly conservative protection level for aquatic ecosystems [22,50]. Therefore, SSDs for 6PPD and 6PPD-Q were constructed using a model-averaging approach, in which weights were assigned to the selected distributions according to their goodness of fit [26].

Based on these results, the mortality-based HC_5 values for 6PPD and 6PPD-Q were 24.33 and 0.0559 μg/L, respectively, and the corresponding mortality-based PNECs were 4.866 and 0.0112 μg/L. In addition, we attempted to predict the chronic mortality of 6PPD-Q using NOEC/LOEC values. The results showed that the HC_5 estimated for chronic mortality was approximately three times higher than the acute lethal concentration. This discrepancy likely reflects the inherent limitations and variability of NOEC/LOEC-based chronic endpoints, highlighting the need for reliable concentration–response data to support SSD construction. For growth data, the "lnorm" distribution provided the best fit for both 6PPD and 6PPD-Q, yielding HC_5 values of 1.622 and 0.932 μg/L, respectively. For biochemical endpoints, the "gamma" distribution was optimal, with corresponding HC_5 values

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of 1.767 and 0.804 μg/L. In both datasets, the HC_5 of 6PPD was approximately twice that of 6PPD-Q. Moreover, the mortality-based HC_5 for 6PPD-Q was approximately one order of magnitude lower than those derived from growth (0.932 μg/L) or biochemical endpoints (0.804 μg/L), and the underlying drivers have been discussed in Section 3.3.

Table 1. SSD parameters of 6PPD and 6PPD-Q based on different toxicity endpoints.

Chemicals	Endpoint	Distribution	N	Mean ± SD (μg/L)	AIC	AICc	ΔAICc	Weight	HC_5 (μg/L))	PNEC (μg/L)
6PPD	morality	llogis	9	917 ± 1881	137	139	0	0.486	29.1	5.81
		lnorm			138	140	0.31	0.415	29.8	5.96
		weibull			141	143	3.18	0.099	6.47	1.29
		gamma			142	144	4.63	0.046	5.72	1.14
		model average							24.3	4.87
6PPD	growth	llogis	7	83.0 ± 512	86	89	0.34	0.297	0.81	0.162
		lnorm			86	89	0	0.353	1.00	0.201
		weibull			87	90	0.88	0.227	0.22	0.0438
		gamma			88	91	2.11	0.123	0.01	0.0028
		model average							1.66	0.332
6PPD	biochemistry	llogis	7	6456 ± 1,454,462	98	101	1.30	0.166	1.54	0.309
		lnorm			97	100	0.89	0.204	1.56	0.311
		weibull			97	100	0.03	0.313	0.88	0.176
		gamma			97	100	0	0.318	0.82	0.164
		model average							1.76	0.352
6PPD-Q	morality	llogis	11	20.3 ± 59.5	59	60	0	0.474	0.032	0.00642
		lnorm			59	60	0.06	0.460	0.041	0.00816
		weibull			63	64	4.18	0.059	0.0031	0.00062
		gamma			67	69	8.48	0.007	0.0005	0.0001
		model average							0.0559	0.0112
6PPD-Q	growth	llogis	11	116 ± 1180	110	112	0.55	0.372	0.571	0.114
		lnorm			110	111	0	0.489	1.377	0.275
		weibull			112	114	2.51	0.139	0.338	0.0676
		gamma			NA	NA	NA	NA	NA	
		model average							0.932	0.186
6PPDQ	biochemistry	llogis	13	715 ± 19,869	146	147	1.52	0.209	0.43	0.086
		lnorm			145	146	0.52	0.344	1.654	0.331
		weibull			144	146	0	0.447	0.324	0.0648
		gamma			NA	NA	NA	NA	NA	
		model average							0.804	0.161

NA indicates data not available.

3.5. Probabilistic Ecological Risk Assessment of 6PPD and 6PPD-Q

The HQ values of 6PPD for mortality, growth, and biochemical endpoints were all below the ecological risk threshold (HQ < 0.05). In contrast, 6PPD-Q exhibited substantially higher risk potential: the proportions of HQ values exceeding the threshold (HQ > 0.1) were 87.2%, 51.2%, and 53.8% for mortality, growth, and biochemical endpoints, respectively, and the proportions exceeding HQ > 1 were 59.0%, 15.4%, and 15.4% (Table 2). Considering the order-of-magnitude differences in HQ values between 6PPD and 6PPD-Q, the combined toxicity risk at each site is predominantly driven by 6PPD-Q. Notably, HQ-Mortality reached 883 in surface runoff in the United States and 183 in river water in Canada. HQ values in wastewater effluents were generally lower than those in surface runoff and river water. Overall, both country and waterbody type influence the HQ values of 6PPD-Q.

For 6PPD, when all concentration data were incorporated into the analysis, the overall risk probability (ORP) derived from mortality, growth, and biochemical endpoints was below the 0.1% risk threshold, indicating that 6PPD poses an acceptable risk to aquatic organisms. In contrast, the ORPs based on mortality, growth, and biochemical endpoints for 6PPD-Q were 11.43%, 4.80%, and 6.41%, respectively (Figure S1 and Table S7). Globally, the ORP values for 6PPD-Q exposure are higher in Europe and North America than in Asia, consistent with regional differences in environmental concentrations (Figure 3a and Table S8). Among water types, the highest risks were observed in surface runoff, followed by river water, whereas effluents from wastewater treatment plants exhibited the lowest risks (Figure 3b and Table S8). Given the substantial variation in DBQ curve behavior across environmental settings (Figure 3c,d), we recommend that individual countries develop watershed-specific regulatory criteria to avoid both overregulation and insufficient protection.

Due to the current scarcity of global environmental data for 6PPD and 6PPD-Q, potential regional variability in toxicity, and inherent limitations of model calculations, uncertainties in risk assessment remain difficult to eliminate even when probabilistic approaches are applied. For example, salmonid species highly sensitive to 6PPD-Q are relatively rare in the Asia-Pacific region, which may lead to overestimation of risk in Asian countries. Therefore, it is necessary to develop localized PNEC values based on regional species toxicity data. Ideally, toxicity datasets should include at least eight species spanning different taxonomic groups. Given the limited availability of such data, the uncertainty in derived toxicity thresholds may be considerable. As new toxicity data become available, SSD

curves should be recalibrated, and HC_5 and PNEC values updated accordingly. In addition, the selection of assessment factors (AFs) involves a degree of subjectivity, which can result in either over- or underestimation of PNEC values. In addition, several 6PPD-Q transformation products identified in the freshwater microalga *Raphidocelis subcapitata*, including TP270, TP312, TP314, and TP328, have been reported to exhibit potentially higher toxicity and environmental risk [51]. Future work should evaluate the toxicity of the broader suite of 6PPD degradation products to fully characterize the potential environmental hazards posed by this class of industrial antioxidants.

Table 2. Hazard quotient (HQ) values of 6PPD-Q in different countries based on mortality, growth, and biochemistry endpoints.

Site	Country	HQ-Mor	HQ-Gro	HQ-Bio	Site	Country	HQ-Mor	HQ-Gro	HQ-Bio
SR1	China	0.13	0.01	0.01	RW1	China	0.21	0.01	0.01
SR2	China	0.10	0.01	0.01	RW2	China	0.15	0.01	0.01
SR3	China	10.89	0.66	0.76	RW3	China	2.74	0.17	0.19
SR4	China	56.79	3.42	3.95	RW4	China	4.17	0.25	0.29
SR5	China	0.14	0.01	0.01	RW5	China	1.86	0.11	0.13
SR6	Norway	11.57	0.70	0.80	RW6	China	3.96	0.24	0.28
SR7	Germany	24.02	1.45	1.67	RW7	China	3.21	0.19	0.22
SR8	Canada	8.93	0.54	0.62	RW8	China	3.38	0.20	0.24
SR9	America	0.44	0.03	0.03	RW9	China	1.92	0.12	0.13
SR11	America	883.93	53.23	61.49	RW10	China	1.16	0.07	0.08
SR12	America	455.36	27.42	31.68	RW11	China	0.63	0.04	0.04
SR13	America	200.89	12.10	13.98	RW12	China	0.02	0.00	0.00
WE1	China	0.00	0.00	0.00	RW13	China	0.83	0.05	0.06
WE2	China	0.19	0.01	0.01	RW14	Canada	7.75	0.47	0.54
WE3	China	0.00	0.00	0.00	RW15	Canada	180.36	10.86	12.55
WE4	China	0.30	0.02	0.02	RW16	Canada	2.13	0.13	0.15
WE5	China	0.08	0.00	0.01	RW17	America	1.09	0.07	0.08
WE6	China	0.15	0.01	0.01	RW18	America	7.98	0.48	0.56
WE7	America	0.33	0.02	0.02	RW19	Australia	1.65	0.10	0.11
WE8	Australia	1.85	0.11	0.13					

Abbreviations: SR, WE, and RW refer to surface runoff, wastewater effluent, and river water, respectively. HQ-Mor, HQ-Gro, and HQ-Bio represent hazard quotient values derived from mortality, growth, and biochemistry endpoints, respectively.

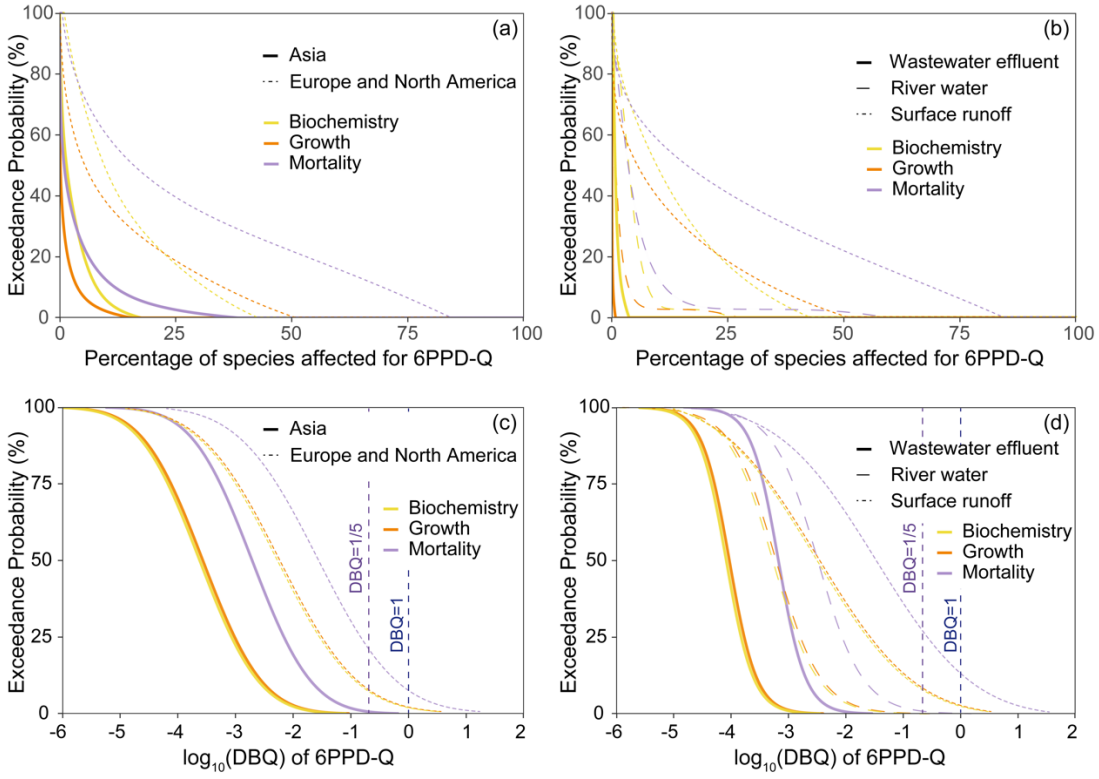

Figure 3. The joint probability curves (**a**,**b**) and exceedance probability of DBQ (**c**,**d**) of 6PPD-Q based on different endpoints. In the legend, black solid and dashed lines represent different regional groups (Asia vs. Europe and North America) or water body types (wastewater effluent, river water, surface runoff), while colored lines denote distinct toxicity endpoints (biochemistry, growth, mortality). Due to limited concentration data from other continents and non-normal exposure distributions, risk comparisons were conducted only between Asia and Europe/North America.

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4. Conclusions

This study developed model-averaged species sensitivity distributions (SSDs) based on published literature and the U.S. EPA ECOTOX database to derive toxicity thresholds for 6PPD and 6PPD-Q and to assess their ecological risks in global surface waters. These results provide scientific support for establishing global pollutant thresholds and informing environmental management. The predicted no-effect concentrations (PNECs) for 6PPD based on mortality, growth, and biochemical endpoints were 4.87, 0.332, and 0.352 μg/L, respectively. For 6PPD-Q, the corresponding PNECs were 0.0112, 0.186, and 0.161 μg/L. Potential high-risk regions were primarily located in Europe and North America, and exposure risks in surface runoff were substantially higher than those in river water. Despite the comprehensive compilation of available data, global exposure concentrations and species-specific toxicity information remain incomplete. In addition, the toxicity of other highly potent transformation products of 6PPD warrants further investigation. Future work should focus on generating more environmental monitoring data and constructing localized SSD curves based on native species to enable region-specific ecological risk assessments.

Supplementary Materials

The additional data and information can be downloaded at: https://media.sciltp.com/articles/others/2601261627012933/GES-25120109-SM-FC1.pdf. Text S1. R scripts for SSD fitting and probabilistic ecological risk assessment. Table S1. Levels of 6PPD and 6PPD-Q in surface runoff around the world. Table S2. Levels of 6PPD and 6PPD-Q in wastewater treatment plants around the world. Table S3. Levels of 6PPD and 6PPD-Q in rivers around the world. Table S4. Compilation of 6PPD and 6PPD-Q concentration (ng/L) data. Table S5. Range of average 6PPD and 6PPD-Q concentrations in various water body types across continents. Table S6. Toxicity data of 6PPD and 6PPD-Q collected from ECOTOX and published literatures. Table S7. ORP of 6PPD and 6PPD-Q around the world based on mortality, growth, and biochemistry. Table S8. ORP of 6PPD-Q based on mortality, growth, and biochemical endpoints in different regions or water types. “SR”, “WE”, and “RW” refer to surface runoff, wastewater effluent, and river water, respectively. Figure S1. The joint probability curves and exceedance probability of DBQ of 6PPD and 6PPD-Q based on different endpoints. References [52–92] are cited in supplementary materials.

Author Contributions

Y.C.: Writing—original draft preparation, Investigation, Conceptualization, Writing—reviewing and editing; Z.-Y.D.: Software, Visualization; X.-Y.L.: Data curation, Investigation; H.-Q.Z.: Investigation; J.-J.W.: Investigation; J.-Y.W.: Investigation; B.P.: Investigation; G.-G.Y.: Supervision; J.-L.Z.: Conceptualization, Methodology, Supervision. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

Not applicable. No original data were generated in this study.

Conflicts of Interest

The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

References

1. Liu, Y.-H.; Mei, Y.-X.; Liang, X.-N.; et al. Small-Intensity Rainfall Triggers Greater Contamination of Rubber-Derived Chemicals in Road Stormwater Runoff from Various Functional Areas in Megalopolis Cities. *Environ. Sci. Technol.* **2024**, *58*, 13056–13064.
2. Liu, Y.-H.; Mei, Y.-X.; Wang, J.-Y.; et al. Precipitation Contributes to Alleviating Pollution of Rubber-Derived Chemicals in Receiving Watersheds: Combining Confluent Stormwater Runoff from Different Functional Areas. *Water Res.* **2024**, *264*, 122240.
3. Chen, X.; He, T.; Yang, X.; et al. Analysis, Environmental Occurrence, Fate and Potential Toxicity of Tire Wear Compounds 6PPD and 6PPD-Quinone. *J. Hazard. Mater.* **2023**, *452*, 131245.
4. Du, B.; Liang, B.; Li, Y.; et al. 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. *Environ. Sci. Technol. Lett.* **2022**, *9*, 1056–1062.
5. Zeng, L.; Li, Y.; Sun, Y.; et al. Widespread Occurrence and Transport of P-Phenylenediamines and Their Quinones in Sediments across Urban Rivers, Estuaries, Coasts, and Deep-Sea Regions. *Environ. Sci. Technol.* **2023**, *57*, 2393–2403.
6. Tian, Z.; Gonzalez, M.; Rideout, C.A.; et al. 6PPD-Quinone: Revised Toxicity Assessment and Quantification with a Commercial Standard. *Environ. Sci. Technol. Lett.* **2022**, *9*, 140–146.

7. Tian, Z.; Zhao, H.; Peter, K.T.; et al. A Ubiquitous Tire Rubber–Derived Chemical Induces Acute Mortality in Coho Salmon. *Science* **2021**, *371*, 185–189.
8. Ge, Y.; Liu, J.; Shi, R.; et al. Environmental Concentrations of 6PPD and 6PPD-Q Cause Oxidative Damage and Alter Metabolism in Eichhornia Crassipes. *Sci. Total Environ.* **2024**, *951*, 175736.
9. Yang, B.; Yang, Q.; Dong, J.; et al. Toxicity of 6PPD to the Stony Coral Pocillopora Damicornis in Different Developmental Stages. *J. Hazard. Mater.* **2025**, *496*, 139214.
10. Peng, W.; Liu, C.; Chen, D.; et al. Exposure to N-(1,3-Dimethylbutyl)-N′-Phenyl-p-Phenylenediamine (6PPD) Affects the Growth and Development of Zebrafish Embryos/Larvae. *Ecotoxicol. Environ. Saf.* **2022**, *232*, 113221.
11. Hua, X.; Feng, X.; Liang, G.; et al. Long-Term Exposure to Tire-Derived 6-PPD Quinone Causes Intestinal Toxicity by Affecting Functional State of Intestinal Barrier in Caenorhabditis Elegans. *Sci. Total Environ.* **2023**, *861*, 160591.
12. Fang, C.; Fang, L.; Di, S.; et al. Characterization of N-(1,3-Dimethylbutyl)-N′-Phenyl-p-Phenylenediamine (6PPD)-Induced Cardiotoxicity in Larval Zebrafish (*Danio rerio*). *Sci. Total Environ.* **2023**, *882*, 163595.
13. Wu, J.; Cao, G.; Zhang, F.; et al. A New Toxicity Mechanism of *N*-(1,3-Dimethylbutyl)-*N*′-Phenyl-*p*-Phenylenediamine Quinone: Formation of DNA Adducts in Mammalian Cells and Aqueous Organisms. *Sci. Total Environ.* **2023**, *866*, 161373.
14. He, W.; Chao, J.; Gu, A.; et al. Evaluation of 6-PPD Quinone Toxicity on Lung of Male BALB/c Mice by Quantitative Proteomics. *Sci. Total Environ.* **2024**, *922*, 171220.
15. Klauschies, T.; Isanta-Navarro, J. The Joint Effects of Salt and 6PPD Contamination on a Freshwater Herbivore. *Sci. Total Environ.* **2022**, *829*, 154675.
16. Cao, G.; Wang, W.; Zhang, J.; et al. Occurrence and Fate of Substituted P-Phenylenediamine-Derived Quinones in Hong Kong Wastewater Treatment Plants. *Environ. Sci. Technol.* **2023**, *57*, 15635–15643.
17. Rauert, C.; Charlton, N.; Okoffo, E.D.; et al. Concentrations of Tire Additive Chemicals and Tire Road Wear Particles in an Australian Urban Tributary. *Environ. Sci. Technol.* **2022**, *56*, 2421–2431.
18. Zhang, R.; Zhao, S.; Liu, X.; et al. Aquatic Environmental Fates and Risks of Benzotriazoles, Benzothiazoles, and p-Phenylenediamines in a Catchment Providing Water to a Megacity of China. *Environ. Res.* **2023**, *216*, 114721.
19. Peter, K.T.; Gilbreath, A.; Gonzalez, M.; et al. Storms Mobilize Organophosphate Esters, Bisphenols, PFASs, and Vehicle-Derived Contaminants to San Francisco Bay Watersheds. *Environ. Sci. Process. Impacts* **2024**, *26*, 1760–1779.
20. Wei, L.-N.; Wu, N.-N.; Xu, R.; et al. First Evidence of the Bioaccumulation and Trophic Transfer of Tire Additives and Their Transformation Products in an Estuarine Food Web. *Environ. Sci. Technol.* **2024**, *58*, 6370–6380.
21. Gredelj, A.; Barausse, A.; Grechi, L.; et al. Deriving Predicted No-Effect Concentrations (PNECs) for Emerging Contaminants in the River Po, Italy, Using Three Approaches: Assessment Factor, Species Sensitivity Distribution and AQUATOX Ecosystem Modelling. *Environ. Int.* **2018**, *119*, 66–78.
22. Yanagihara, M.; Hiki, K.; Iwasaki, Y. Which Distribution to Choose for Deriving a Species Sensitivity Distribution? Implications from Analysis of Acute and Chronic Ecotoxicity Data. *Ecotoxicol. Environ. Saf.* **2024**, *278*, 116379.
23. Zhang, J.; Ye, Y.; Wang, Z.; et al. Derivation of Hexachlorocyclohexane Toxicity Thresholds for Assessing Ecological Risks in the Surface Waters of China. *Water Res.* **2026**, *288*, 124651.
24. Zeng, X.; Yu, J.; Zhang, S.; et al. Ecological Risk of Phenol on Typical Biota of the Northern Chinese River from an Integrated Probability Perspective: The Hun River as an Example. *Env. Monit. Assess.* **2023**, *195*, 1512.
25. Tao, H.; Shi, J.; Zhang, J.; et al. ICE-SSD Model: Bridging the Ecological Risk Assessment Gap between Plasticizer and the Substitute. *ACS EST Water* **2025**, *5*, 727–737.
26. Binet, M.T.; Golding, L.A.; Adams, M.S.; et al. Advantages of Model Averaging of Species Sensitivity Distributions Used for Regulating Produced Water Discharges. *Integr. Environ. Assess. Manag.* **2024**, *20*, 498–517.
27. Fox, D.R.; Van Dam, R.A.; Fisher, R.; et al. Recent Developments in Species Sensitivity Distribution Modeling. *Environ. Toxicol. Chem.* **2020**, *40*, 293–308.
28. Belanger, S.E.; Carr, G.J. SSDs Revisited: Part II—Practical Considerations in the Development and Use of Application Factors Applied to Species Sensitivity Distributions. *Environ. Toxicol. Chem.* **2019**, *38*, 1526–1541.
29. Yang, Y.; Zhang, X.; Jiang, J.; et al. Which Micropollutants in Water Environments Deserve More Attention Globally? *Environ. Sci. Technol.* **2022**, *56*, 13–29.
30. Karjalainen, J.; Hu, X.; Mäkinen, M.; et al. Sulfate Sensitivity of Aquatic Organism in Soft Freshwaters Explored by Toxicity Tests and Species Sensitivity Distribution. *Ecotoxicol. Environ. Saf.* **2023**, *258*, 114984.
31. Yang, Y.; Zhao, X.-M.; Lai, R.W.S.; et al. Decoding Adverse Effects of Organic Contaminants in the Aquatic Environment: A Meta-Analysis of Species Sensitivity, Hazard Prediction, and Ecological Risk Assessment. *Environ. Sci. Technol.* **2024**, *58*, 18122–18132.
32. Jiang, Y.; Wang, C.; Ma, L.; et al. Environmental Profiles, Hazard Identification, and Toxicological Hallmarks of Emerging Tire Rubber-Related Contaminants 6PPD and 6PPD-Quinone. *Environ. Int.* **2024**, *187*, 108677.
33. Yan, X.; Xiao, J.; Kiki, C.; et al. Unraveling the Fate of 6PPD-Q in Aquatic Environment: Insights into Formation, Dissipation, and Transformation under Natural Conditions. *Environ. Int.* **2024**, *191*, 109004.
34. Zhang, P.; Tang, X.; Qin, N.; et al. Advanced Understanding of the Natural Forces Accelerating Aging and Release of Black Microplastics (Tire Wear Particles) Based on Mechanism and Toxicity Analysis. *Water Res.* **2024**, *266*, 122409.

35. Wang, J.; Li, Y.; Nie, C.; et al. Occurrence, Fate and Chiral Signatures of p-Phenylenediamines and Their Quinones in Wastewater Treatment Plants, China. *Water Res.* **2025**, *276*, 123272.
36. Maurer, L.; Carmona, E.; Machate, O.; et al. Contamination Pattern and Risk Assessment of Polar Compounds in Snow Melt: An Integrative Proxy of Road Runoffs. *Environ. Sci. Technol.* **2023**, *57*, 4143–4152.
37. Wang, B.; Sun, W.; Ye, X.; et al. Occurrence, Analytical Methods, and Ecotoxicological Effects of 6PPD-Quinone in Aquatic Environments: A Review. *TrAC Trends Anal. Chem.* **2025**, *193*, 118449.
38. Liu, J.; Yu, M.; Shi, R.; et al. Comparative Toxic Effect of Tire Wear Particle-Derived Compounds 6PPD and 6PPD-Quinone to Chlorella Vulgaris. *Sci. Total Environ.* **2024**, *951*, 175592.
39. Liu, Z.; Feng, Y.; Sun, W.; et al. Environmental Concentrations of 6PPD and 6PPD-Quinone Induce Hepatic Lipid Metabolism Disorders in Male Black-Spotted Frogs. *J. Hazard. Mater.* **2024**, *480*, 136400.
40. Shen, D.; Shi, Q.; Zhang, J.; et al. Transformations of 6PPD and 6PPD-Quinone in Soil under Redox-Driven Conditions: Kinetics, Product Identification, and Environmental Implications. *Environ. Int.* **2025**, *200*, 109532.
41. Di, S.; Liu, Z.; Zhao, H.; et al. Chiral Perspective Evaluations: Enantioselective Hydrolysis of 6PPD and 6PPD-Quinone in Water and Enantioselective Toxicity to Gobiocypris Rarus and Oncorhynchus Mykiss. *Environ. Int.* **2022**, *166*, 107374.
42. Grasse, N.; Seiwert, B.; Massei, R.; et al. Reemtsma, T. Uptake and Biotransformation of the Tire Rubber-Derived Contaminants 6-PPD and 6-PPD Quinone in the Zebrafish Embryo (*Danio rerio*). *Environ. Sci. Technol.* **2023**, *57*, 15598–15607.
43. Greer, J.B.; Dalsky, E.M.; Lane, R.F.; et al. Establishing an *In Vitro* Model to Assess the Toxicity of 6PPD-Quinone and Other Tire Wear Transformation Products. *Environ. Sci. Technol. Lett.* **2023**, *10*, 533–537.
44. Ahi, E.P.; Lindeza, A.S.; Miettinen, A.; et al. Transcriptional Responses to Changing Environments: Insights from Salmonids. *Rev. Fish. Biol. Fish.* **2025**, *35*, 681–706.
45. Greer, J.B.; Dalsky, E.M.; Lane, R.F.; et al. Tire-Derived Transformation Product 6PPD-Quinone Induces Mortality and Transcriptionally Disrupts Vascular Permeability Pathways in Developing Coho Salmon. *Environ. Sci. Technol.* **2023**, *57*, 10940–10950.
46. Li, R.; Barrett, H.; Nair, P.; et al. Enantioselectivity in Metabolism and Toxicity of 6PPD-Quinone in Salmonids. *Environ. Sci. Technol.* **2025**, *59*, 12878–12888.
47. Hiki, K.; Yamamoto, H. The Tire-Derived Chemical 6PPD-Quinone Is Lethally Toxic to the White-Spotted Char *Salvelinus Leucomaenis Pluvius* but Not to Two Other Salmonid Species. *Environ. Sci. Technol. Lett.* **2022**, *9*, 1050–1055.
48. Brinkmann, M.; Montgomery, D.; Selinger, S.; et al. Acute Toxicity of the Tire Rubber-Derived Chemical 6PPD-Quinone to Four Fishes of Commercial, Cultural, and Ecological Importance. *Environ. Sci. Technol. Lett.* **2022**, *9*, 333–338.
49. Jhwueng, D.-C.; Huzurbazar, S.; O'Meara, B.C.; et al. Investigating the Performance of AIC in Selecting Phylogenetic Models. *Stat. Appl. Genet. Mol. Biol.* **2014**, *13*, 459–475.
50. Iwasaki, Y.; Yanagihara, M. Comparison of Model-Averaging and Single-Distribution Approaches to Estimating Species Sensitivity Distributions and Hazardous Concentrations for 5% of Species. *Environ. Toxicol. Chem.* **2025**, *44*, 834–840.
51. Yan, X.; Zhang, Y.; Kiki, C.; Zhao, S.; Zhao, G.; Xiao, J.; Sun, Q. Stepwise Screening with LC-Q/TOF-MS and Signal Subtraction with GC ×GC-TOFMS Enables Rapid Identification of 6PPD-Q and 6PPD Metabolites in Raphidocelis Subcapitata. *J. Hazard. Mater.* **2025**, *495*, 138805.
52. Cao, G.; Wang, W.; Zhang, J.; et al. New Evidence of Rubber-Derived Quinones in Water, Air, and Soil. *Environ. Sci. Technol.* **2022**, *56*, 4142–4150.
53. Zhang, H.-Y.; Huang, Z.; Liu, Y.-H.; et al. Occurrence and risks of 23 tire additives and their transformation products in an urban water system. *Environ. Int.* **2023**, *171*, 107715.
54. Kryuchkov, F.; Foldvik, A.; Sandodden, R.; et al. Presence of 6PPD-quinone in runoff water samples from Norway using a new LC–MS/MS method. *Front. Environ. Chem.* **2023**, *4*, 1194664.
55. Jaeger, A.; Monaghan, J.; Tomlin, H.; et al. Intensive Spatiotemporal Characterization of the Tire Wear Toxin 6PPD Quinone in Urban Waters. *ACS EST Water* **2024**, *4*, 5566–5574.
56. Black, G.P.; De Parsia, M.; Uychutin, M.; et al. 6PPD-quinone in water from the San Francisco-San Joaquin Delta, California, 2018–2024. *Environ. Monit. Assess.* **2025**, *197*, 369.
57. Lane, R.F.; Smalling, K.L.; Bradley, P.M.; et al. Tire-derived contaminants 6PPD and 6PPD-Q: Analysis, sample handling, and reconnaissance of United States stream exposures. *Chemosphere* **2024**, *363*, 142830.
58. Li, Z.-M.; Kannan, K. Mass Loading, Removal, and Emission of 1,3-Diphenylguanidine, Benzotriazole, Benzothiazole, *N*-(1,3-Dimethylbutyl)-*N*′-phenyl-*p*-phenylenediamine, and Their Derivatives in a Wastewater Treatment Plant in New York State, USA. *ACS EST Water* **2024**, *4*, 2721–2730.
59. Geng, N.; Hou, S.; Sun, S.; et al. A Nationwide Investigation of Substituted p-Phenylenediamines (PPDs) and PPD-Quinones in the Riverine Waters of China. *Environ. Sci. Technol.* **2025**, *59*, 3183–3192.
60. Zhu, J.; Guo, R.; Ren, F.; et al. Occurrence and partitioning of p-phenylenediamine antioxidants and their quinone derivatives in water and sediment. *Sci. Total Environ.* **2024**, *914*, 170046.
61. Wei, L.-N.; Wu, N.-N.; Xu, R.; et al. Rainfall, seasonal variation, and stream type governing the multi-media fate and ecological risks of tire additives and their transformation products in mega-urban streams. *Water Res.* **2025**, *287*, 124366.

62. Johannessen, C.; Helm, P.; Lashuk, B.; et al. The Tire Wear Compounds 6PPD-Quinone and 1,3-Diphenylguanidine in an Urban Watershed. *Arch. Environ. Contam. Toxicol.* **2022**, *82*, 171–179.
63. Helm, P.A.; Raby, M.; Kleywegt, S.; et al. Assessment of Tire-Additive Transformation Product 6PPD-Quinone in Urban-Impacted Watersheds. *ACS EST Water* **2024**, *4*, 1422–1432.
64. Halama, J.J.; McKane, R.B.; Barnhart, B.L.; et al. Watershed analysis of urban stormwater contaminant 6PPD-Quinone hotspots and stream concentrations using a process-based ecohydrological model. *Front. Environ. Sci.* **2024**, *12*, 1364673.
65. Zhao, H.N.; Hu, X.; Tian, Z.; et al. Transformation Products of Tire Rubber Antioxidant 6PPD in Heterogeneous Gas-Phase Ozonation: Identification and Environmental Occurrence. *Environ. Sci. Technol.* **2023**, *57*, 5621–5632.
66. Zhou, L.J., Liu, S., Wang, M.; et al. Nationwide Occurrence and Prioritization of Tire Additives and Their Transformation Products in Lake Sediments of China. *Environ. Int.* **2024**, *193*, 109139.
67. Liang, C., Hou, Y.Y., Li, J.Y.; et al. Occurrences, Profiles, And Mass Inventories of Sediment *p*-Phenylenediamine and Their Quinones in the Northern South China Sea. *Mar. Pollut. Bull.* **2025**, *221*, 118482.
68. Gwak, J., Cha, J., Lee, S.; et al. Spatial Distribution, Compositional Profiles, and Potential Ecological Risks of Rubber Additives in Sediments of Lake Sihwa, South Korea: Insights into Industrial and Road-Derived Toxic Substances. *ACS EST Water* **2025**, *5*, 3953–3962.
69. Varshney, S.; Gora, A.H.; Siriyappagouder, P.; et al. Toxicological effects of 6PPD and 6PPD quinone in zebrafish larvae. *J. Hazard. Mater.* **2022**, *424*, 127623.
70. Yan, X.; Kiki, C.; Xu, Z.; et al. Comparative growth inhibition of 6PPD and 6PPD-Q on microalgae Selenastrum capricornutum, with insights into 6PPD-induced phototoxicity and oxidative stress. *Sci. Total Environ.* **2024**, *957*, 177627.
71. Li, X.; Liu, W.; Ge, Y.; et al. Response of *Ceratophyllum demersum* L. and its epiphytic biofilms to 6PPD and 6PPD-Q exposure: Based on metabolomics and microbial community analysis. *J. Hazard. Mater.* **2024**, *480*, 136420.
72. Shi, C.; Wu, F.; Zhao, Z.; et al. Effects of environmental concentrations of 6PPD and its quinone metabolite on the growth and reproduction of freshwater cladoceran. *Sci. Total Environ.* **2024**, *948*, 175018.
73. Hiki, K.; Asahina, K.; Kato, K.; et al. Acute Toxicity of a Tire Rubber-Derived Chemical, 6PPD Quinone, to Freshwater Fish and Crustacean Species. *Environ. Sci. Technol. Lett.* **2021**, *8*, 779–784.
74. Ackerly, K.L.; Roark, K.J.; Lu, K.; et al. Acute toxicity testing of 6PPD-quinone on the estuarine-dependent sport fish, *Sciaenops ocellatus*. *Ecotoxicology* **2024**, *33*, 582–589.
75. Montgomery, D.; Ji, X.; Cantin, J.; et al. Interspecies Differences in 6PPD-Quinone Toxicity Across Seven Fish Species: Metabolite Identification and Semiquantification. *Environ. Sci. Technol.* **2023**, *57*, 21071–21079.
76. Lo, B.P.; Marlatt, V.L.; Liao, X.; et al. Acute Toxicity of 6PPD-Quinone to Early Life Stage Juvenile Chinook (*Oncorhynchus tshawytscha*) and Coho (*Oncorhynchus kisutch*) Salmon. *Environ. Toxicol. Chem.* **2023**, *42*, 815–822.
77. Obanya, H.E.; Couceiro, F.; Ford, A.T.; et al. Toxicological assessment of tyre-derived compounds: Effects of 6PPD-quinone, diphenylguanidine, and mercaptobenzothiazole on *Phaeodactylum tricornutum*. *Aquat. Toxicol.* **2025**, *286*, 107457.
78. Prosser, R.S.; Salole, J.; Hang, S. Toxicity of 6PPD-quinone to four freshwater invertebrate species. *Environ. Pollut.* **2023**, *337*, 122512.
79. Maji, U.J.; Kim, K.; Yeo, I.-C.; et al. Toxicological effects of tire rubber-derived 6PPD-quinone, a species-specific toxicant, and dithiobisbenzanilide (DTBBA) in the marine rotifer *Brachionus koreanus*. *Mar. Pollut. Bull.* **2023**, *192*, 115002.
80. Foldvik, A.; Kryuchkov, F.; Sandodden, R.; et al. Acute Toxicity Testing of the Tire Rubber–Derived Chemical 6PPD-quinone on Atlantic Salmon (*Salmo salar*) and Brown Trout (*Salmo trutta*). *Environ. Toxicol. Chem.* **2022**, *41*, 3041–3045.
81. Calle, L.; Le Du-Carrée, J.; Martínez, I.; et al. Toxicity of tire rubber-derived pollutants 6PPD-quinone and 4-tert-octylphenol on marine plankton. *J. Hazard. Mater.* **2025**, *484*, 136694.
82. Botelho, M.T.; Militão, G.G.; Brinkmann, M.; et al. Toxicity and mutagenicity studies of 6PPD-quinone in a marine invertebrate species and bacteria. *Environ. Mol. Mutagen.* **2023**, *64*, 335–341.
83. Shankar, P.; Dalsky, E.M.; Salzer, J.E.; et al. Evaluation of 6PPD-Quinone Lethal Toxicity and Sublethal Effects on Disease Resistance and Swimming Performance in Coastal Cutthroat Trout (*Oncorhynchus clarkii clarkii*). *Environ. Sci. Technol.* **2025**, *59*, 11505–11514.
84. Philibert, D.; Stanton, R.S.; Tang, C.; et al. The lethal and sublethal impacts of two tire rubber-derived chemicals on brook trout (Salvelinus fontinalis) fry and fingerlings. *Chemosphere* **2024**, *360*, 142319.
85. Anderson-Bain, K.; Roberts, C.; Kohlman, E.; et al. Apical and mechanistic effects of 6PPD-quinone on different life-stages of the fathead minnow (*Pimephales promelas*). *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2023**, *271*, 109697.
86. Huang, Z.; Chen, C.; Guan, K.; et al. Protective role of ghrelin against 6PPD-quinone-induced neurotoxicity in zebrafish larvae (*Danio rerio*) via the GHSR pathway. *Ecotoxicol. Environ. Saf.* **2024**, *285*, 117031.
87. Zhou, H.; Wu, Z.; Wang, X.; et al. 6PPD-quinone exposure induces oxidative damage and physiological disruption in Eisenia fetida: An integrated analysis of phenotypes, multi-omics, and intestinal microbiota. *J. Hazard. Mater.* **2025**, *493*, 138334.
88. Roberts, C.; Kohlman, E.; Jain, N.; et al. Subchronic and Acute Toxicity of 6PPD-Quinone to Early Life Stage Rainbow Trout (*Oncorhynchus mykiss*). *Environ. Sci. Technol.* **2025**, *59*, 6771–6777.

89. Nair, P.; Sun, J.; Xie, L.; et al. Synthesis and Toxicity Evaluation of p-Phenylenediamine-Quinones. *Environ. Sci. Technol.* **2025**, *59*, 7485–7494.
90. Foldvik, A.; Kryuchkov, F.; Ulvan, E.M.; et al. Acute Toxicity Testing of Pink Salmon (*Oncorhynchus gorbuscha*) with the Tire Rubber–Derived Chemical 6PPD-Quinone. *Environ. Toxicol. Chem.* **2024**, *43*, 1332–1338.
91. Roberts, C.; Lin, J.; Kohlman, E.; et al. Acute and Subchronic Toxicity of 6PPD-Quinone to Early Life Stage Lake Trout (*Salvelinus namaycush*). *Environ. Sci. Technol.* **2025**, *59*, 791–797.
92. Di, S.; Xu, H.; Yu, Y.; et al. Environmentally Relevant Concentrations of S-6PPD-Quinone Caused More Serious Hepatotoxicity Than R-Enantiomer and Racemate in Oncorhynchus mykiss. *Environ. Sci. Technol.* **2024**, *58*, 17617–17628.