Table 2-3. Summary of toxicity studies reviewed for the evaluation of human health effects

Chemical	Endpoint	Dose/Duration	Species (number of test subjects)	Key Findings	Reference
6PPD	ADME	Hydrolysis rate of 6PPD in simulated gastric juice	Not indicated	At the 48h observation period, 60% of 6PPD was hydrolyzed. The major hydrolysis product observed was aniline with two minor intermediate hydrolysis products identified: benzoquinone imine-N-phenyl and N-1,3 dimethyl-butylamine p-phenol. hydrolysis rate: -0.0188 half-life: 36.9 hours	Anonymous (1986), as referenced in (ECHA 2023)
6PPD	ADME	Urine biomonitoring was conducted in April 2019 (one spot morning fasting sample per person).	Human (n=151 adults in Quzhou, China; 58% were female; mean age 50 years [male] and 46 years [female])	Nine PPDs, including 6PPD, were measured in human urine samples. Total concentrations of PPDs ranged from 0.41 to 38 ng/mL. 6PPD was detected at the highest concentration (mean 1.2 ng/mL, range < LOD–3.8 ng/mL) and exhibited the highest detection frequency (82%). Female adults exhibited significantly higher mean urinary concentrations than their male counterparts (1.4 ± 0.29 ng/mL compared to 1.0 ± 0.18 ng/mL). In addition, urinary concentrations showed a general decreasing trend with the age of participants. Daily excretion rates for 6PPD were estimated as 34 ng/kg-bw/day (mean value).	(Mao et al. 2024) DOI: 10.1016/j.scitotenv.2024.170045
6PPD	Acute toxicity	In an OECD Guideline 401 (acute toxicity study) male and female Sprague-Dawley rats were exposed to 6PPD in corn oil via oral gavage at the following doses: 0, 250, 500, 1,000, and 2,000 mg/kg-bw.	Sprague-Dawley rat (n=5 per sex per dose)	The acute oral LD_{50} was reported as 893 mg/kg-bw/day for females and 1,005 mg/kg-bw/day for males based on clinical signs and pathological lesions in digestive organs and respiratory system. Clinical signs included reduced volume of feces (500 mg/kg dose for male and females); hypoactivity, diarrhea, bradypnea, hypothermia, and prone position (1,000 mg/kg dose); and abnormal gait, lacrimation, and weakness of limbs (2,000 mg/kg dose). Male rats were less sensitive than female rats.	(Hatano Research Institute 1999), as cited in (ECHA 2023) as "Anonymous, 1999b" and (OECD 2004)
6PPD	Acute toxicity	An acute oral toxicity study was conducted under Good Laboratory Practice according to USEPA OTS 798.1175 (Acute Oral Toxicity). A dose-range finding study was conducted up to 5,000 mg/kg-bw. Based on the range-finding test, a single oral dose of 5,000 mg/kg-bw was tested. Following dosing, the rats were observed daily and weighed weekly.	Sprague-Dawley rat (n=5 per sex per dose)	Based on findings from this study, an acute oral LD_{50} greater than 5,000 mg/kg-bw was estimated for males and females. The basis of the LD_{50} was clinical signs including decreased fecal output, fecal/urine stains, rough coat, piloerection, diarrhea/soft stools, and dark material around the facial area.	Anonymous (1991), as referenced in (ECHA 2023)
6PPD	Acute toxicity	In an acute dermal toxicity study, male and female New Zealand White rabbits were exposed to 2,150, 3,160, 5,010, or 7,940 mg/kg-bw of 6PPD undiluted via semiocclusive dermal exposure for 24 hours.	New Zealand White rabbits	The dermal LD_{50} was reported as >7,940 mg/kg-bw/day based on reduced appetite and reduced activity for three to seven days. At necropsy viscera appeared normal.	Anonymous (1973) and Randall and Bannister (1990), as referenced in (ECHA 2023).
6PPD	Skin irritation	In a dermal irritation study, male and female albino New Zealand White rabbits were exposed to undiluted 6PPD (500 mL warmed to 46°C to liquefy) via semi-occlusive dressings to clipped intact and abraded skin at a concentration of 0.5 mL for 24h. The rabbits were observed for 7 days.	New Zealand White rabbits (n=6)	The mean overall irritation score was reported as 0/8 based on no effects observed.	Anonymous (1973) and Randall and Bannister (1990), as referenced in (ECHA 2023).

Chemical	Endpoint	Dose/Duration	Species (number of test subjects)	Key Findings	Reference
6PPD	Skin irritation	A Draize test was conducted using rabbits (strain not specified) exposed to undiluted 6PPD via occlusive dermal exposure for 24h. The rabbits were observed for 120h.	Unspecified strain of rabbits (n=3)	The mean overall irritation score was reported as 0.6/8 for the 4h observation timepoint. At the 24h observation timepoint, very slight to well-defined erythema was observed with a mean overall irritation score of 1.6. At the 48h observation timepoint, erythema was observed with a mean irritation score of 1.3. At the 72h observation timepoint, very slight erythema was observed with a mean irritation score of 1. All of the observed effects at each time point were fully reversible within 120h.	Anonymous (1962), as referenced in (ECHA 2023).
6PPD	Eye irritation	An undiluted 0.1 mL dose of 6PPD (warmed to 46°C to liquefy) was applied into the conjunctiva sac of the rabbit eye for 24 hours. A 7-day observation period followed the application of the 6PPD.	New Zealand White rabbits	6PPD was slightly irritating to the rabbit eye (irritation mean score at 24h, 48h, and 72h: 1.2/110.0). Effects on the conjunctivae were noted in all animals at 24h (mean score 24h: 5.6/110) but were reversible within 72h.	Anonymous (1973), as referenced in (ECHA 2023).
6PPD	Eye irritation	An undiluted 6PPD (0.1 mL) applied to the eyes of rabbits (strain not specified) for 24h followed by a 5-day post-exposure period.	Unspecified strain of rabbits (n=3)	6PPD was slightly irritating to the rabbit eye (average score of 20.6/110). After 1h, slight edema and erythema, copious discharge and slight dullness of the corneal area were observed. Iris and cornea cleared somewhat in 24h, and within 72h iris clarity was normal. Very slight redness and edema disappeared by the fifth day.	Anonymous (1962), as referenced in (ECHA 2023).
6PPD	Skin sensitization	A guinea pig maximization test was conducted using doses of 50 ppm and 5,000 ppm. Acetone was used as the vehicle for dosing.	Hartley guinea pig, female (n=4)	6PPD was reported as sensitizing to the skin of four female guinea pigs in a guinea pig maximization test. All four guinea pigs showed positive skin reactions for both doses of 6PPD (50 ppm [0.005%] and 5,000 ppm [0.5%]).	(Yamano and Shimizu 2009), as referenced in (ECHA 2023) DOI: 10.1111/j.1600-0536.2008.01500.x
6PPD	Skin sensitization	Female BALB/c mice were exposed to the following doses of 6PPD: 0%, 0.1%, 0.3%, 1%, and 3%.	BALB/c mice (n=4)	6PPD was reported as a positive skin sensitizer in a mouse local lymph node assay. The stimulation index score was reported as 2.34 for the 1% dose and 5.06 for the 3% dose.	(Yamano and Shimizu 2009), as referenced in (ECHA 2023) DOI: 10.1111/j.1600-0536.2008.01500.x
6PPD	Skin sensitization	Repeated insult patch test (method of Shelanski)—induction by 15 repeated treatments followed by one challenge application) or a modified Schwartz patch test	Human	In older studies reported between 1964 and 1997, 6PPD was found to induce dermal sensitization in humans. Positive results were noted in repeated insult patch tests where sensitization was reported for groups of 17/50, 16/50, 4/50, and 5/50 individuals and in modified Schwartz patch tests where sensitization was reported for groups of 3/10, 5/10, 9/10, and 3/5 individuals. In these studies, volunteer subjects had been previously sensitized to rubber samples. Where test subjects (n=50) were not previously exposed to 6PPD, three studies all showed negative patch-test results. In three additional patch test studies, patients with sensitization to 6PPD showed cross-sensitization to other rubber additives.	(OECD 2004) https://hpvchemicals.oecd.org/UI/handler.axd?id=5e1a446c-5969-479c-9270-7ced8726952e.
6PPD	Repeat dose (chronic)	An OECD Guideline 452 (Chronic Toxicity) study was conducted using male and female Sprague-Dawley rats exposed to 0 ppm, 50 ppm, 250 ppm, or 1,500 ppm 6PPD in their feed daily. At the beginning of the experiment all of the rats in the group were dosed with 6PPD. At 12 months of treatment approximately 20 rats/sex/group were sacrificed. After 24 months of treatment all survivors were sacrificed.	Sprague-Dawley rat (n=70)	Treatment-related effects were observed in males and females related to body weight, body weight changes, food consumption and liver weight. Mean body weight was consistently lower in males and females at the high dose (1,500 ppm; 84.8–109.5 mg/kg/day) (mean difference to control −9.9% and −18.4%, respectively) and lower in females in the mid dose (250 ppm; 13.5–16.5 mg/kg/day) (−5.4%). Mean food consumption was increased in males and females at 1,500 ppm (mean difference to control +5.5% and +17.3%, respectively) and in females at 250 ppm (+4.1%). Mean absolute and relative liver weights were increased in males and females after 12 and 24 months in the high-dose groups and in the mid-dose groups at termination. **NOAEL:** 2.6 mg/kg/day in males, 3.2 mg/kg/day females **Basis:** based on reduced body weight in females, increased food consumption in females and increased liver weight at 250 ppm in both sexes (13.5–16.5 mg/kg/day)	Anonymous (1993), as referenced in (ECHA 2023).

Chemical	Endpoint	Dose/Duration	Species (number of test subjects)	Key Findings	Reference
6PPD	Repeat dose (subchronic)	A Good Laboratory Practice-compliant Japanese guideline 28-day repeat dose toxicity study was conducted using male and female Sprague-Dawley rats. Test subjects were exposed to 0, 4, 20, or 100 mg/kg-bw/day of 6PPD followed by a 14-day recovery period. The vehicle was corn oil.	Sprague-Dawley rat (five animals per sex per dose group)	No effects on survival or body weight gain were reported. Relative liver weights were significantly increased in both sexes but were reversible during recovery. In female rats, following recovery, the increase was still significant, and a histopathological liver change was observed. There were no effects on weights or histopathologic findings of any other organs. **NOEL: 4 mg/kg-bw/day** **Basis:** For female rats only. **LOEL: 20 mg/kg-bw/day** **Basis:** Reversible periportal fatty changes of the liver in females without an increase of liver weight and increased total serum protein in females. **NOAEL:** 20 mg/kg-bw/day **Basis:** Based on effects to the liver and hematological and chemistry parameters observed at the high dose (100 mg/kg-bw/day)—reversible periportal fatty changes of the liver in females without an increase of liver weight and increased total serum protein in females. **LOAEL:** 100 mg/kg-bw/day **Basis:** An increase in relative liver weights in both sexes, accompanied by changes in periportal fatty change, clinical chemistry, hematological parameters, indicating an existing anemia.	(OECD 2004) https://hpvchemicals.oecd.org/UI/handler.axd?id=5e1a446c-5969-479c-9270-7ced8726952e.
6PPD	Genotoxicity	*In vitro* OECD Guideline 471(bacterial mutation assay) µg/plate: TA1535, TA1537/-S9mix: up to 19.5; TA98, 100/-S9mix: up to 78.1; TA100, TA1535, TA1537/+S9mix: up to 156; TA98/+S9mix: up to 625 *E. coli:* 313, 625, 1,250, 2,500, 5,000 µg/plate	*S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100 *E. coli* strain WP2uvrA	No genotoxic potential of 6PPD was indicated. 6PPD did not induce gene mutation in *S. typhimurium* tester strains or *E. coli* strain WP2uvrA with and without metabolic activation.	Shibuya T, Hara T, Kawakami K, 1999, as referenced in (ECHA 2023)
6PPD	Genotoxicity	*In vitro* mammalian cell gene mutation assay pre-test: 0.33 to 333 µg/mL, pre-test II −S9: 0.5 to 5 µg/mL, +S9: 1 to 20 µg/mL, main experiment I: −S9 1 to 5 µg/mL, +S9: 1 to 24 µg/mL, main experiment II and III: −S9 1 to 5 µg/mL, +S9: 3 to 15 µg/mL	Chinese hamster ovary	6PPD did not induce mutagenicity in the presence or absence of metabolic activation. The ranges of concentrations for all experiments (including the cytotoxicity-range finding experiments) that induced significant cytotoxicity were 3.3–5 µg/mL, 6–10 µg/mL, 8–10 µg/mL, 9–15 µg/mL and 10–24 µg/mL in the absence of S) and in the presence of 1%, 2%, 5%, and 10% S9, respectively.	Monsanto Chemical Co. (1987) unpublished study Project MSL No. 6346 1/87, as cited in (OECD 2004).
6PPD	Genotoxicity	*In vitro* OECD Guideline 473 (In Vitro Mammalian Chromosome Aberration Test)continuous treatment/-S9mix: 0, 0.0025, 0.0050, 0.010 mg/mL; short-term treatment: −S9-mix: 0, 0.00063, 0.0013, 0.0025 mg/mL; +S9mix: 0, 0.0038, 0.0075, 0.015 mg/mL	Chinese hamster lung cells	A chromosomal aberration test with Chinese hamster lung cells was performed with harvest times of 6 hours (short-term treatment) both with and without metabolic activation (S9-mix) and with harvest times of 24 and 48 hours (continuous treatment) in the absence of a metabolic activation system. No chromosomal aberrations were observed ,with metabolic activation, after 6 hours of exposure to 6PPD. For the continuous treatment, without metabolic activation, the data indicated a dose-dependent increase in chromosomal aberrations after 24 hours (0.005 mg/mL) and 48 hours (0.01 mg/mL). The study authors concluded that 6PPD showed clastogenic activity in CHL cells in vitro. This finding has not been confirmed in vivo (OECD 2004).	Tanaka N, Kusakabe H, Nakagawa Y, Takahasi T, Hashimoto K, 1999. In vitro chromosomal aberration test of N-(1,3- dimethylbutyl)-N'-phenyl-p-phenylenediamine on cultured Chinese hamster cells.Tox Testing Rep Environ Chemicals 7: 537–541, as cited in (OECD 2004).

Chemical	Endpoint	Dose/Duration	Species (number of test subjects)	Key Findings	Reference
6PPD	Genotoxicity	In an in vivo bone marrow chromosome aberration assay, male and female Sprague-Dawley rats were exposed to one dose of 900, 1,300, or 1,790 mg/kg-bw/day (preliminary study) or 1,000 mg/kg-bw/day (main study) of 6PPD.	Sprague-Dawley rat (five rats per sex per dose)	6PPD (1,000 mg/kg-bw/day) did not induce structural chromosomal aberrations to hemopoietic cells of the rat bone marrow. Clinical signs observed at 6h, 18h, and 30h after application in animals treated with 1,000 mg/kg-bw/day were abnormal gait and decreased body tone with most of the rats also exhibiting decreased activity and abnormal stance. Several animals also exhibited piloerection, diarrhea, lacrimation, tremor, body drop, staining of the anal-genital region, and poor grooming around the oral and nasal region. No pharmacotoxic signs were observed in rats administered the vehicle or positive controls except for one male in the 6h corn oil group, which exhibited diarrhea prior to colchicine administration. Results from the 6h, 18h, and 30h sacrifice data show that no statistically significant increase in the frequency of chromosome aberrations compared to control values was seen in the groups treated with 6PPD.	Pharmakon Research International (1988) In Vivo Bone Marrow Cytogenetics Rat Metaphase Analysis PH 315-MO-001-87 (Monsanto PK 87-316), Santoflex 13. Unpublished study of Monsanto Chemical Co. NTIS/OTS 546286, EPA/OTS document # 88-920008736, as cited in (OECD 2004).
6PPD	Carcinogenicity	A two-year OECD Guideline 451 (carcinogenicity) study was conducted using male and female Sprague-Dawley rats exposed to 0, 50 (2.6 mg/kg-bw/day for males and 3.2 mg/kg-bw/day for females), 250 (13.5 mg/kg-bw/day for males and 16.5 mg/kg-bw/day for females), or 1,500 (84.8 mg/kg-bw/day for males and 109.5 mg/kg-bw/day females) ppm of 6PPD via their feed. At the beginning of the experiment, all rats in the group were dosed with 6PPD. At 12 months of treatment, approximately 20 rats/sex/group were sacrificed, and the remaining animals were sacrificed after 24 months.	Sprague-Dawley rat (n=70 per sex)	Slightly non-statistically significant increase in thyroid follicular carcinoma was observed in males of the mid- and high-dose groups (250 ppm and 1,500 ppm). The incidences in males in the mid-dose group (2/69; 2.9%) and high-dose group (3/69; 4.4%) were slightly above the historical control incidences observed in that laboratory (4/501; 0.8%). No increase was observed in females. The mechanism of potential thyroid gland follicular carcinoma was reviewed in literature by the study authors. The findings of the review suggested that the effects on the thyroid gland are most likely to be high-dose secondary effects caused by increased liver activity and that these effects are not directly relevant to humans. Therefore, the study authors concluded that it is unlikely that 6PPD possesses a carcinogenic risk for humans. **NOEL:** 50 ppm in males and females (2.6 mg/kg/day in males, 3.2 mg/kg/day in females) **Basis:** Reduced body weight in females, increased food consumption in females, and increased liver weight at 250 ppm in both sexes (13.5–16.5 mg/kg/day).	Anonymous (1993), as referenced in (ECHA 2023).
6PPD	Carcinogenicity	A two-year chronic feeding study was conducted using male and female CD Outbred Charles River rats exposed to 0, 100, 300, or 1,000 ppm (approx. 0, 8, 23, or 75 mg/kg-bw/day) of 6PPD in their diet.	Charles River (CD Outbred) rat (n=50 per sex)	No treatment-related lesions were identified. **NOAEL (systemic toxicity)**: 300 ppm (23 mg/kg-bw/day) in males and females **Basis:** No adverse effects. **LOAEL (systemic toxicity):** 1,000 ppm (75 mg/kg-bw/day) in males and females **Basis:** Reduced body weight; reduced body weight gain, reduced erythrocyte counts, reduced hemoglobin concentrations, and reduced hematocrit values. **NOAEL (carcinogenicity):** 1,000 ppm (75 mg/kg-bw/day) in males and females **Basis:** Neoplasms found in treated mice compared to control and/or within the historical control range.	Stevens MW, Levinskas GJ, Graham PR, 1981. Chronic toxicity and reproduction studies on rubber antiozonants (substituted paraphenylenediamines). Toxicologist 1: 58, as cited in (ECHA 2023).
6PPD	Carcinogenicity	An in vitro cell transformation assay was conducted using BALB/3T3 cells exposed to 0.165, 0.33, 0.495, 0.66, or 0.99 µg/mL of 6PPD.	BALB/3T3 cells	There was no significant increase in the number of transformed foci; therefore, 6PPD was considered negative for carcinogenicity in this assay.	Litton Bionetics (1982) Evaluation of 8048-69-5 WTR No. 2 6PPD in the in vitro transformation of BALB/3T3 cells assay, final report. Litton Bionetics Inc. Project No.: 20992, unpublished study of Goodyear Tire & Rubber Co., as cited in (OECD 2004).

Chemical	Endpoint	Dose/Duration	Species (number of test subjects)	Key Findings	Reference
6PPD	Reproductive toxicity	An OECD Guideline 443 (extended one-generation reproductive toxicity) study was conducted using male and female Sprague-Dawley rats exposed to 7, 20, or 60 mg/kg-bw/day of 6PPD via oral gavage (corn oil as vehicle). F0 males were dosed orally by gavage for 70 consecutive days prior to mating and continuing through the day prior to euthanasia (for a minimum of 10 weeks). F0 females were dosed orally by gavage for 70 consecutive days prior to mating and continuing throughout mating, gestation, and lactation, through the day prior to euthanasia (following completion of weaning for all F1 litters). The offspring selected for the F1 generation began dosing on the day of weaning until the day prior to euthanasia. All animals were dosed at approximately the same time each day.	Sprague-Dawley rat	No adverse systemic effects were observed in F0 (parental) males and females at dosages up to 60 mg/kg/day (the highest dosage tested). The key effect for reproductive toxicity is dystocia, which was found in two females in the 20 mg/kg/day group and in five females in the 60 mg/kg/day group. No male reproductive effects were observed. No test substance-related effects were found on F1 survival at any dosage level. After weaning, lower body weights and lower body weight gain than controls was observed in the pups. **NOAEL (systemic):** 60 mg/kg/day for females and males. **Basis:** Mortality. **NOAEL (reproductive)**: 7 mg/kg/day for females and 60 mg/kg/day for males **Basis:** Reproductive performance and dystocia for females; reproductive function (sperm measures) and reproductive performance for males. **NOAEL:** 20 mg/kg/day for neonatal toxicity **Basis:** Pup survival, body weight, and weight gain.	Anonymous (2019), as cited in (ECHA 2023).
6PPD	Developmental toxicity	6PPD was administered orally by gavage to three groups of bred Sprague-Dawley female rats. The rats were treated once daily from gestation day 6 through 15. Dose levels of 50, 100 and 250 mg/kg-bw/day were selected. The control group was composed of 25 bred females dosed with corn oil, the vehicle control material.	Sprague-Dawley rat (n=25)	6PPD administered to pregnant rats during the period of major organogenesis, was neither teratogenic nor embryo/fetotoxic at any treatment level. An increase in clinical signs in dams occurred at dose levels of 100 and 250 mg/kg/day in a dose-dependent manner. Clinical signs seen in mid to high doses were salivation prior to dosing, soft stool, diarrhea, decreased defecation, and green fecal discoloration. **NOAEL (maternal toxicity):** 50 mg/kg-bw/day **Basis:** No adverse effects. **LOAEL (maternal toxicity):** 100 mg/kg-bw/day **Basis:** Clinical signs. **NOAEL (teratogenicity):** 250 mg/kg-bw/day **Basis:** No adverse effects at highest dose tested.	Monsanto Chemical Co. (1987) A teratology study in rats with Santoflex 13, as cited in (ECHA 2023).
6PPD	Developmental toxicity	An OECD Guideline 414 (Prenatal Developmental Toxicity) study was conducted using New Zealand White rabbits exposed to 0, 25, 50, or 100 mg/kg-bw/day of 6PPD (vehicle 1% methylcellulose in deionized water) via oral gavage for 28 days (gestation day 7–28 inclusively).	New Zealand White rabbit	Test substance–related abortions were observed at the highest-dose test (100 mg/kg-bw/day). Clinical observations included a thin body, decreased defecation, and/or brown material on various body surfaces. Mean body weight losses, lower mean body weight gains, and lower food consumption were noted at 50 mg/kg/day. In addition, higher maternal liver weights were noted at 50 and 100 mg/kg/day. Test substance–related lower mean fetal body weights (male, female, and combined) were noted in the 50 (39.3 g, 38.6 g, and 39.0 g, respectively) and 100 (33.9 g, 34.6 g, and 35.0 g, respectively) mg/kg/day groups when compared to the control groups. **NOAEL (maternal):** 25 mg/kg-bw/day **Basis:** Body weight and weight gain. **NOAEL (developmental)**: 25 mg/kg-bw/day **Basis:** Fetal/pup body weight changes.	Anonymous (2018). An Oral (Gavage) Prenatal Developmental Toxicity Study of N-1,3-DimethylbutylN'-Phenyl-p-Phenylenediamine in Rabbits, as cited in (ECHA 2023).

Chemical	Endpoint	Dose/Duration	Species (number of test subjects)	Key Findings	Reference
6PPD	Developmental toxicity	In an early teratogenicity study, New Zealand Albino rabbits were exposed to the 0, 10, or 30 mg/kg-bw/day of 6PPD from gestation day 6 to 18 (organogenesis) and sacrificed on day 29 of gestation. The results of a pilot study showing high maternal toxicity at 100 and 300 mg/kg-bw/day determined the doses used.	New Zealand Albino rabbit	The application of 6PPD did not cause test-substance–related increased incidences of external, visceral, or skeletal abnormalities. **NOAEL (maternal toxicity):** 30 mg/kg-bw/day **Basis:** No adverse effects. **NOAEL (teratogenicity):** 30 mg/kg-bw/day **Basis:** No adverse effects.	Anonymous (1976). Teratogenicity study with Santoflex 13 in Albino rats with cover sheets and letter, as cited in (ECHA 2023).
6PPD-q	ADME, repeat dose (subchronic)	Two exposure modes were performed: single and repeated intraperitoneal injections. 0.4 mg/kg-bw, 4 mg/kg-bw. Repeated dose was every 4 days for 28 days	Male BALB/c mice (n=36)	Serum, liver, kidney, lung, spleen, testis, brain, and heart were collected for injury evaluation by organ index, histopathology analysis, and biochemical parameters for liver (ALT, AST, and ALP) and kidney (urea and creatine). Repeated exposure indicated alterations in organs of liver, kidney, lung, spleen, and testis; significant pathological changes in liver, kidney, lung, spleen, testis, and brain; and altered liver and kidney biochemical parameters. Accumulation was highest in the lung (23.09 ± 4.13 ng/g) and liver (19.41 ± 3.37 ng/g), followed by the kidney (10.93 ± 2.04 ng/g). Lesser amounts were accumulated in the spleen, testis, and brain. 6PPD-q was not detected in the heart.	(He, Gu, and Wang 2023) https://doi.org/10.1016/j.scitotenv.2023.164842.
6PPD-q	ADME, neurotoxicity	Testing concentrations of 10 nM (2.98 ng/mL) or 100 nM (29.8 ng/mL) 6PPD-q were used to evaluate the potential to exacerbate Lewy neutrite formation. α-syn PFF and 6PPD-q were added to neurons at 10 days in vitro for 7 days. To determine whether 6PPD-q increased α-syn PFF-induced p-α-synSer129 levels, the authors exposed primary dopaminergic cells to 10 nM or 100 nM 6PPD-q and/or 1 µg/mL of α-syn PFF. When measuring effects on mitochondrial respiration, the cells were exposed to 10 nM or 100 nM 6PPD-q and/or 2 µg/mL of α-syn PFF.	Human (n=24 from Shenzhen, South China) and primary dopaminergic neurons from the ventral midbrain of E13.5 C57BL/6 mice	Cerebrospinal fluid samples from Parkinson's disease patients and non-Parkinson's disease subjects (controls) were analyzed. The average age of all participants was 57.71 years old, and the age of Parkinson's disease patients and the control population did not differ significantly. The Parkinson's disease patients had an average of 7 years of disease. Average concentrations of 6PPD-q were found to be twice as high in Parkinson's disease patients (11.18 ng/mL and 100% detection frequency) compared to controls (5.07 ng/mL and 64% detection frequency). Treatment of the murine neurons at both 10 nM (2.98 ng/mL) and 100 nM (29.8 ng/mL) of 6PPD-q resulted in a dose-dependent decrease in adenosine triphosphate (ATP) production. The neurons demonstrated a decrease in the basal respiratory capacity after treatment with 100 nM 6PPD-q. In addition, 6PPD-q [presumably at 100 nM] caused changes in the concentrations of some components of the citric acid cycle. The study authors found some synergistic effects on mitochondrial membrane potential and mitochondrial processes when pre-seeding the cells with misfolded a-synuclein protein, a hallmark of Parkinson's disease.	(J. Fang et al. 2024) https://doi.org/10.1016/j.jhazmat.2023.133312.
6PPD-q	Reproductive toxicity	L1-larval nematodes were exposed to environmentally relevant concentrations of 0.1, 1 and 10 µg/L 6PPD-q for 4.5 days (until development of adult day-1) to assess long-term exposure.	*C. elegans*: Wild-Type N2 and transgenic strain WS1433 (30 nematodes per treatment)	Reproductive capacity: Exposure to 1 and 10 µg/L 6PPD-q significantly decreased both the number of fertilized eggs in the gonad and the number of hatched eggs. Gonad development: At concentrations of 1 µg/L 6PPD-q the number of mitotic cells per gonad and area of gonad arm were significantly decreased. At concentrations of 10 µg/L 6PPD-q the number of mitotic cells per gonad, length of gonad arm, and area of gonad arm were significantly reduced. The study authors concluded that these results indicated that 6PPD-q exposure caused impairments on gonad development in *C. elegans*. Germline apoptosis: Exposure to 1 and 10 µg/L 6PPD-q significantly increased the induction of germline apoptosis compared to the control. Expressions of *ced-3*, *ced-4* and *egl-1* were also significantly increased, while *ced-9* expression was significantly decreased. Germline DNA damage: Indication of germline DNA damage was also enhanced. Exposure to 1 and 10 µg/L 6PPD-q caused significant increase in the expression of DNA-damage checkpoints genes *clk-2*, *hus-1* and *mrt-2* and an increase in expression of *ced-1* and *ced-6*, which govern the cell corpse engulfment process.	(Hua et al. 2023) https://doi.org/10.1016/j.jhazmat.2023.131495.

Chemical	Endpoint	Dose/Duration	Species (number of test subjects)	Key Findings	Reference
6PPD-q	ADME	Mice were fed with a single human-relevant dose (400 µg/kg) of deuterated D5-6PPD-q dissolved in corn oil by gavage.	To determine a human-relevant dose, human serum samples were collected from 30 healthy volunteers. Participants were selected randomly from the general population without any occupational exposure to 6PPD-q or other rubber chemicals. In total, 30 volunteers, of which 12 were males and 18 were females (mean age 40.5 years, age range 23–66 years), were sampled. Adult male and female ICR mice (7–8 weeks old) (n=4 for each sex).	In human serum, 6PPD-q was found at concentrations ranging from 0.11 to 0.43 ng/mL (mean: 0.21 ng/mL, median: 0.24 ng/mL), which was similar to concentrations measured in adult urine (median: 0.4 ng/mL, geometric mean: 0.43 ng/mL) as reported by Du et al. 2022. 6PPD-q was found to be primarily distributed in the adipose tissue of mice (110.5 ± 13.3 ng/g) followed by the kidney, lung, testis, liver, spleen, heart, and muscle. 6PPD-q was also demonstrated to penetrate the blood–brain barrier of mice within 0.5 h after exposure. Rapid elimination of 6PPD-q in the liver was observed with a half-life ($t_{1/2}$) of 6.7 ± 0.6 h. Half-lives of 6PPD-q in serum, lung, kidney, and spleen of mice were slower, measured at 12.7 ± 0.3 h, 20.7 ± 1.4 h, 21.6 ± 5.3 h, and 20.6 ± 2.8 h, respectively. Possible metabolites of 6PPD-q were screened in mice liver using stable isotope-assisted high-resolution mass spectrometry. Two novel hydroxylated metabolites (D5-6PPD-q-OH and D5-6PPD-q-2OH) were identified. Fecal excretion as compared to urinary excretion was the main excretory pathway for 6PPD-q (35.1 ± 1.9% of the initial dose at one week after administration) and its hydroxylated metabolites.	(Zhang et al. 2024) https://doi.org/10.1016/j.scitotenv.2023.169291.
6PPD or 6PPD-q	ADME, developmental	Urinary Excretion: 4 mg/kg (dissolved in corn oil) via oral gavage in mice aged 10–15 weeks; urine samples collected prior to treatment and at 1, 5 and, 24 hours post-treatment. Maternal Transfer: 4 mg/kg (dissolved in corn oil); pregnant dams treated from E11.5 to E15.5 once per day via oral gavage.	Male/Female C57Bl/6 mice (n=12/group)	Female and male mice exhibited sex difference in excretion profiles of 6PPD and 6PPD-q. Urine concentrations of 6PPD-q (7.9 ± 2.2 ng/mL, 4.3 ± 1.1 ng/mgL and <LOQ at 1h, 5h, and 24h post-treatment, respectively) were one order of magnitude lower than those of 6PPD (45 ± 45 ng/mL, 110 ± 48 ng/mgL, and 5.9 ± 3.6 ng/mL at 1h, 5h, and 24h post-treatment, respectively), suggesting lower excretion and higher bioaccumulation of 6PPD-q. 6PPD concentrations were significantly higher in female urine samples than male samples at 1h and 5h post-treatment. 6PPD-q concentrations were higher in male urine samples measured 1h post-treatment, though the finding lacked statistical significance due to the small sample size. Urine concentrations of 6PPD-q measured in mice were similar to those measured in humans (<LOQ–4.3 ng/mL compared to 0.08–2.9 ng/mL), whereas urine concentrations of 6PPD in mice were two orders of magnitude higher than concentrations measured in humans (5.9 ng/mL compared to 0.015–0.068 ng/mL). The study authors noted that this discrepancy was due to the sample collection and analytical uncertainties. In pregnant mice treated with 6PPD or 6PPD-q from embryonic day 11.5 to 15.5, 6PPD-q showed ~1.5–8 times higher bioaccumulation than 6PPD in placenta, embryo body, and embryo brain, suggesting higher placental transfer of 6PPD-q. Lower concentrations of 6PPD-q and 6PPD were measured in the fetal brain as compared with the dam brain, which indicates partial protection effect of the placenta. Using in vitro dual-luciferase reporter assays, the study authors determined that 6PPD-q activated the human retinoic acid receptor α (RARα) and retinoid X receptor α (RXRα) at concentrations as low as 0.3 µM, which was ~10-fold higher than the concentrations detected in human urine. 6PPD activated the RXRα in a dose-dependent manner at concentrations as low as 1.2 µM.	(Zhao et al. 2023) https://pubs.acs.org/doi/10.1021/acs.est.3c05026

Chemical	Endpoint	Dose/Duration	Species (number of test subjects)	Key Findings	Reference
6PPD or 6PPD-q	Repeat dose (subchronic)	6PPD and 6PPD-q were dissolved in acetone (five parts per thousand by volume) and mixed with corn oil. Mice in the six treatment groups were orally administered 10 mg/kg, 30 mg/kg, or 100 mg/kg 6PPD or 6PPD-q for six weeks	Male C57BL/6 mice (n = 56)	A high dose (100 mg/kg) of 6PPD and 6PPD-q exposure increased liver weights and liver triglyceride levels, indicating that 6PPD and 6PPD-q exposure induced hepatotoxicity in mice. After 6 weeks of treatment, 6PPD and 6PPD-q were detected and measured in treated mouse livers, with measured concentrations generally increasing with dose. Transcriptomic analysis revealed that 6PPD and 6PPD-q induced differential expression of genes mainly enriched in glucolipid metabolism, immune-related, and glutathione metabolism pathways. Therefore, 6PPD and 6PPD-q altered hepatic metabolism in mice. 6PPD-q could induce an immune response by upregulating the transcription of immune-related genes and promoting macrophage infiltration in the liver.	(L. Fang et al. 2023) https://doi.org/10.1016/j.scitotenv.2023.161836
6PPD or 6PPD-q	ADME	N/A	Human (n=150 from Guangzhou, South China; adult [50], children [50], pregnant women [50])	Both 6PPD and 6PPD-q were detected in the urine samples (detection frequency between 60% and 100%, respectively). Urinary 6PPD-q concentrations were significantly higher than those of 6PPD and correlated well with those of 6PPD ($p < 0.01$), indicating coexposure to 6PPD and 6PPD-q in humans. 6PPD-q levels in urine were determined to be 0.40, 0.076, and 2.91 ng/mL in adults, children, and pregnant women, respectively. 6PPD measured in urine was 0.018, 0.015, and 0.068 ng/mL in adults, children and pregnant women, respectively. Women had higher urinary concentrations of 6PPD and 6PPD-q than did men. In pregnant women, median concentrations of 6PPD-q were nearly two orders of magnitude higher than that of 6PPD. In vitro metabolic experiments demonstrated rapid depletion of 6PPD by human liver microsomes, which could be responsible for the lower concentrations of 6PPD in human urine. In all, 65% of 6PPD was metabolized after three hours of incubation, but the transformation ratio of 6PPD-q was less than 2%, which indicates that the biotransformation of 6PPD to 6PPD-q is not the main metabolic pathway for 6PPD in liver metabolism system. Estimated daily excretion rates of 6PPD-q in urine of adults, children, and pregnant women (median 11.3, 2.18, 90.9 ng/kg-bw/day, respectively) were higher than its parent 6PPD (median 0.51, 0.43, and 2.13 ng/kg-bw/day, respectively). Pregnant women had higher daily excretion of 6PPD and 6PPD-q than did adults and children, and the high daily excretion of 6PPD-q in urine of pregnant women was up to 247 ng/kg-bw/day.	(Du et al. 2022) https://doi.org/10.1021/acs.estlett.2c00821

Notes: µg/kg=micrograms per kilogram, µg/mL=micrograms per milliliter, µM=micromolar ADME=absorption, distribution, metabolism, and excretion, ALP=alkaline phosphatase, ALT=alanine aminotransferase, AST=aspartate aminotransferase, ATP=adenosine triphosphate, DNA= deoxyribonucleic acid, LOAEL=lowest observed adverse effect level, mg/kg-bw=milligrams per kilogram of body weight, mg/L=milligrams per liter, ng/mL=nanograms per milliliter, nM=nanomolar, NOAEL=no observed adverse effect level, NOEL= no observed effect level, PFF=preformed fibrils, ppm=parts per million, RARα=retinoic acid receptor α, RXRα=retinoid X receptor α

References

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. 2023. “CLH Report for N-1,3-Dimethylbutyl-N’-Phenyl-p-Phenylenediamine.” Registry of CLH Intentions Until Outcome. Helsinki, Finland: ECHA: European Chemicals Agency. https://echa.europa.eu/documents/10162/2d0a7ea4-0e57-fff1-3412-92c929f4129e.

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.

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.

Hatano Research Institute. 1999. “N-(1,3- Dimethylbutyl)-N’-Phenyl-p-Phenylenediamine – Summary.” Toxicity Testing Reports of Environmental Chemicals 7. https://dra4.nihs.go.jp/mhlw_data/home/file/file793-24-8.html.

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.

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.

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.

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.

Yamano, Tetsuo, and Mitsuru Shimizu. 2009. “Skin Sensitization Potency and Cross-reactivity of *p* -phenylenediamine and Its Derivatives Evaluated by Non-radioactive Murine Local Lymph Node Assay and Guinea-pig Maximization Test.” *Contact Dermatitis* 60 (4): 193–98. https://doi.org/10.1111/j.1600-0536.2008.01500.x.

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.

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,* August. https://doi.org/10.1021/acs.est.3c05026.