CHAPTER 3. TOXICOKINETICS, SUSCEPTIBLE POPULATIONS, BIOMARKERS, CHEMICAL INTERACTIONS

3.1 TOXICOKINETICS

Inhalation exposure is the major route of human exposure to benzene, although oral and dermal exposure can also occur.

- Benzene is readily absorbed following inhalation or oral exposure. Although benzene is also readily absorbed from the skin, much of the benzene applied to the skin evaporates from the skin surface.
- Absorbed benzene is rapidly distributed throughout the body and tends to accumulate in fatty tissues.
- Benzene is metabolized in liver and other tissues, including lymphocyte progenitor cells in bone marrow. Benzene metabolism results in the production of several reactive metabolites that are thought to contribute to benzene toxicity.
- At low exposure levels, benzene is rapidly metabolized and excreted predominantly as conjugated urinary metabolites. At higher exposure levels, metabolic pathways appear to become saturated and a large portion of an absorbed dose of benzene is excreted as parent compound in exhaled air.
- Benzene metabolism appears to be qualitatively similar among humans and various laboratory animal species. However, there are quantitative differences in the relative amounts of benzene metabolites.

3.1.1 Absorption

Inhalation exposure is probably the major route of human exposure to benzene. Several studies have estimated absorption from measurements of respiratory extraction of inhaled benzene (the difference between the concentration of benzene in inhaled and exhaled air (Laitinen et al. 1994; Lindstrom et al. 1994; Nomiyama and Nomiyama 1974a; Srbova et al. 1950; Yu and Weisel 1996). Existing evidence indicates that benzene is rapidly absorbed by humans following inhalation exposure. Results from a study of 23 subjects who inhaled 47–110 ppm benzene for 2–3 hours showed that absorption was highest in the first few minutes of exposure, but decreased rapidly thereafter (Srbova et al. 1950). In the first 5 minutes of exposure, absorption was 70–80%, but by 1 hour, it was reduced to approximately 50% (range, 20–60%). Respiratory extraction (the net amount of benzene removed from inhaled air following inhalation of benzene) in six volunteers including males and females exposed to 52–62 ppm benzene for 4 hours was determined to be approximately 47% (Nomiyama and Nomiyama 1974a). In a similar study, three healthy nonsmoking volunteers were exposed to benzene at levels of 1.6 or 9.4 ppm for 4 hours

(Pekari et al. 1992). The amount of benzene absorbed was estimated from the difference between the concentration inhaled and the concentration exhaled. Respiratory extraction was 48% for the high dose and 52% for the low dose, supporting the evidence of Nomiyama and Nomiyama (1974a). Yu and Weisel (1996) measured the extraction of benzene from inhaled air by three female subjects exposed to benzene in smoke generated by burning cigarettes, which resulted in airborne benzene concentrations in the range of 32–69 ppm. The average extraction for exposure periods of 30 or 120 minutes was 64% and did not appear to be influenced by exposure duration.

In occupational exposure settings, benzene can be absorbed by inhalation and skin (Hostynek et al. 2012). Studies of occupational exposure to benzene suggest that absorption occurs both by inhalation and dermally in many workplace settings. In a study conducted in 1992 in Finland, car mechanics' exposure to benzene was evaluated (Laitinen et al. 1994). Different work phases were measured at five Finnish garages. Blood samples from car mechanics (eight nonsmokers) were taken 3–9 hours after exposure to benzene. The results were approximated to the time point of 16 hours after exposure. Fourteen air samples were taken from the breathing zone and five stationary samples were collected from the middle of the garage for background concentration levels. The average background concentration (stationary samples) of gasoline vapors was 6±7 cm^3/m^3 (2±2 ppm) and the concentration of benzene was under the detection limit of 0.2 cm^3/m^3 (0.1 ppm). The concentrations of benzene in the breathing zone varied from the detection limit of 0.2 cm^3/m^3 to 1.3 cm^3/m^3 (0.1–0.4 ppm) for unleaded gasoline and from the detection limit to 3.7 cm^3/m^3 (1.2 ppm) for leaded gasoline. The highest benzene exposure levels (2.4–3.7 cm^3/m^3 or 0.8–1.2 ppm) were measured when changing the filter to the fuel pump. The mechanics worked without protective gloves, and penetration through the skin was likely. During carburetor renewal and gathering, benzene concentrations were 0.5–1.1 cm^3/m^3 (0.2–0.3 ppm). During changing of the fuel filter to electronic fuel-injection system, benzene concentrations were 0.9–3.4 cm^3/m^3 (0.3–1.1 ppm). The approximated benzene concentrations in blood corresponding to the time point of 16 hours after the exposure showed much higher levels of exposure than could be expected according to the corresponding air measurements (8-hour TWA). The comparison of expected benzene concentrations in blood, if no dermal exposures were present, to the levels at the time point of 16 hours after the exposure showed that the dermal route can be the source of exposure (range 1.1–88.2%). Two of eight workers had minimal exposure through the skin (0–1.1%). The other six workers showed high dermal exposure (79.4%).

Exposure to benzene-contaminated water can also provide an opportunity for both inhalation and dermal absorption. Lindstrom et al. (1994) reported benzene exposures resulting from a showering scenario that occurred in a single-family residence whose water was contaminated with benzene. From 1986 to 1991,

benzene concentrations in water were between 33 and 673 µg/L (ppb). The exposure scenario involved 20-minute showers, air sampling within the bathroom and other rooms, personal breathing zone sampling of the monitoring team, and estimating the benzene doses of the person who showered. The shower scenario indicated exposure to benzene from the showerhead at 185–367 µg/L (ppb), while drain-level samples ranged from below the detectable limit (0.6 µg/L or ppb) to 198 µg/L (ppb). Benzene air concentrations were 758–1,670 µg/m^3 (235–518 ppb) in the shower stall, 366–498 µg/m^3 (113–154 ppb) in the bathroom, 81–146 µg/m^3 (25–45 ppb) in the bedroom, and 40–62 µg/m^3 (12–19 ppb) in the living room. The individual who took a 20-minute shower had estimated inhalation doses of 79.6, 105, and 103 µg (mean=95.9 µg) for 3 consecutive sampling days, which were 2.1–4.9 times higher than corresponding 20-minute bathroom exposures. Another inhalation dose estimate for the showered individual, based on syringe benzene levels, was 113 µg. The average dermal dose was estimated to be 168 µg. The total benzene dose resulting from the shower was estimated to be approximately 281 µg (40% via inhalation and 60% via dermal), suggesting a higher potential exposure to benzene via dermal contact from the water than through vaporization and inhalation. The estimated inhalation and dermal doses reported by Lindstrom et al. (1994) have not been validated by others and are therefore of questionable value for quantitative analysis.

Additional evidence of benzene absorption following inhalation exposure comes from data on cigarette smokers. Benzene levels were significantly higher in the venous blood of 14 smokers (median: 493 ng/L) than in a control group of 13 nonsmokers (median: 190 ng/L) (Hajimiragha et al. 1989). Cigarette smoke is known to contain benzene (Brunnemann et al. 1989; Byrd et al. 1990), and the subjects had no known exposure to other sources of benzene (Hajimiragha et al. 1989). Kok and Ong (1994) reported blood and urine levels of benzene as 110.9 and 116.4 ng/L, respectively, for nonsmokers, and 328.8 and 405.4 ng/L, respectively, for smokers. In a study based on workers at burning oil wells in Kuwait, benzene concentrations in blood were higher in smokers than in nonsmokers in the firefighter group (Etzel and Ashley 1994). Benzene-exposed ceramic workers who smoked had higher concentrations of benzene metabolites in their urine than nonsmokers (Ibrahim et al. 2014).

Benzene released from fires can also be absorbed. The National Association of Medical Examiners Pediatric Toxicology (PedTox) Registry reported blood benzene concentrations of 0.2–4.9 mg/L in eight children who died in fires and were dead at the scene, indicating absorption of benzene from burning materials (Hanzlick 1995). Blood benzene levels taken from U.S. engineers and firefighters working at burning oil wells in Kuwait were compared to blood benzene levels from non-exposed U.S. citizens (Etzel and Ashley 1994). The median concentrations of benzene in whole blood from engineers,

firefighters, and the U.S. reference group were 0.035 µg/L (range: not detected–0.055 µg/L), 0.18 µg/L (range: 0.063–1.1 µg/L), and 0.066 µg/L (range: not detected–0.54 µg/L), respectively. The median concentration in firefighters was generally higher than the median concentration in engineers.

Animal data confirm that benzene is rapidly absorbed through the lungs. Inhalation studies with laboratory dogs indicate that distribution of benzene throughout the animal's body is rapid, with tissue values dependent on blood supply. A linear relationship existed between the concentration of benzene in air (200–1,300 ppm) and the equilibrium concentration in blood (Schrenk et al. 1941). At these exposures, the concentrations of benzene in the blood of dogs exposed to benzene reached a steady state within 30 minutes.

In rodents, the extent of uptake increased linearly with concentration for exposures up to 200 ppm. At concentrations of >200 ppm, zero-order kinetics were observed (i.e., uptake became nonlinear, indicating saturation of the metabolic capacity). The percentage of inhaled benzene that was absorbed and retained during a 6-hour exposure period decreased from 33 to 15% in rats and from 50 to 10% in mice as the exposure concentration was increased from about 10 to 1,000 ppm (Sabourin et al. 1987). When rats and mice were exposed to approximately 300 ppm, mice had greater uptake than rats. Mice and rats had different absorption characteristics; the cumulative inhaled dose in mice was greater than that in rats (Eutermoser et al. 1986; Sabourin et al. 1987). Purebred Duroc-Jersey pigs were exposed to 0, 20, 100, and 500 ppm benzene vapors 6 hours/day, 5 days/week for 3 weeks (Dow 1992). The average concentration of phenol in the urine increased linearly with dose.

In animals, benzene appears to be efficiently absorbed following oral dosing. Oral absorption of benzene was first demonstrated by Parke and Williams (1953). After radiolabeled (^{14}C) benzene was administered orally to rabbits (340–500 mg/kg), the total radioactivity eliminated in exhaled air and urine accounted for approximately 90% of the administered dose, indicating that at least this much of the administered dose was absorbed.

The amount of benzene absorbed in the gastrointestinal tract may vary by species, dose, and dosing vehicle (e.g., food, water, oil). Studies in rats and mice showed that gastrointestinal absorption was greater than 97% in both species when the animals were administered benzene by gavage (in corn oil) at doses of 0.5–150 mg/kg/day (Sabourin et al. 1987). In many animal studies, benzene is administered orally in oil to ensure predictable solubility and dose concentration control. This is unlike the predicted human oral exposure, which is likely to be in drinking water. There are a number of studies in which

benzene has been administered to animals in the drinking water, which more closely resembles predicted human oral exposure (Lindstrom et al. 1994). Although no information was located regarding the extent of oral absorption of benzene in aqueous solutions, it is reasonable to assume that oral absorption from water solutions would be nearly 100%.

The bioavailability of pure as opposed to soil-adsorbed benzene was conducted in adult male rats. Turkall et al. (1988) estimated bioavailability and kinetics of absorption of benzene in rats. Adult male rats received a gavage dose of ^{14}C-benzene, either as benzene alone or adsorbed to clay or sandy soil. Plasma concentrations of radiolabel were monitored for determining absorption half-times and area under the curve (AUC). Absorption half-times were estimated to be 7.1 minutes for benzene, 3.8 minutes for benzene adsorbed to sandy soil, and 6.1 minutes for benzene adsorbed to clay. Peak plasma concentrations and AUCs of radioactivity were higher following dosing with benzene adsorbed to sandy soil or clay, compared to benzene alone.

Studies conducted *in vivo* in humans and *in vitro* using human skin indicate that benzene can be absorbed dermally. Modjtahedi and Maibach (2008) estimated absorption of benzene after direct application of radiolabeled benzene to the forearm or palm of four adults. The mean fractions of applied dose absorbed were 0.13% when applied to the palm and 0.07% when applied to the forearm (estimated from measurements of radiolabel excreted in urine for 7 days following exposure). The above estimates were for benzene applied skin without occlusion to prevent evaporation. Occluding the application site increased absorption of benzene through excised human cadaver skin (Hui et al. 2009; Petty et al. 2011). Dermal absorption is influenced by a variety of factors including evaporation, deposition region, and solvents in which benzene is applied and is higher when applied in water relative to more volatile solvents (Blank and McAuliffe 1985; Gajjar and Kasting 2014; Lodén 1986; Petty et al. 2011).

In vivo experiments on four volunteers, to whom 0.0026 mg/cm^2 of ^{14}C-benzene was applied to forearm skin, indicated that approximately 0.05% of the applied dose was absorbed (Franz 1984). Absorption was rapid, with >80% of the total excretion of the absorbed dose occurring in the first 8 hours after application. Estimation of dermal absorption from radioactivity excreted in urine required an assumption about the fraction of the absorbed dose of radiolabel excreted in urine. A value of 45.3% was estimated from studies conducted in Rhesus monkeys that received a subcutaneous dose of radiolabeled benzene (Franz 1984). The estimate of 0.05% absorbed did not account for evaporation of the applied dose from the skin surface. In another study, 35–43 cm^2 of the forearm was exposed to approximately 0.06 g/cm^2 of liquid benzene for 1.25–2 hours (Hanke et al. 1961). The absorption was estimated from the amount of

phenol eliminated in the urine. The absorption rate of liquid benzene by the skin (under the conditions of complete saturation) was calculated to be approximately 0.4 mg/cm^2/hour. In the same study, absorption from benzene in air was negligible. The estimate of absorption may be biased from sources other than benzene contributing to urinary phenol.

In vitro experiments using human skin support the fact that benzene can be absorbed dermally. An experiment on the permeability of excised human skin with regard to benzene (specific activity 99.8 mCi/mmol; total volume of applied benzene not reported) resulted in the absorption of 0.17 mg/cm^2 after 0.5 hours and 1.92 mg/cm^2 after 13.5 hours (Lodén 1986). Following application of 5, 120, 270, and 520 µL/cm^2 of benzene to human skin, total absorption was found to be 0.01, 0.24, 0.56, and 0.9 µL/cm^2, respectively. The study author indicated that evaporation of benzene did not exceed 5%. When exposure time (i.e., the time to complete evaporation) at each dose was measured and plotted as the ordinate of absorption, total absorption was found to increase linearly with exposure time. The percentage of the applied dose absorbed at each concentration was constant at about 0.2% (Franz 1984).

McDougal et al. (1990) estimated permeability constants of 0.15 and 0.08 cm/hour for rat and human skin, respectively, based on the appearance of benzene in the blood of rats dermally exposed to benzene vapors at a concentration of 40,000 ppm for 4 hours. A physiologically based pharmacodynamic (PBPK) model was used to estimate the permeability of the vapor in rat and human skin. These results indicated that dermal absorption of benzene may be greater in rats than humans.

Adami et al. (2006) applied benzene to human skin *in vitro* and recovered 0.43% of the applied dose in the receptor fluid; a permeability coefficient of 0.000438 cm/hour was determined. Hui et al. (2009a, 2009b) observed dose-related increased absorption of radiolabeled benzene in cleaning solvents through human skin *in vitro* and reported that occlusion increased absorption.

Using results from an *in vitro* study, it was estimated that an adult working in ambient air containing 10 ppm benzene would absorb 7.5 µL/hour from inhalation and 1.5 µL/hour from whole-body (2 m^2) dermal exposure (Blank and McAuliffe 1985). It was also estimated that 100 cm^2 of smooth and bare skin in contact with gasoline containing 5% benzene would absorb 7.0 µL/hour. Diffusion through the stratum corneum was considered the most likely rate-limiting step for dermal absorption because of benzene's low water solubility (Blank and McAuliffe 1985).

Based on an observational study of workers in a tire factory, it was estimated that a worker exposed to benzene as a result of direct skin contact with petroleum naphtha containing 0.5% benzene could absorb 4–8 mg benzene/day through intact skin (Susten et al. 1985). This absorbed amount was compared with an estimated 14 mg of benzene absorbed from inhalation of 1 ppm for an 8-hour day. The estimate for dermal absorption is theoretical since in many facilities the concentration of benzene in rubber solvents such as petroleum naphtha is <0.5% and may be as low as 0.09%.

Benzene is also absorbed dermally by animals. In rhesus monkeys, minipigs, and hairless mice, dermal absorption was <1% following a single direct (unoccluded) application of liquid benzene (Franz 1984; Maibach and Anjo 1981; Susten et al. 1985). As with humans, absorption appeared to be rapid, with the highest urinary excretion of the absorbed dose observed in the first 8 hours following exposure (Franz 1984). Multiple applications, as well as application to stripped skin, resulted in greater skin penetration (Maibach and Anjo 1981). The percentage of absorption of the applied dose of benzene in each of these animals was approximately 2–3-fold higher than that of humans.

Data indicate that soil adsorption decreases the dermal bioavailability of benzene. A study in which male rats were treated dermally with 0.004 mg/cm^2 ^{14}C-benzene, with or without 1 g of clay or sandy soil, reported benzene absorption half-lives of 3.1, 3.6, and 4.4 hours for pure benzene, sandy soil, and clay soil, respectively (Skowronski et al. 1988).

Benzene in air was rapidly absorbed through the skin of hairless mice that were attached to respirators to avoid pulmonary uptake of benzene vapors (Tsuruta 1989). The rate of benzene absorption through the skin increased linearly with dose. The skin absorption rate for 200 ppm was 4.11 nmol/cm^2/hour (0.31 µg/cm^2/hour); at 1,000 ppm, the rate was 24.2 nmol/cm^2/hour (1.89 µg/cm^2/hour), and at 3,000 ppm, the rate was 75.5 nmol/cm^2/hour (5.90 µg/cm^2/hour). The skin absorption coefficient was 0.619 cm/hour.

In an *in vitro* experiment using Fischer 344 rat skin, the partition coefficient for skin:air and skin permeability coefficient were determined at a benzene air concentration of 203 ppm (Mattie et al. 1994). The skin:air partition coefficient was estimated to be 34.5 and the permeability coefficient was estimated to be 1.52 mm/hour.

Based on data for skin absorption of benzene vapors in mice and occupational exposure data, Tsuruta (1989) estimated the ratio of skin absorption rate to pulmonary uptake for humans exposed to benzene to be 0.037. Dermal absorption could account for a relatively higher percentage of total benzene uptake in

occupational settings where personnel, using respirators but not protective clothing, are exposed to high concentrations of benzene vapor.

Modjtahedi and Maibach (2008) reported absorption of benzene through forearm skin and palm of volunteers. Absorption through the forearm skin and palm averaged 0.7 and 0.13%, respectively, of the applied dose, based on recovery in the urine. Fent et al. (2014) reported increased benzene levels in the breath of firefighters exposed to benzene and other substances during controlled structure burns, indicating that some degree of dermal absorption of benzene had occurred because the subjects wore their protective breathing systems during the burns.

3.1.2 Distribution

Information on the distribution of benzene in humans comes primarily from case studies. The data suggest that benzene is distributed throughout the body following absorption into blood. Since benzene is lipophilic, a high distribution to fatty tissue might be expected. Following inhalation exposure to benzene, the chemical has been detected in the biological fluids and tissues of the subjects (Pekari et al. 1992; Tauber 1970; Winek and Collom 1971; Winek et al. 1967). Fluid and tissue levels of benzene have been reported in cases of both accidental and intentional lethal exposures. Levels of 0.38 mg% in blood, 1.38 mg% in the brain, and 0.26 mg% in the liver were reported in a worker who died from exposure to very high air concentrations of benzene (Tauber 1970). Tauber (1970) did not report the exact units of the mg% (i.e., whether it was expressed as mg/100 mL or mg/100 g). An autopsy (time after death not indicated) performed on a youth who died while sniffing reagent-grade benzene revealed benzene concentrations of 2.0 mg% in blood, 3.9 mg% in brain, 1.6 mg% in liver, 1.9 mg% in kidney, 1 mg% in stomach, 1.1 mg% in bile, 2.23 mg% in abdominal fat, and 0.06 mg% in urine (Winek and Collom 1971). Winek and Collom (1971) did not report the exact units of the mg% (i.e., whether it was expressed as mg/100 mL or mg/100 g).

Results from animal studies indicate that absorbed benzene is distributed among several compartments. The parent compound is preferentially stored in the fat, although the relative uptake in tissues also appears to be dependent on the perfusion rate of tissues by blood. Because benzene distributes preferentially to fat, factors that affect body fat content (e.g., sex, lifestage) may affect body burdens and exposure-response relationships for health effects associated with exposure to benzene (Zhang et al. 2020).

Following a 10-minute inhalation exposure of pregnant mice to 2,000 ppm benzene, the parent compound and its metabolites were found to be present in lipid-rich tissues, such as brain and fat, and in well-perfused tissues, such as liver and kidney. Benzene was also found in placentas and fetuses immediately following inhalation of benzene (Ghantous and Danielsson 1986).

Rickert et al. (1979) measured the kinetics of distribution of inhaled benzene in rats. During inhalation exposure of rats to 500 ppm, steady-state concentrations were 11.5 µg/mL in blood, 164.4 µg/g in fat, 37.0 µg/g in bone marrow, 25.3 µg/g in kidney, 15.1 µg/g in lung 9.9 µg/g in liver, 6.5 µg/g in brain, and 4,9 µg/k in spleen. The half-time to steady state in blood was estimated to be 1.4 hours but was considerably less (not measurable) in bone marrow. Half-times to steady state ranged from 0.9 to 2.6 hours for other tissues. Elimination half-time from blood was 0.7 hours and ranged from 0.4 to 1.6 hours (fat) for other tissues. In this same study, benzene metabolites, phenol, catechol, and hydroquinone, were detected in blood and bone marrow following 6 hours of exposure to benzene, with levels in bone marrow exceeding the respective levels in blood. The levels of phenol in blood and bone marrow decreased much more rapidly after exposure ceased than did those of catechol or hydroquinone, suggesting the possibility of accumulation of the latter two compounds.

Benzene was rapidly distributed throughout the bodies of dogs exposed via inhalation to concentrations of 800 ppm for up to 8 hours/day for 8–22 days (Schrenk et al. 1941). Fat, bone marrow, and urine contained about 20 times the concentration of benzene in blood; benzene levels in muscles and organs were 1–3 times that in blood; and erythrocytes contained about twice the amount of benzene found in plasma. During inhalation exposure of rats to 1,000 ppm (2 hours/day for 12 weeks), benzene was stored longer (and eliminated more slowly) in female and male rats with higher body fat content than in leaner animals (Sato et al. 1975).

Benzene was detected in the liver, lung, and blood of rats and mice examined immediately following a 6-hour exposure to benzene vapors at a concentration of 50 ppm (Sabourin et al. 1988). Sabourin and coworkers (Sabourin et al. 1987, 1988) also examined effects, exposure concentration, exposure rate, and route of administration on the comparative metabolism of benzene in rats and mice. Results of these studies are summarized in Section 3.1.3, Metabolism.

No studies were located regarding distribution in humans after oral exposure to benzene.

Low et al. (1989) studied the kinetics of distribution of absorbed benzene in Sprague-Dawley rats. One hour after rats were dosed with 0.15 mg/kg of ^{14}C-benzene (gavage), tissue distribution of radiolabel was highest in liver and kidney, intermediate in blood, and lowest in the Zymbal gland, nasal cavity tissue, and mammary gland. One hour after exposure to 1.5 mg/kg benzene, the highest concentrations of radiolabel were found in bone marrow, kidney, and liver. Elimination of radiolabel from tissues was biphasic with rapid phase half-times ranging from 2.1 hours (blood) to 4.2 hours (kidney), following the 0.15 mg/kg dose. The elimination half-time for bone marrow was 3.2 hours. The slower-phase half-times following the 0.15 mg/kg dose ranged from 11 hours (bone marrow) to 29 hours (blood).

Low et al. (1989) also measured benzene metabolites in tissues. The highest tissue concentrations of benzene's metabolite hydroquinone 1 hour after administration of 15 mg/kg of ^{14}C-benzene (gavage) were in the liver, kidney, and blood, while the highest concentrations of the metabolite phenol were in the oral cavity, nasal cavity, and kidney. The major tissue sites of conjugated metabolites of benzene (phenyl sulphate and hydroquinone glucuronide) were blood, bone marrow, oral cavity, kidney, and liver. Muconic acid was also detected in these tissues. Additionally, the Zymbal gland and nasal cavity were depots for phenyl glucuronide, another conjugated metabolite of benzene. The Zymbal gland is a specialized sebaceous gland and a site for benzene-induced tumors. Therefore, it is reasonable to expect that lipophilic chemicals like benzene would partition readily into this gland. Radiolabel in the Zymbal gland constituted <0.0001% of the administered dose.

The bioavailability of pure as opposed to soil-adsorbed benzene was conducted in adult male rats (Turkall et al. 1988). Animals were gavaged with an aqueous suspension of ^{14}C-benzene alone, or adsorbed to clay or sandy soil. Stomach tissue then fat had the highest amounts of radioactivity 2 hours after exposure in all treatment groups. Tissue concentrations of radiolabel were not statistically different when benzene was administered alone or adsorbed to sandy soil or clay. Elimination half-times of radiolabel from plasma were 806 minutes for benzene alone, 648 minutes for benzene adsorbed to sandy soil, and 82.8 minutes for benzene adsorbed to clay.

No studies were located regarding distribution in humans after dermal exposure to benzene.

A study of male rats treated dermally with 0.004 mg/cm^2 of ^{14}C-benzene, with and without 1 g of clay or sandy soil, revealed soil-related differences in tissue distribution following treatment. The ^{14}C activity (expressed as a percentage of initial dose per g of tissue) 48 hours after treatment with soil-adsorbed benzene was greatest in the treated skin (0.059–0.119%), followed by the kidney (0.024%) and liver

(0.013–0.015%), in both soil groups. In the pure benzene group, the kidney contained the largest amount of radioactivity (0.026%), followed by the liver (0.013%) and treated skin (0.011%) (Skowronski et al. 1988). In all three groups, <0.01% of the radioactivity was found in the following tissues: fat, bone marrow, esophagus, pancreas, lung, heart, spleen, blood, brain, thymus, thyroid, adrenal, testes, untreated skin, and remaining carcass.

3.1.3 Metabolism

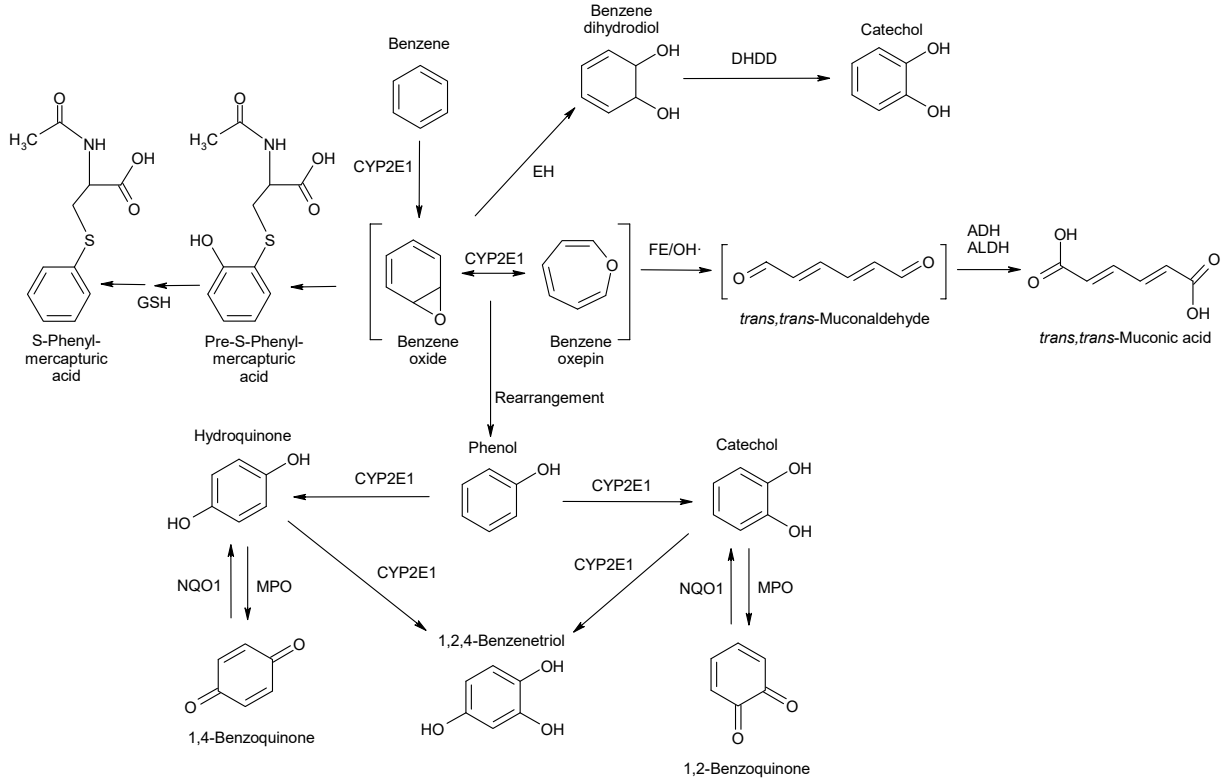
The metabolism of benzene has been studied extensively. It is generally understood that both cancer and noncancer effects are caused by one or more reactive metabolites of benzene. Available data indicate that metabolites produced in the liver are distributed to the bone marrow where benzene toxicity is expressed. Benzene metabolism also occurs in the bone marrow and other tissues.

Data regarding metabolism of benzene in humans are derived primarily from studies using inhalation exposures. Benzene is excreted both unchanged via the lungs and as metabolites (but also as parent compound in small amounts) in the urine. The rate and percentage of excretion via the lungs are dependent on exposure dose and route. Qualitatively, the metabolism and elimination of benzene appear to be similar in humans and laboratory animals (Henderson et al. 1989; Sabourin et al. 1988).

The metabolic scheme shown in Figure 3-1 is based on the results of numerous mechanistic studies of benzene metabolism (Henderson et al. 1989; Ross 1996, 2000). The first step is the CYP2E1-catalyzed oxidation of benzene to form benzene oxide (Lindstrom et al. 1997), which is in equilibrium with benzene oxepin (Vogel and Günther 1967). Several pathways are involved in the metabolism of benzene oxide. The predominant pathway involves nonenzymatic rearrangement to form phenol (Jerina et al. 1968), the major initial product of benzene metabolism (Parke and Williams 1953). Phenol is oxidized in the presence of CYP2E1 to catechol or hydroquinone, which are oxidized via MPO to the reactive metabolites, 1,2- and 1,4-benzoquinone, respectively (Nebert et al. 2002). The reverse reaction (reduction of 1,2- and 1,4-benzoquinone to catechol and hydroquinone, respectively) is catalyzed by NQO1 (Nebert et al. 2002). Both catechol and hydroquinone may be converted to the reactive metabolite, 1,2,4-benzenetriol, via CYP2E1 catalysis. Alternatively, benzene oxide may undergo epoxide hydrolase-catalyzed conversion to benzene dihydrodiol and subsequent dihydrodiol dehydrogenase-catalyzed conversion to catechol (Nebert et al. 2002; Snyder et al. 1993a, 1993b). Other pathways of benzene oxide metabolism that have been proposed include: (1) reaction with glutathione to form PhMA (Nebert et al. 2002; Sabourin et al. 1988; Schafer et al. 1993; Schlosser et al. 1993; Schrenk et al. 1992; van Sittert et al.

1993), and (2) iron-catalyzed ring-opening conversion to *trans*,*trans*-muconic acid, presumably via the reactive *trans*,*trans*-muconaldehyde intermediate (Bleasdale et al. 1996; Nebert et al. 2002; Ross 2000; Witz et al. 1990a, 1990b, 1996). PhMA is formed from the acid-catalyzed dehydration of the *in vivo* pre-phenylmercapturic acid (pre-PhMA) during sample preparation (Bowman et al. 2023; Sabourin et al. 1998).

Figure 3-1. Metabolic Pathways for Benzene



ADH = alcohol dehydrogenase; ALDH = aldehyde dehydrogenase; CYP2E1 = cytochrome P450 2E1; DHDD = dihydrodiol dehydrogenase; EH = epoxide hydrolase; GSH = glutathione; MPO = myeloperoxidase; NQO1 = NAD(P)H:quinone oxidoreductase

Sources: Bowman et al. 2023; Nebert et al. 2002; Ross 2000; Sabourin et al. 1998

Each of the phenolic metabolites of benzene (phenol, catechol, hydroquinone, and 1,2,4-benzenetriol) can undergo sulfonic or glucuronic conjugation (Nebert et al. 2002; Schrenk and Bock 1990; not shown in Figure 3-1). The conjugates of phenol and hydroquinone are major urinary metabolites of benzene (Sabourin et al. 1989a; Wells and Nerland 1991).

Results of several studies provide strong evidence for the involvement of CYP2E1 in the oxidation of benzene. For example, no signs of benzene-induced toxicity were observed in transgenic CYP2E1 knockout mice (that do not express hepatic CYP2E1 activity) following exposure to benzene vapors

(200 ppm, 6 hours/day for 5 days) that caused severe genotoxicity and cytotoxicity in wild-type mice (Valentine et al. 1996a, 1996b). Pretreatment of mice with cytochrome P450 (CYP) inhibitors (toluene, propylene glycol, β-diethyl amino ethyl diphenyl propyl acetate hydrogen chloride [SKF-525A]) has been demonstrated to reduce both benzene metabolite formation (Andrews et al. 1977; Gill et al. 1979; Ikeda et al. 1972; Tuo et al. 1996) and resulting genotoxicity in mice (Tuo et al. 1996). Pretreatment with CYP inducers (3-methylcholanthrene and β-naphthoflavone) increased both benzene metabolism and benzene clastogenicity (Gad-El-Karim et al. 1986).

Other studies provided additional information about CYP2E1 involvement and suggest that other CYPs may metabolize benzene. Immunoinhibition studies in rat and rabbit hepatic microsomes provide additional support to the major role of CYP2E1 in benzene metabolism (Johansson and Ingelman-Sundberg 1988; Koop and Laethem 1992). Polymorphisms of CYP2E1 are associated with changes in urinary metabolites of benzene (Kim et al. 2007a). In human lymphocyte cultures, higher CYP2E1 expression was associated with increased numbers of DNA strand breaks (Zhang et al. 2011). Occupationally exposed workers with a phenotype corresponding to rapid CYP2E1 metabolism were more susceptible to benzene hematotoxicity than workers not expressing this phenotype (Rothman et al. 1997; Ye et al. 2015). *In vitro* studies using human liver microsomes demonstrate a positive correlation between benzene metabolism and CYP2E1 activity (Nedelcheva et al. 1999; Seaton et al. 1994). Although CYP2E1 appears to be the major catalyzing agent in initial benzene metabolism, other CYPs, such as CYP2B1 and CYP2F2, may also be involved (Gut et al. 1996a, 1996b; Powley and Carlson 2000, 2001; Sheets and Carlson 2004; Sheets et al. 2004; Snyder et al. 1993a, 1993b).

Although CYP2E1 is expressed in a variety of tissues, the liver is considered to be the primary site of production of toxic benzene metabolites. Partial hepatectomy diminished both the rate of metabolism of benzene and its toxicity in rats exposed to benzene via subcutaneous injection (Sammett et al. 1979).

Bone marrow of mice, rats, rabbits, and humans also expresses CYP2E1 (Bernauer et al. 1999, 2000; Schnier et al. 1989) and *in vitro* studies have shown that bone marrow obtained from mice, rabbits, and rats can metabolize benzene (Andrews et al. 1979; Ganousis et al. 1992; Gollmer et al. 1984; Irons et al. 1980; Schnier et al. 1989). Bone marrow expresses MPO, which can contribute to production of reactive metabolites in bone marrow, including quinones and semiquinone radicals (Ross et al. 1996; Smith 1999). Bone marrow fibroblasts and macrophages also express glutathione-S-transferase, uridine 5'-diphospho-glucuronosyltransferase (UDP-glucuronyltransferase), and peroxidase activities (Ganousis et al. 1992), which may contribute to metabolism of benzene in bone marrow.

Glutathione and quinone reductases play critical roles in modulating hydroquinone-induced toxicity. Bone marrow stromal cells obtained from rats had higher activities of glutathione reductase and quinone reductase than marrow stromal cells from mice (Zhu et al. 1995).

It remains unclear how much bone marrow, relative to the liver, contributes to the production of reactive intermediates of benzene metabolism in marrow. Irons et al. (1980) demonstrated that the isolated perfused rat femur was capable of metabolizing benzene (approximately 0.0002% of ^{14}C-benzene was recovered as metabolites); however, benzene oxide and phenol were not detected as metabolites of benzene in *in vitro* preparations of microsomes obtained from rat bone marrow (Lindstrom et al. 1999). An interpretation of this observation is that marrow may not be a major contributor to the initial steps in the oxidative metabolism of benzene. No studies were located regarding the potential for human bone marrow tissue to metabolize benzene.

In vitro studies have demonstrated that pulmonary microsomes of humans and laboratory animals are capable of metabolizing benzene, which appears to be catalyzed by both CYP2E1 and CYP2F2 (Powley and Carlson 1999, 2000; Sheets et al. 2004).

Mouse liver microsomes and cytosol have been shown to catalyze ring opening in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) *in vitro*, producing *trans*,*trans*-muconaldehyde, a six-carbon diene dialdehyde also referred to as muconic dialdehyde (Goon et al. 1993; Latriano et al. 1986), a known hematotoxin (Witz et al. 1985) and toxic metabolite of benzene (Henderson et al. 1989).

Metabolism of benzene and *trans,trans*-muconaldehyde in the isolated perfused rat liver indicated that benzene was metabolized to muconic acid, a ring-opened metabolite of benzene (Grotz et al. 1994). *trans,trans*-Muconaldehyde was metabolized to muconic acid and three other metabolites. Furthermore, mouse liver microsomes incubated with benzene produced the following metabolites: phenol, hydroquinone, *trans,trans*-muconaldehyde, *6*-oxo-*trans,trans*-2,4-hexadienoic acid, 6-hydroxy-*trans,trans*-2,4-hexadienal, and 6-hydroxy-*trans,trans*-2,4-hexadienoic acid (Zhang et al. 1995a). β-Hydroxymuconaldehyde, a new metabolite, was also identified.

The precursor of muconic acid is thought to be the precursor of muconic dialdehyde. Zhang et al. (1995b) suggested that *cis,cis*-muconaldehyde is formed first, followed by *cis,trans*-muconaldehyde, and finally

converted to *trans,trans*-muconaldehyde. Muconic dialdehyde has been shown to be metabolized *in vivo* in mice to muconic acid (Witz et al. 1990b).

Small amounts of muconic acid were found in the urine of rabbits and mice that received oral doses of ^{14}C-benzene (Gad-El-Karim et al. 1985; Parke and Williams 1953). The percentage of this metabolite formed varied with the administered benzene dose and was quite high at low doses (17.6% of 0.5 mg/kg benzene administered to C57BL/6 mice) (Witz et al. 1990b). Other studies in animals support these results (Brondeau et al. 1992; Ducos et al. 1990; McMahon and Birnbaum 1991; Sabourin et al. 1989a; Schad et al. 1992).

The muconic acid pathway also appears to be active in humans (Bechtold and Henderson 1993; Ducos et al. 1990, 1992; Lee et al. 1993; Melikian et al. 1993, 1994). Muconic acid has been detected in urine from male and female smokers and nonsmokers (Melikian et al. 1994). Melikian et al. (1994) also found that the amount of muconic acid produced varied by sex, pregnancy status, and smoking level. Because of its relative importance in benzene toxicity, additional modeling studies have been conducted to further describe how *trans,trans*-muconaldehyde is transformed to muconic acid (Bock et al. 1994).
A study of 131 participants showed that the median *trans,trans*-muconic acid concentration among smokers was approximately 2.5 times higher compared to nonsmokers, and participants who smoked >20 cigarettes per day had a higher median *trans,trans*-muconaldehyde concentration compared to participants who smoked no more than 10 or 11–20 cigarettes per day (Buratti et al. 1996). A study of 136 smokers showed that urinary *trans,trans*-muconaldehyde concentrations were correlated with cigarettes smoked per day (Taniguchi et al. 1999). In a study of 177 participants in Iran's Golestan Province with a high incidence of cancer, cigarette and hookah smokers had significantly higher levels of *trans,trans*-muconic acid, with median creatinine-adjusted levels of 65.1 and 82.4μg/g, respectively, than nonsmokers who had a median level of 30.2 μg/g (Bhandari et al. 2023).

Kenyon et al. (1995) compared urinary metabolites in B6C3F1 mice after oral dosing with phenol to results of Sabourin et al. (1989a) who administered a comparable oral dose of benzene to B6C3F1 mice. Phenol administration resulted in lower urinary levels of hydroquinone glucuronide and higher levels of phenol sulfate and phenol glucuronide (Kenyon et al. 1995) compared to benzene administration (Sabourin et al. 1989a). Kenyon et al. (1995) hypothesized that the differences in the urinary metabolite profiles between phenol and benzene after oral dosing were due to zonal differences in the distribution of metabolizing enzymes within the liver.

Conjugating enzymes are more concentrated in the periportal area of the liver, the first region to absorb the compound, whereas oxidizing enzymes are more concentrated in the pericentral region of the liver. Based on this hypothesis, during an initial pass through the liver after oral administration, phenol would have a greater opportunity to be conjugated as it was absorbed from the gastrointestinal tract into the periportal region of the liver, thus resulting in less free phenol being delivered into the pericentral region of the liver to be oxidized. With less free phenol available for oxidation, less hydroquinone would be produced, relative to conjugated phenol metabolites.

In contrast to phenol metabolism, benzene must be oxidized before it can be conjugated. Therefore, metabolism of benzene would be minimal in the periportal region of the liver, with most of the benzene reaching the pericentral region to be oxidized to hydroquinone. Based on this scheme, the study authors suggested that benzene administration would result in more free phenol being delivered to oxidizing enzymes in the pericentral region of the liver than administration of phenol itself (Kenyon et al. 1995).

Benzene has been found to stimulate its own metabolism, thereby increasing the rate of toxic metabolite formation. Pretreatment of mice, rats, and rabbits subcutaneously with benzene increased benzene metabolism *in vitro* without increasing CYP2E1 concentrations (Arinc et al. 1991; Gonasun et al. 1973; Saito et al. 1973). In contrast, there was no significant effect on the metabolism of benzene when Fischer 344 rats and B6C3F1 mice, pretreated with repeated inhalation exposure to 600 ppm of benzene, were again exposed to 600 ppm benzene (Sabourin et al. 1990).

The rate of benzene metabolism can be altered by pretreatment with various compounds. Benzene is a preferential substrate of CYP2E1, which also metabolizes ethanol. CYP2E1 can be induced by these substrates and is associated with the generation of hydroxyl radicals, probably via cycling of the cytochrome (Chepiga et al. 1991; Parke 1989; Snyder et al. 1993a, 1993b). It is possible that hydroxy radical formation by CYP2E1 may play a role in the benzene ring-opening pathway, leading to the formation of *trans,trans*-muconaldehyde. Phenol, hydroquinone, benzoquinone, and catechol have also been shown to induce CYPs in human hematopoietic stem cells (Henschler and Glatt 1995). Therefore, exposure to chemicals that stimulate the activity of this enzyme system prior to exposure to benzene could increase the rate of benzene metabolism.

Both NADPH-linked and ascorbate-induced lipid peroxidation activities induced *in vitro* were lowered 5.5 and 26%, respectively, in rats following oral administration of 1,400 mg/kg/day of benzene for 3 days, followed by intraperitoneal injection of phenobarbital. These results suggest that benzene alters hepatic

drug metabolism and lipid peroxidation. The decrease in lipid peroxidation could be due to the antioxidant property of the metabolites (Pawar and Mungikar 1975).

The ultimate disposition and metabolic fate of benzene depends on animal species, dose, and route of exposure. The dose of benzene affects both the total metabolism and the concentrations of individual metabolites formed. In mice, the percentage of hydroquinone glucuronide decreased as the dose increased. In both rats and mice, the percentage of muconic acid decreased as the dose increased. The shift in metabolism may affect the dose-response relationship for toxicity and has been observed in all animal species studies thus far (Sabourin et al. 1989a, 1992; Witz et al. 1990a, 1990b).

Species differences in benzene metabolism have been observed. Mice have a higher minute volume per kg body weight than rats (1.5 times higher) when benzene is inhaled. As a result, equilibrium between the concentration of benzene in inhaled air and blood was more rapid in mice than rats, although the steady-state level in blood was not influenced (Sabourin et al. 1987). Benzene metabolism is saturable in mice and rats; however, rats have a higher capacity to metabolize benzene than mice (Sabourin et al. 1987). In this study, complete saturation of benzene metabolism occurred in mice at oral doses >50 mg/kg, whereas rats continued to metabolize benzene at oral doses >50 mg/kg.

Species differences in benzene metabolism following oral exposure were observed in rats and mice administered benzene by gavage at doses of 0.5–150 mg/kg/day (Sabourin et al. 1987). Metabolism was dose dependent. For rats and mice, doses <15 mg/kg, >90% of the benzene was metabolized, while at doses >15 mg/kg, an increasing percentage of orally administered benzene was exhaled unmetabolized. Additionally, total metabolites per unit body weight were equal in rats and mice at doses up to 50 mg/kg/day. However, total metabolites in mice did not increase at doses >50 mg/kg/day, suggesting saturation of metabolic pathways (Sabourin et al. 1987).

The integrated dose to a tissue over a 14-hour period (6-hour exposure, 8 hours following exposure) was calculated for benzene metabolites in rats and mice that were exposed to 50 ppm of radiolabeled (^{3}H) benzene (Sabourin et al. 1988). The major metabolic products in rats were detoxification products that were marked by phenyl conjugates. In contrast, mice had substantial quantities of the markers for toxification pathways (muconic acid, hydroquinone glucuronide, and hydroquinone sulfate) in their tissues. Muconic acid and hydroquinone glucuronide were also detected in mouse bone marrow.

In vitro studies of benzene metabolism by mouse and rat liver microsomes also indicate species differences in benzene metabolism (Schlosser et al. 1993). Quantitation of metabolites from the microsomal metabolism of benzene indicated that after 45 minutes, mouse liver microsomes from male B6C3F1 mice had converted 20% of the benzene to phenol, 31% to hydroquinone, and 2% to catechol. In contrast, rat liver microsomes from male Fischer 344 rats converted 23% to phenol, 8% to hydroquinone, and 0.5% to catechol. Mouse liver microsomes continued to produce hydroquinone and catechol for 90 minutes, whereas rat liver microsomes had ceased production of these metabolites by 90 minutes. Muconic acid production by mouse liver microsomes was <0.04 and <0.2% from phenol and benzene, respectively, after 90 minutes.

There are qualitative and quantitative differences in rodent benzene metabolism. Benzene metabolism has been studied in isolated hepatocytes (Orzechowski et al. 1995). In this study, mouse hepatocytes incubated with benzene produced two metabolites (1,2,4-trihydroxybenzene sulfate and hydroquinone sulfate) that were not found in rat hepatocyte incubations. These sulfate metabolites were also produced by mouse hepatocytes incubated with the benzene metabolites, hydroquinone and 1,2,4-benzenetriol. Mouse hepatocytes were almost 3 times more effective in metabolizing benzene, compared to rat hepatocytes. This difference was accounted for in the formation of hydroquinone, hydroquinone sulfate, and 1,2,4-trihydroxybenzene sulfate.

Sabourin et al. (1988) compared metabolites of benzene in tissues of Fischer 344 rats and B6CF1 mice following exposure to 50 ppm benzene. In rats, phenol, catechol, and hydroquinone were not detected in in the liver, lungs, and blood. The major water-soluble metabolites in rat tissues were muconic acid, phenyl sulfate, prephenyl mercapturic acid, and an unknown metabolite. The unknown was present in amounts equal to the amounts of phenyl sulfate in the liver; phenyl sulfate and the unknown were the major metabolites in the liver. In contrast to rats, phenol and hydroquinone were detected in the liver, lungs, and blood of mice, and catechol was detected in the liver, but not in the lungs or blood. As in the rat, the unknown was present in amounts equal to the amounts of phenyl sulfate in the liver. Mice had more muconic acid in the liver indicating a greater risk for toxicity than from phenyl conjugated metabolites, which are less toxic and water soluble (Sabourin et al. 1988).

The effect of exposure rate on benzene metabolism was studied in Fischer 344 rats and B6C3F1 mice that were exposed to several different combinations of concentrations and durations that resulted in the same total amount of benzene (Sabourin et al. 1989a). The benzene inhalation exposures evaluated in the study were 600 ppm for 0.5 hour, 150 ppm for 2 hours, or 50 ppm for 6 hours. In rats, the area under the curve

(AUC) for water-soluble metabolites in tissues (blood, liver, lung) was not affected by the benzene exposure rate. However, in mice, exposure rate effects were observed that indicated a shift from markers of toxicity (hydroquinone glucuronide, muconic acid, phenylglucuronide, and prephenylmercapturic acid) to less toxic metabolites. As compared with exposures with lower benzene concentrations but longer durations, the fastest exposure rate (0.5 hour times 600 ppm) had lower AUCs for muconic acid and hydroquinone glucuronide in the blood, liver, and lungs. In blood and lung tissues there were decreased AUC ratios for muconic acid relative to phenylsulfate and decreased AUC for hydroquinone glucuronide relative to phenylsulfate. These changes indicate that at the faster exposure rate, mice tended to shift a greater portion of their benzene metabolism toward phenyl conjugation, which produces less toxic metabolites.

The detoxification pathways for benzene appear to be low-affinity, high-capacity pathways, whereas pathways leading to the putative toxic metabolites appear to be high-affinity, low-capacity systems (Henderson et al. 1989). Accordingly, if the exposure dose regimen, via inhalation, extends beyond the range of linear metabolism rates of benzene (200 ppm by inhalation) (Sabourin et al. 1989b), then the fraction of toxic metabolites formed relative to the amount administered will be reduced.

Bois and Paxman (1992) used a PBPK model to assess effects of dose rate on the disposition of benzene metabolites. Simulations were performed for rats exposed either for 15 minutes to 32 ppm or for 8 hours to 1 ppm (equivalent 8-hour TWAs). The amount of metabolites (hydroquinone, catechol, and muconaldehyde) formed was 20% higher after the 15-minute exposure at the higher level than after the 8-hour exposure at the lower level. Differences between the model predictions (Bois and Paxman 1992) and the empirical data of Sabourin et al. (1989a, 1989b) may be related, at least in part, to the higher benzene exposure levels (50, 150, and 600 ppm) used by Sabourin and coworkers. The pattern of urinary metabolites observed in exposed workers has provided evidence of a high- and low-affinity pathway for metabolite production (Kim et al. 2006a, 2006b; Rappaport et al. 2009, 2010). In theory, a high-affinity pathway could result in nonlinear metabolic clearance of benzene, with faster clearance at lower exposures (<0.1 ppm). Evidence of nonlinear metabolic clearance at low exposure concentrations remains equivocal, with some studies finding non-linearities and other studies not observing non-linearity (Cox et al. 2017; McNally et al. 2017; Price et al. 2012).

Covalent binding of benzene metabolites to cellular macromolecules is thought to be related to benzene's mechanism of toxicity, although the relationship between adduct formation and toxicity is not clear. Benzene metabolites have been found to form covalent adducts with proteins from blood in humans

(Bechtold et al. 1992b). Benzene metabolites form covalent adducts with nucleic acids and proteins in rats and mice (Norpoth et al. 1988; Rappaport et al. 1996). Covalent binding of benzene metabolites to proteins has been observed in mouse or rat liver, bone marrow, kidney, spleen, blood, and muscle *in vivo* (Bechtold and Henderson 1993; Bechtold et al. 1992a, 1992b; Creek et al. 1997; Longacre et al. 1981a, 1981b; Sun et al. 1990). Metabolite binding to proteins has also been observed in perfused bone marrow preparations (Irons et al. 1980) and in rat and mouse liver DNA *in vivo* (Creek et al. 1997; Lutz and Schlatter 1977). Binding of benzene metabolites to DNA also has been observed in *in vitro* preparations of rabbit and rat bone marrow mitochondria (Rushmore et al. 1984). Exposure-related increases in blood levels of albumin adducts of benzene oxide and 1,4-benzoquinone were noted among workers occupationally exposed to benzene air concentrations of 0.07–46.6 ppm (Rappaport et al. 2002a, 2002b). Several reactive metabolites of benzene have been proposed as agents of benzene hematotoxic and leukemogenic effects. These metabolites include benzene oxide, reactive products of the phenol pathway (catechol, hydroquinone, and 1,4-benzoquinone), and *trans*,*trans*-muconaldehyde.

3.1.4 Excretion

Available human data indicate that following inhalation exposure to benzene, the major route for elimination of unmetabolized benzene is via exhalation. Benzene has also been detected in samples of human breast milk (Fabietti et al. 2004). Absorbed benzene is also excreted in humans via metabolism to phenol and muconic acid followed by urinary excretion of conjugated derivatives (sulfates and glucuronides). In six male and female volunteers exposed to 52–62 ppm benzene for 4 hours, respiratory excretion (the amount of absorbed benzene excreted via the lungs) was approximately 17%; no gender-related differences were observed (Nomiyama and Nomiyama 1974a, 1974b). Results from a study of 23 subjects who inhaled 47–110 ppm benzene for 2–3 hours showed that 16.4–41.6% of the retained benzene was excreted by the lungs within 5–7 hours (Srbova et al. 1950). The rate of excretion of benzene was the greatest during the first hour.

Results of a study involving a single human experimental subject exposed to concentrations of benzene of 6.4 and 99 ppm for 8 hours and 1 hour, respectively, suggested that excretion of benzene in breath has three phases and could possibly have four phases. The initial phase is rapid and is followed by two (or three) slower phases (Sherwood 1988). The initial phase with a high exposure concentration (99 ppm) and a short-term exposure duration (1 hour) had a more rapid excretion rate (half-life=42 minutes) and a greater percentage of the total dose excreted (17%) than did the initial phase with a low exposure concentration (6.4 ppm) and longer exposure duration (8 hours) (half-life=1.2 hours, percentage of total

dose excreted=9.3%). Subsequent phases showed an increase in the half-lives. Benzene metabolites were excreted in urine. This limited study indicated a greater proportion of the total dose was excreted in urine than in breath (Sherwood 1988).

Absorbed benzene is also eliminated in humans by metabolism to phenol and muconic acid followed by urinary excretion of conjugated derivatives (sulfates and glucuronides). Srbova et al. (1950) found that only 0.07–0.2% of benzene extracted from inhaled air was excreted in the urine as benzene. Sherwood (1988) showed that urinary excretion of phenol conjugate was biphasic, with an initial rapid excretion phase, followed by a slower excretion phase. Ghittori et al. (1993) stated that benzene in urine may be a useful biomarker of occupational exposure.

The urinary excretion of phenol in workers was measured following a 7-hour work shift exposure to 1–200 ppm benzene. A correlation of 0.881 between exposure level and urinary phenol excretion was found (Inoue et al. 1986). Urine samples were collected from randomly chosen subjects not exposed to known sources of benzene, from subjects exposed to side stream cigarette smoke, or from supermarket workers presumed exposed to benzene from unknown sources (Bartczak et al. 1994). Samples analyzed for muconic acid found concentrations ranging from 8 to 550 ng/mL.

The primary excretory pathway for benzene and its metabolites is the urine. Excretory products that are known to correlate with blood benzene levels include muconic acid, PhMA, and 8-hydroxy-deoxyguanosine. Blood benzene and urinary benzene levels have been correlated ($r=0.61$, $p<0.001$) in a smoker and nonsmoker group (Kok and Ong 1994). Furthermore, urinary muconic acid and PhMA levels have been correlated (0.40–0.81) with benzene blood levels (mean=3.3 µg/L) and benzene air levels, which reached a maximum of 13 mg/m^3 (Popp et al. 1994).

Smoking tobacco can increase the concentrations of benzene or benzene-correlated biomarkers excreted into blood and urine. Blood and urine levels of benzene were 110.9 and 116.4 ng/L, respectively, in nonsmokers, and 328.8 and 405.4 ng/L, respectively, in smokers (Kok and Ong 1994). Melikian et al. (1994) compared urinary muonic acid levels in smokers and nonsmokers and in pregnant and nonpregnant woman. The mean urinary levels of muconic acid in groups of male, female-nonpregnant, and female-pregnant smokers were 3–5 times higher than urinary levels in the corresponding nonsmoking groups (Melikian et al. 1994). Similar mean urinary concentrations of muconic acid were observed in males who smoke and nonpregnant female smokers. On a creatinine basis, urinary muconic acid levels were similar in pregnant and nonpregnant smokers.

Animal data show that exhalation is the main route for excretion of unmetabolized benzene and that metabolized benzene is excreted primarily in urine. Only a small amount of an absorbed dose is eliminated in feces.

A biphasic pattern of excretion of unmetabolized benzene in expired air was observed in rats exposed to 500 ppm for 6 hours, with half-times for expiration of 0.7 hour for the rapid phase and 13.1 hours for the slow phase (Rickert et al. 1979). Excretion of exhaled benzene was also biphasic in mice following an intraperitoneal dose of 0.13 mg/kg (Zhang et al. 2017). The half-life for the slow phase of benzene elimination suggests the accumulation of benzene.

The major route of excretion following a 6-hour, nose-only inhalation exposure of rats and mice to various concentrations of ^{14}C-benzene (10–1,000 ppm) appeared to be dependent on the inhaled concentration (Sabourin et al. 1987). When exposed to the same concentrations, the inhaled dose per kg body weight was 150–200% higher in mice compared to rats. At all concentrations, fecal excretion accounted for <3.5% of the radioactivity for rats and <9% for mice. At lower exposure concentrations (i.e., 13–130 ppm in rats and 11–130 ppm in mice), <6% of the radioactivity was excreted in expired air.

At the highest exposure concentrations (rats, 870 ppm; mice, 990 ppm), the percentages of exhaled unmetabolized benzene were 48 and 14% in rats and mice, respectively, following termination of the exposure. Most of the benzene-associated radioactivity that was not exhaled was found in the urine and in the carcass 56 hours after the end of exposure to these high concentrations. The radioactivity in the carcass was associated with the pelt of the animals. The study authors assumed that this was due to contamination of the pelt with urine since the inhalation exposure had been nose-only. Further investigation confirmed that the radioactivity was associated with the fur of the animals. Accordingly, the percentage of the total radioactivity excreted by these animals (urine and urine-contaminated pelt) that was not exhaled or associated with feces was 47–92% for rats and 80–94% for mice. At exposures of 260 ppm in rats, 85–92% of the radioactivity was excreted as urinary metabolites, while at exposures of 130 ppm in mice, 88–94% of the radioactivity was excreted as urinary metabolites. The total urinary metabolite formation was 5–37% higher in mice than in rats at all doses. This may be explained by the greater amount of benzene inhaled by mice per kg of body weight (Sabourin et al. 1987).

In mice exposed to 50 ppm benzene (6 hours/day for 8 days), the following metabolites were detected in urine above levels in unexposed mice: phenol, *trans,trans*-muconic acid, hydroquinone, and

s-phenylmercapturic acid (Bird et al. 2010). Purebred Duroc-Jersey pigs were exposed to 0, 20, 100, and 500 ppm benzene vapors 6 hours/day, 5 days/week for 3 weeks (Dow 1992). The average concentration of phenol in the urine increased linearly with dose.

No studies were located regarding excretion in humans after oral exposure to benzene. Data on excretion of benzene or its metabolites in human breast milk after oral exposure were not found.

Radiolabeled benzene (340 mg/kg) was administered by oral intubation to rabbits; 43% of the label was recovered as exhaled unmetabolized benzene and 1.5% was recovered as carbon dioxide (Parke and Williams 1953). Urinary excretion accounted for about 33% of the dose. The isolated urinary metabolites were mainly in the form of conjugated phenols. Phenol was the major metabolite accounting for about 23% of the dose or about 70% of the benzene metabolized and excreted in the urine. The other phenols excreted (percentage of dose) were hydroquinone (4.8%), catechol (2.2%), and trihydroxy-benzene (0.3%). L-Phenyl-N-acetyl cysteine accounted for 0.5% of the dose. Muconic acid accounted for 1.3%; the rest of the radioactivity (5–10%) remained in the tissues or was excreted in the feces (Parke and Williams 1953).

Mice received a single oral dose of either 10 or 200 mg/kg radiolabeled benzene (McMahon and Birnbaum 1991). Radioactivity was monitored in urine, feces, and breath. At the low dose, urinary excretion was the major route of elimination. Hydroquinone glucuronide, phenylsulfate, and muconic acid were the major metabolites at this dose, accounting for 40, 28, and 15% of the dose, respectively. At 200 mg/kg, urinary excretion decreased to account for 42–47% of the administered dose, while respiratory excretion of volatile components increased to 46–56% of the administered dose. Fecal elimination was minor and relatively constant over both doses, accounting for 0.5–3% of the dose.

The effect of dose on the excretion of radioactivity, including benzene and metabolites, following oral administration of ^{14}C-benzene (0.5–300 mg/kg) has been studied in rats and mice (Sabourin et al. 1987). At doses of <15 mg/kg for 1 day, 90% of the administered dose was excreted in the urine of both species. There was a linear relationship for the excretion of urinary metabolites up to 15 mg/kg; above that level, there was an increased amount of ^{14}C eliminated in the expired air. Mice and rats excreted equal amounts up to 50 mg/kg; above this level, metabolism apparently became saturated in mice. In rats, 50% of the 150 mg/kg dose of ^{14}C was eliminated in the expired air; in mice, 69% of the 150 mg/kg dose of ^{14}C was eliminated in expired air (Sabourin et al. 1987). The label recovered during exhalation was largely in the form of unmetabolized benzene, suggesting that saturation of the metabolic pathways had occurred. Dose

also affected the metabolite profile in the urine. At low doses, a greater fraction of the benzene was converted to putative toxic metabolites than at high doses, as reflected in urinary metabolites.

Mathews et al. (1998) reported similar results following gavage administration of ^{14}C-benzene to rats, mice, and hamsters in single doses from as low as 0.2 mg/kg and up to 100 mg/kg. For example, >95% of a 0.5 mg/kg dose was recovered in the urine of rats; a small amount (3%) was recovered in expired air. At benzene doses of 10 and 100 mg/kg, elimination in the breath rose to 9 and 50%, respectively, indicating the likely saturation of benzene metabolism. Excretion in the feces was minimal at all dose levels. Similar results were noted for mice and hamsters. Both dose and species differences were noted in the composition of urinary metabolites. Phenyl sulfate was the major metabolite in rat urine at all dose levels, accounting for 64–73% of urinary radioactivity. Phenyl sulfate (24–32%) and hydroquinone glucuronide (27–29%) were the predominant urinary metabolites in mice. At a dose of 0.1 mg/kg, mice produced a considerably higher proportion of muconic acid than rats (15 versus 7%). In hamsters, hydroquinone glucuronide (24–29%) and muconic acid (19–31%) were the primary urinary metabolites. Two additional metabolites (1,2,4-trihydroxybenzene and catechol sulfate) were recovered from the urine of hamsters, but not rats or mice.

Limited data on excretion of benzene after dermal exposure in humans were found. Four human male subjects were given a dermal application of 0.0024 mg/cm^2 ^{14}C benzene (Franz 1984). A mean of 0.023% (range: 0.006–0.054%) of the applied radiolabel was recovered in the urine over a 36-hour period. Urinary excretion of the radiolabel was greatest in the first two hours following skin application. More than 80% of the total excretion occurred in the first 8 hours. In another study, 35–43 cm^2 of the forearm were exposed to approximately 0.06 g/cm^2 of liquid benzene for 1.25–2 hours (Hanke et al. 1961). The absorption was estimated from the amount of phenol eliminated in the urine. The absorption rate of liquid benzene by the skin (under the conditions of complete saturation) was calculated to be low, approximately 0.4 mg/cm^2/hour. The absorption due to vapors in the same experiment was negligible. Although there was a large variability in the physiological values, the amount of excreted phenol was 8.0–14.7 mg during the 24-hour period after exposure. It is estimated that approximately 30% of dermally absorbed benzene is eliminated in the form of phenol in the urine.

Data on excretion of benzene or its metabolites in human breast milk after dermal exposure were not found.

Monkeys and minipigs were exposed dermally to 0.0026–0.0036 mg/cm^2 of ^{14}C-benzene (Franz 1984). After application, the urine samples were collected over the next 2–4 days at 5-hour intervals. The rate of excretion was highest in the first two collection periods. The total urinary excretion of radioactivity was found to be higher in monkeys than in minipigs with the same exposure. Mean excretion in monkeys was 0.065% (range: 0.033–0.135%) of the applied dose compared to 0.042% (range: 0.030–0.054%) in minipigs.

Results of a study in which male rats were dermally treated with 0.004 mg/cm^2 of ^{14}C-benzene, with or without 1g of clay or sandy soil, showed that for all treatment groups, the major routes of excretion were the urine and, to a lesser extent, the expired air (Skowronski et al. 1988). The highest amount of radioactivity in urine appeared in the first 12–24 hours after treatment (58.8, 31.3, and 25.1% of the absorbed dose, respectively, for pure benzene, sandy soil–adsorbed benzene, and clay soil-adsorbed benzene). In the group treated with pure benzene, 86.2% of the absorbed dose was excreted in the urine. Sandy soil and clay soil significantly decreased urinary excretion to 64.0 and 45.4%, respectively, of the absorbed dose during the same time period. Rats receiving pure benzene excreted 12.8% of the absorbed dose in expired air within 48 hours. Only 5.9% of the radioactivity was collected in expired air 48 hours after treatment with sandy soil–adsorbed benzene, while experiments with clay soil–adsorbed benzene revealed that 10.1% of the radioactivity was located in expired air. Less than 1% of the absorbed dose was expired as $^{14}CO_2$ in all groups. The ^{14}C activity in the feces was small (<0.5% of the applied radioactivity) in all groups 48 hours after treatment. Phenol was the major urinary metabolite detected in the 0–12-hour urine samples of all treatment groups. The percentage of total urinary radioactivity associated with phenol was 37.7% for benzene alone, 44.2% for benzene adsorbed to sandy soil, and 45.5% for benzene adsorbed to clay soil. Smaller quantities of hydroquinone, catechol, and benzenetriol were also detected (Skowronski et al. 1988).

The metabolic fate of benzene can be altered in fasted animals. In nonfasted rats that received an intraperitoneal injection of 88 mg of benzene, the major metabolites present in urine were total conjugated phenols (14–19% of dose), glucuronides (3–4% of dose), and free phenol (2–3% of dose). However, in rats fasted for 24 hours preceding the same exposure, glucuronide conjugation increased markedly (18–21% of dose) (Cornish and Ryan 1965). Free phenol excretion (8–10% of dose) was also increased in fasted, benzene-treated rats. There was no apparent increase in total conjugated phenol excretion in fasted rats given benzene.

When ^{14}C-benzene (0.5 and 150 mg/kg) was injected intraperitoneally into rats and mice, most of the ^{14}C-benzene and ^{14}C-metabolites were excreted in the urine and in the expired air. A smaller amount of ^{14}C-benzene was found in the feces due to biliary excretion (Sabourin et al. 1987). Monkeys were dosed intraperitoneally with 5–500 mg/kg radiolabeled benzene, and urinary metabolites were examined (Sabourin et al. 1992). The proportion of radioactivity excreted in the urine decreased with increasing dose, whereas as the dose increased, more benzene was exhaled unchanged. This indicated saturation of benzene metabolism at higher doses. Phenyl sulfate was the major urinary metabolite. Hydroquinone conjugates and muconic acid in the urine decreased as the dose increased. When C57BL/6 mice and DBA/2 mice were given benzene subcutaneously in single doses (440, 880, or 2,200 mg/kg) for 1 day, or multiple doses (880 mg/kg) 2 times daily for 3 days, no strain differences were observed in the total amount of urinary ring-hydroxylated metabolites (Longacre et al. 1981a). Although each strain excreted phenol, catechol, and hydroquinone, differences in the relative amounts of these metabolites were noted. The more sensitive DBA/2 mice excreted more phenol but less hydroquinone than the more resistant C57BL/6 mice, while both strains excreted similar amounts of catechol. DBA/2 mice excreted more phenyl glucuronide but less sulfate conjugate. Both strains excreted similar amounts of phenyl mercapturic acid (Longacre et al. 1981a).

3.1.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Models are simplified representations of a system with the intent of reproducing or simulating its structure, function, and behavior. PBPK models are more firmly grounded in principles of biology and biochemistry. They use mathematical descriptions of the processes determining uptake and disposition of chemical substances as a function of their physicochemical, biochemical, and physiological characteristics (Andersen and Krishnan 1994; Clewell 1995; Mumtaz et al. 2012a; Sweeney and Gearhart 2020). PBPK models have been developed for both organic and inorganic pollutants (Ruiz et al. 2011) and are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Mumtaz et al. 2012b; Ruiz et al. 2011; Sweeney and Gearhart 2020; Tan et al. 2020). PBPK models can also be used to more accurately extrapolate from animal to human, high dose to low dose, route to route, and various exposure scenarios and to study pollutant mixtures (El-Masri et al. 2004). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic endpoints (Clewell 1995).

Several PBPK models have been developed that simulate the disposition of benzene in humans (Bois et al. 1996; Brown et al. 1998; Fisher et al. 1997; Knutsen et al. 2013a, 2013b; Majumdar et al. 2016; Medinsky et al. 1989c; Ruiz et al. 2020; Sinclair et al. 1999; Travis et al. 1990), mice (Cole et al. 2001; Medinsky et al. 1989a, 1989b; Sun et al. 1990; Travis et al. 1990), and rats (Bois et al. 1991a; Medinsky et al. 1989a, 1989b; Sun et al. 1990; Travis et al. 1990). A comparative summary of the models is provided in Table 3-1. All of the models have the same general structure (Figure 3-2). Most of the models simulate inhalation and oral exposures; one model provides a simulation of dermal absorption (Sinclair et al. 1999). Physiological parameters and partition coefficients for simulating benzene biokinetics of human females were reported for the Brown et al. (1998) and Fisher et al. (1997) models. Flow-limited exchange of benzene between blood and tissues is assumed in all models, with excretion of benzene in exhaled air and, in one case, to breast milk (Fisher et al. 1997). All models include simulations of blood, fat, liver, lung, and lumped compartments representing other slowly-perfused tissues (e.g., skeletal muscle) and rapidly-perfused tissues (e.g., kidneys, other viscera). Simulation of bone marrow, the primary target for benzene toxicity, is included in the models reported by Bois et al. (1991a, 1996), Knutsen et al. (2013a, 2013b), Sinclair et al. (1999), and Travis et al. (1990). The Knutsen et al. (2013a, 2013b) model includes a bladder compartment that accumulates benzene metabolites circulating in blood.

Table 3-1. Summary Comparison of Physiologically Based Pharmacokinetic Models for Benzene

		Simulated areas				
Reference	Species	Absorption pathways	Tissues[a]	Metabolic pathways[b]	Excretion pathways[c]	Comment
Bois et al. 1991a	Rat	Inhalation, oral	BL, BM, FA, LI, LU, RP, SP	BM, LI: BZ→BO(c) BO→BG(c) BO→PH(f) BO→GSH(c) BG→DI(c) PH→HQ(c) PH→CA(c) BM, LI, LU, GI: PH→PHCO(c)	EH: BZ UR: PH	Simulates metabolic pathways in bone marrow, and phenol conjugation in lung and gastrointestinal tract

Table 3-1. Summary Comparison of Physiologically Based Pharmacokinetic Models for Benzene

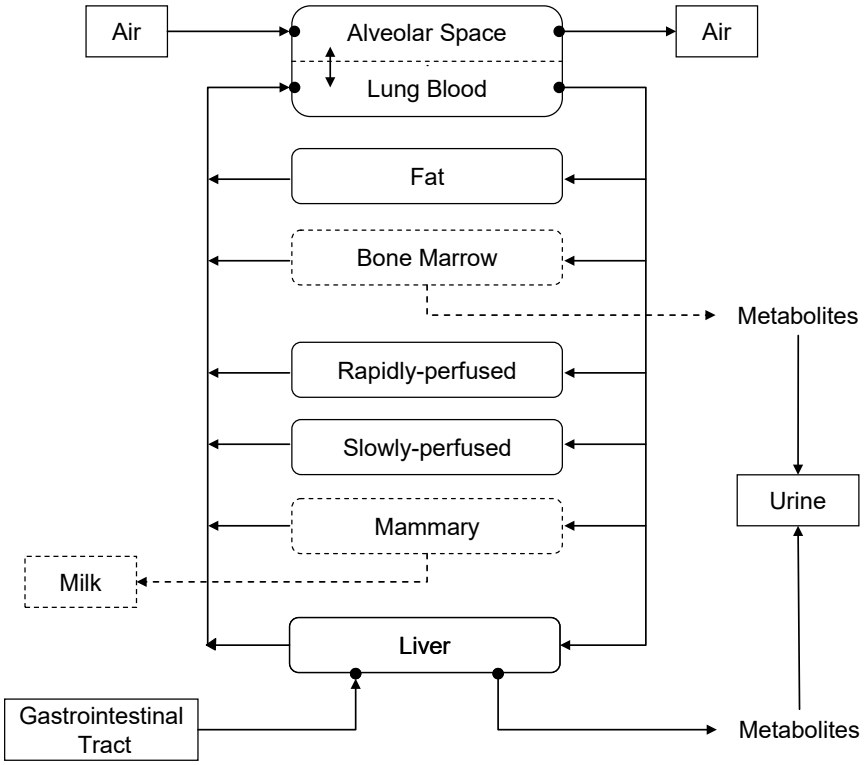
	Simulated areas					
Reference	Species	Absorption pathways	Tissues[a]	Metabolic pathways[b]	Excretion pathways[c]	Comment
Bois et al. 1996	Human	Inhalation	BL, BM, FA, LI, LU, RP, SP	BM, LI: $BZ \rightarrow M_{tot}(c)$ LI: $PHX_{end} \rightarrow PH(z)$	EH: BZ UR: M_{tot} PH	Simulates metabolic pathways in bone marrow, and endogenous production of phenolic metabolites
Brown et al. 1998	Human (males, females)	Inhalation	BL, FA, LI, LU, RP, SP	LI: $BZ \rightarrow M_{tot}(f)$	EH: BZ	Simulates males or females
Cole et al. 2001	Mouse	Inhalation, oral	BL, FA, LI, LU, RP, SP	LI: BZ→BO(c) BO→PH(f) BO→PMA(f) BO→MA(f) PH→HQ(c) PH→PHCO(c) PH→CA(c) CA→THB(c) HQ→HQCO(c)	EH: BZ UR: CA MA PHCO PMA HQCO THB	All metabolism is assigned to the liver
Fisher et al. 1997	Human	Inhalation	FA, LI, LU, RP, SP, MI	LI: $BZ \rightarrow M_{tot}(c)$	EH: BZ MI: BZ	Simulates transfer of benzene to breast milk
Knutsen et al. 2013a, 2013b	Human, mouse	Inhalation	BL, BM, FA, LI, LU, RP, SP, UB	LI, BM: BZ→BO(c) BO→PH(f) BO→PMA(f) BO→MA(f) PH→HQ(c) PH→PHCO(c) PH→CA(c) CA→THB(c) HQ→HQCO(c)	EH: BZ UR: CA HQ MA PHCO PMA	Metabolism assigned to liver and marrow (4% of liver)
Majumdar et al. 2016	Human	Inhalation	FA, LI, LU, RP, SP	LI: $BZ \rightarrow M_{tot}(c)$	UR: M_{tot} MA	All metabolism is assigned to the liver
Medinsky et al. 1989a, 1989b, 1989c	Human, mouse, rat	Inhalation, oral	FA, LI, LU, RP, SP	LI: BZ→BO(c) BO→PHCO(c) BO→PMA(c) BO→HQCO(c) BO→MA(c)	EH: BZ	All metabolism is assigned to the liver

Table 3-1. Summary Comparison of Physiologically Based Pharmacokinetic Models for Benzene

	Simulated areas					
Reference	Species	Absorption pathways	Tissues[a]	Metabolic pathways[b]	Excretion pathways[c]	Comment
Sinclair et al. 1999	Human	Inhalation, oral, dermal	BL, BM, LI, LU, MU, RP	BM, LI: BZ→M_{tot}(c)	EH: BZ UR: M_{tot} PH	Simulates dermal exposure and absorption
Sun et al. 1990	Mouse, rat	Inhalation, oral	BL, FA, LI, LU, RBC, RP, SP	LI: BZ→BO(c) BO→PHCO(c) BO→PMA(c) BO→HQCO(c) BO→MA(c) RBC: BO→HBA(c,f)	EH: BZ	Simulates formation of hemoglobin adducts in RBCs derived from benzene oxide
Travis et al. 1990	Human, mouse, rat	Inhalation, oral	BL, BM, FA, LI, LU, MU, RP	BM, LI: BZ→M_{tot}(c)	EH: BZ	Total metabolism of benzene in the bone marrow and liver

[a]Tissues simulated: BL = blood; BM = bone marrow; FA = fat; GI = gastrointestinal; LI = liver; LU = lung; MU = muscle; RBC = red blood cells; RP = other rapidly-perfused tissues; SP = other slowly-perfused tissues; UB = urinary bladder.
[b]Metabolic pathways simulated: BG = benzene glycol; BO = benzene oxide; BZ = benzene; CA = catechol; DI = diols; GSH = glutathione; HBA = hemoglobin adduct; HQ = hydroquinone; HQCO = hydroquinone conjugates; MA = muconic acid; M_{tot} = total metabolites; PH = phenol; PHCO = phenol conjugates; PMA = phenylmercapturic acid; PHX_{end} = endogenous phenolic metabolites; THB = trihydroxybenzene; (c) = capacity-limited; (f) = first-order; (z) = zero-order.
[c]Excretion pathways simulated: EH = exhalation; MI = breast milk; UR = urine.

Figure 3-2. General Structure of Physiologically Based Pharmacokinetic Models of Benzene*



*Tissues shown with dashed lines are not simulated in all models. Flow-limited exchange of benzene between blood and tissues is assumed. Metabolism is simulated to varying degrees of complexity (Table 3-1).

Simulations of metabolism in the various models vary in complexity. In the simplest representation, metabolic elimination of benzene is simulated as a single capacity-limited process, represented with Michaelis-Menten function of benzene concentration in tissue (Bois et al. 1996; Brown et al. 1998; Fisher et al. 1997; Sinclair et al. 1999; Travis et al. 1990). In the more complex representations, the major pathways of metabolism of benzene, including conjugation reactions, are simulated as capacity-limited or first-order processes (Bois et al. 1991a; Cole et al. 2001; Medinsky et al. 1989a, 1989b, 1989c; Sun et al. 1990). In most of the models, all metabolic pathways are attributed to the liver; however, four of the models include simulations of metabolism in bone marrow (Bois et al. 1991a, 1996; Sinclair et al. 1999; Travis et al. 1990) and one model includes simulations of the formation of sulfate and glucuronide conjugates of phenol in the gastrointestinal and respiratory tracts (Bois et al. 1991a). The Sun et al. (1990) model includes a simulation of the formation of hemoglobin adducts derived from benzene oxide. In models that simulate the disposition of the metabolites, metabolites are assumed to be excreted in urine either at a rate equal to their formation (Cole et al. 2001), or in accordance with a first-order excretion rate constant (Bois et al. 1991a, 1996; Sinclair et al. 1999); the difference being, in the latter, the mass balance

for formation and excretion of metabolites is simulated, allowing predictions of metabolite levels in tissues. All of the models use typical parameters and values for species-specific blood flows and tissue volumes.

Brief summaries of the models presented in Table 3-1 are provided below, with emphasis on unique features that are applicable to risk assessment.

Medinsky et al. 1989a, 1989b, 1989c

Description of the Model. The Medinsky et al. (1989a, 1989b, 1989c) model simulates absorption and disposition of benzene in the human, mouse, and rat. Tissues simulated include the blood, bone marrow, fat, liver, lung, other slowly-perfused tissues, and other rapidly-perfused tissues. Gastrointestinal absorption of benzene is simulated as a first-order process; absorption and excretion of benzene in the lung are assumed to be flow-limited. Exchange of benzene between blood and tissues is assumed to be flow-limited. The model simulates capacity-limited (i.e., Michaelis-Menten) metabolism of benzene to benzene oxide as a function of the concentration of benzene in liver. Conversion of benzene oxide to phenol conjugates, phenylmercapturic acid, hydroquinone conjugates, and muconic acid are simulated as parallel, capacity-limited reactions in liver. The model simulates rates of formation of metabolites, but not the disposition (e.g., excretion) of metabolites. Metabolism parameter values (V_{max}, K_m) for the mouse and rat models were estimated by optimization of the model to observations of total metabolites formed in mice and rats exposed by inhalation or oral routes to benzene (Medinsky et al. 1989b; Sabourin et al. 1987). Human metabolism parameter values were derived from allometric scaling of the values for mice (Medinsky et al. 1989c).

Risk Assessment. The model has been used to predict the amounts of benzene metabolites formed in rats and mice after inhalation or oral exposures (Medinsky et al. 1989a, 1989b). For inhalation concentrations up to 1,000 ppm, mice were predicted to metabolize at least 2–3 times more benzene than rats. For oral doses >50 mg/kg, rats were predicted to metabolize more benzene on a kg-body weight basis than mice. The model also predicts different metabolite profiles in the two species: mice were predicted to produce primarily hydroquinone glucuronide and muconic acid, metabolites linked to toxic effects, whereas rats were predicted to produce primarily phenyl sulfate, a detoxification product. These predictions agree with experimental data and provide a framework for understanding the greater sensitivity of the mouse to benzene toxicity.

Validation of the Model. The model was calibrated with data from Sabourin et al. (1987). Bois et al. (1991b) compared predictions made to observations of benzene exhaled by rats following exposures to 490 ppm benzene, reported by Rickert et al. (1979), as well as the data from which the model was calibrated (Sabourin et al. 1987). In general, the model tended to overestimate observations to which it was not specifically fitted.

Target Tissues. The model simulates amounts and concentrations of benzene in blood, liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues as well as amounts of metabolites formed. It does not simulate concentrations of metabolites in these tissues. It does not simulate bone marrow, a target of benzene metabolites.

Species Extrapolation. The model has been applied to simulations of mice, rats, and humans (Medinsky et al. 1989a, 1989b, 1989c).

High-low Dose Extrapolation. The model has been evaluated for simulating inhalation exposures in rodents ranging from 1 to 1,000 ppm and gavage doses of 0.1–300 mg/kg (Bois et al. 1991b; Medinsky et al. 1989a, 1989b, 1989c).

Inter-route Extrapolation. The model simulates inhalation and oral exposures and has been applied to predicting internal dose metrics (e.g., amounts of metabolites formed) resulting from exposures by these routes (Medinsky et al. 1989a, 1989b, 1989c).

Strengths and Limitations. Strengths of the model are that it simulates disposition of inhaled and ingested (single dose) benzene, including rates and amounts of major metabolites formed in mice, rats, and humans. Limitations include: (1) the model has not been evaluated for multiple exposures; (2) the model attributes all metabolism to the liver; (3) the model does not simulate the fate of metabolites formed and, therefore, cannot be used to predict concentrations of metabolites (e.g., muconaldehyde) in tissues; and (4) the model does not simulate bone marrow, a major target tissue for benzene metabolites.

Sun et al. 1990

Description of the Model. The Sun et al. (1990) model is an extension of the mouse and rat models developed by Medinsky et al. (1989a, 1989b, 1989c). The Sun et al. (1990) model includes a simulation of the formation of hemoglobin adducts derived from benzene oxide. Adduct formation is represented as

the sum of capacity-limited and first-order functions of the concentration of benzene oxide in the liver. Parameter values were estimated by optimization to measurements of hemoglobin adduct formations in rats and mice exposed to single gavage doses of benzene (Sun et al. 1990).

Risk Assessment. The model has been applied to predicting the levels of hemoglobin adducts in mice and rats following inhalation or oral exposures to benzene. This approach could be potentially useful for predicting exposure levels that correspond to measured hemoglobin adduct levels, for use of adducts as an exposure biomarker.

Validation of the Model. The model was calibrated against measurements of hemoglobin adduct formation in mice and rats that received single gavage doses of benzene of 0.008–800 mg/kg (Sun et al. 1990). The model was evaluated by comparing predictions to observations of amounts of hemoglobin adducts formed in mice and rats exposed to benzene vapor concentrations of 5, 50, or 600 ppm for 6 hours (Sabourin et al. 1989a).

Target Tissues. The model predicts hemoglobin adduct formation after oral and inhalation exposure (Sun et al. 1990).

Species Extrapolation. The model has been applied to simulations for mice and rats.

High-low Dose Extrapolation. The model was calibrated with observations made in mice and rats exposed to single gavage doses of 0.1–10,000 μmol/kg (0.008–800 mg/kg) and evaluated for predicting observations in mice and rats exposed by inhalation to 600 ppm benzene.

Inter-route Extrapolation. The model examined two routes of exposure: oral and inhalation. The model was found to be useful in predicting the concentrations of hemoglobin adducts in blood in rodents after oral and inhalation exposure.

Strengths and Limitations. Strengths of the model are that it extends the Medinsky et al. (1989a, 1989b, 1989c) models to simulate hemoglobin adduct formation secondary to formation of benzene oxide. A limitation of the adduct model is that it simulates production of adducts as a function of benzene oxide concentration in liver and does not consider other potential pathways of adduct formation through hydroquinone, phenol, or muconaldehyde.

Travis et al. 1990

Description of the Model. The Travis et al. (1990) model simulates the absorption and disposition of benzene in the human, mouse, and rat. Tissues simulated include the blood, bone marrow, fat, liver, lung, other slowly-perfused tissues, and rapidly-perfused tissues. Gastrointestinal absorption of benzene is simulated as a first-order process. Absorption and excretion of benzene in the lung are assumed to be flow-limited, as are exchanges of benzene between blood and tissues. The model simulates capacity-limited (i.e., Michaelis-Menten) metabolic elimination of benzene as a function of the concentration of benzene in bone marrow and liver. The model simulates rates of metabolic elimination of benzene, but not the rates of formation of specific metabolites or their disposition (e.g., excretion). For the purpose of comparing model predictions to observations, 80% of the total metabolite formed in 24 hours (and excreted in urine) was assumed to be phenol. Metabolism parameter values (V_{max}, K_m) were estimated by optimization of the model to observations of total metabolites formed (i.e., excreted in urine) in humans, mice, and rats exposed to benzene by inhalation or oral routes. The V_{max} for metabolism in bone marrow in humans was assumed to be 4% of that of liver, consistent with optimized values for rodents.

Risk Assessment. This model has been used to predict the amounts of benzene in expired air, concentrations of benzene in blood, and total amount of benzene metabolized following inhalation exposures to humans and inhalation, intraperitoneal, gavage, or subcutaneous exposures in mice or rats (Travis et al. 1990). Cox (1996) applied the model to derive internal dose-response relationships for benzene in humans.

Validation of the Model. The model was evaluated by comparing predictions with observations made in mouse and rat inhalation studies (Rickert et al. 1979; Sabourin et al. 1987; Sato et al. 1975; Snyder et al. 1981); mouse gavage studies (Sabourin et al. 1987); mouse subcutaneous injection studies (Andrews et al. 1977); and rat intraperitoneal injection studies (Sato and Nakajima 1979). Predictions of benzene in expired air and/or blood concentrations were also compared to observations made in humans who inhaled concentrations of 5–100 ppm benzene (5 ppm: Berlin et al. 1980; Sherwood 1972; 25–57 ppm: Sato et al. 1975; Sherwood 1972; Nomiyama and Nomiyama 1974a, 1974b; 99–100 ppm: Sherwood 1972; Teisinger and Fišerová-Bergerová 1955). Further evaluations of predictions of benzene in workers are reported in Sinclair et al. (1999) and Sherwood and Sinclair (1999), who compared model predictions with observations of benzene in exhaled breath and urinary excretion of phenol in workers who were exposed to benzene at concentrations of 1–1,100 ppm.

Target Tissues. The model simulates amounts and concentrations of benzene in blood, bone marrow (a target tissue), liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues; and amounts of metabolites formed in liver and bone marrow. It does not simulate concentrations of metabolites in these tissues.

Species Extrapolation. The model has been applied to simulations for mice, rats, and humans (Sherwood and Sinclair 1999; Sinclair et al. 1999; Travis et al. 1990).

High-low Dose Extrapolation. The model has been evaluated for simulating inhalation exposures in humans ranging from 1 to 1,110 ppm (Sherwood and Sinclair 1999; Sinclair et al. 1999; Travis et al. 1990). Evaluations of predictions in rodents included observations made during inhalation exposures of 11–1,000 ppm and gavage doses of 0.5–300 mg/kg.

Inter-route Extrapolation. The model simulates inhalation and oral exposures and has been applied to predicting internal dose metrics (e.g., benzene concentration in blood, amount of benzene metabolized) resulting from exposures by these routes (Travis et al. 1990).

Strengths and Limitations. Strengths of the model are that it simulates: (1) disposition of inhaled and ingested (single dose) benzene in mice, rats, and humans; and (2) concentrations of benzene, and rates and amount of benzene metabolized in bone marrow, a target tissue for benzene metabolites. Limitations of the model include: (1) the model simulates metabolic elimination of benzene, but not the rates of formation of major metabolites; and (2) the model does not simulate fate of metabolites formed and, therefore, cannot be used to predict concentrations of metabolites in tissues.

Fisher et al. 1997

Description of the Model. The Fisher et al. (1997) model extends the model reported by Travis et al. (1990) to include a simulation of lactational transfer of benzene to breast milk in humans. Other tissues simulated include blood, fat, liver, lung, other slowly-perfused tissues, and rapidly-perfused tissues. Absorption and excretion of benzene in the lung and exchange of benzene between blood and tissues are assumed to be flow-limited, as is excretion of benzene in breast milk. The lactational transfer model includes simulations of breast milk production and loss from nursing; the latter is represented as a first-order process. Estimates of blood:air and blood:milk partition coefficients during lactation (from which the milk:blood partition coefficient could be calculated) were measured in nine lactating subjects (Fisher

et al. 1997). The model simulates capacity-limited (i.e., Michaelis-Menten) metabolism of metabolic elimination of benzene as a function of the concentration of benzene in liver. Rates of formation of specific metabolites and their disposition (e.g., excretion) are not simulated. Metabolism parameter values (K_m,V_{max}) and tissue:blood partition coefficients were derived from Travis et al. (1990).

Risk Assessment. This model has been used to predict benzene concentrations in breast milk and lactational transfers to breastfeeding infants (Fisher et al. 1997). Exposures to the threshold limit value (TLV) (10 ppm, 8 hours/day, 5 days/week) were predicted to yield 0.053 mg of benzene in breast milk per 24 hours. This approach has potential applicability to assessing lactational exposures to infants resulting from maternal exposures.

Validation of the Model. The lactation model was evaluated (Fisher et al. 1997) by comparing predictions for perchloroethylene (not benzene) with those predicted by a perchloroethylene model developed by Schreiber (1993). Other components of the biokinetics model were derived from the Travis et al. (1990) model, which has undergone evaluations against data obtained from studies in humans.

Target Tissues. The model simulates concentrations of benzene in blood, breast milk, liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues as well as rates of metabolic elimination of benzene. It does not simulate concentrations of metabolites in these tissues and does not simulate metabolism in bone marrow, a major target of benzene metabolites.

Species Extrapolation. The model has been applied to simulations for humans (Fisher et al. 1997).

High-low Dose Extrapolation. The lactational model has not been evaluated for simulating inhalation exposures to benzene in humans; therefore, applicability to high-low dose extrapolations cannot be assessed.

Inter-route Extrapolation. The model was developed to simulate inhalation exposures. Extrapolation to other routes (e.g., oral, dermal) would require the extension of the model to include simulations of absorption from these routes.

Strengths and Limitations. Strengths of the model are that it simulates the disposition of inhaled benzene in females during lactation, including transfers of benzene to breast milk and nursing infants; concentrations of benzene in blood and tissues; and rates of elimination of benzene metabolites.

Limitations of the model include that the model does not simulate rates of formation of major metabolites and that the model does not simulate kinetics of uptake or metabolism of benzene in bone marrow, a major target of benzene toxicity.

Sinclair et al. 1999

Description of the Model. The Sinclair et al. (1999) model is an extension of the human model developed by Travis et al. (1990) to include a simulation of first-order urinary excretion of total metabolites and phenol, and dermal absorption of benzene.

Risk Assessment. The model has been applied to predicting the levels of benzene in exhaled air and phenol in urine in workers exposed to benzene (Sherwood and Sinclair 1999; Sinclair et al. 1999).

Validation of the Model. The model was evaluated against measurements of benzene in exhaled breath and urinary excretion of phenol in workers who were exposed to benzene at concentrations of 1–1,100 ppm (Sherwood and Sinclair 1999; Sinclair et al. 1999).

Target Tissues. The model simulates amounts and concentrations of benzene in blood, bone marrow (a target tissue), liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues; rates of metabolic elimination of benzene in liver and bone marrow; and excretion of total metabolites formed and phenol.

Species Extrapolation. The model has been applied to simulations for humans (Sinclair et al. 1999).

High-low Dose Extrapolation. The model was evaluated against observations of benzene in exhaled breath and urinary excretion of phenol in workers who were exposed to benzene at concentrations of 1–1,100 ppm (Sherwood and Sinclair 1999; Sinclair et al. 1999).

Inter-route Extrapolation. The model simulates inhalation, oral, and dermal exposures.

Strengths and Limitations. Strengths of the model are that it extends the Travis et al. (1990) model to include simulation of dermal absorption of benzene.

Bois et al. 1991a

Description of the Model. The Bois et al. (1991a) model simulates absorption and disposition of benzene and the benzene metabolite, phenol, in the rat. Tissues simulated include the blood, bone marrow, fat, liver, lung, other slowly-perfused tissues, and other rapidly-perfused tissues. Gastrointestinal absorption of benzene and phenol are simulated as a first-order function for dose. Absorption and excretion of benzene in the lung are assumed to be flow-limited as are exchanges of benzene and phenol between blood and tissues. Excretion of phenol is simulated as a first-order transfer to urine. The model simulates capacity-limited (i.e., Michaelis-Menten) and first-order metabolism of benzene and metabolites in bone marrow, liver, gastrointestinal tract, and respiratory tract (see Table 3-1). All pathways are assumed to be capacity-limited reactions, except for the spontaneous hydrolysis of benzene oxide to form phenol, which is simulated as a first-order process. The model simulates rates of formation of metabolites and first-order excretion of phenol; however, disposition (e.g., excretion) of other metabolites is not simulated. Parameter values, including metabolism parameter values, were optimized to a reference set of observations of metabolites formed in rats exposed by inhalation or to single gavage doses of benzene (see below).

Risk Assessment. This model has been used to predict amounts of benzene and phenol metabolites formed in rats during gavage exposures to benzene equivalent to those administered in NTP (1986) and to inhalation exposures equivalent to the Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) (Bois and Paxman 1992; Bois et al. 1991a). Model simulations indicate that dose rate may be an important factor in benzene toxicity. For example, when the model was applied to simulations for rats exposed either for 15 minutes to a benzene vapor concentration of 32 ppm or for 8 hours to 1 ppm (equivalent 8-hour TWAs), the amount of metabolites (hydroquinone, catechol, and muconaldehyde) formed was 20% higher after the 15-minute exposure at the higher level than after the 8-hour exposure at the lower level (Bois and Paxman 1992). These metabolites have been identified as being important in the genesis of bone marrow toxicity after benzene exposure (Eastmond et al. 1987). These types of analyses, if extended to humans, would be applicable to evaluations of the adequacy of short-term exposure limits.

Validation of the Model. The model was calibrated (Bois and Paxman 1992; Bois et al. 1991a) with observations made in rats exposed to single gavage doses of benzene, or to inhalation exposures of 13–870 ppm (Sabourin et al. 1987, 1989b), in rats administered single parenteral doses of phenol (Cassidy

and Houston 1984), and in *in vitro* metabolism studies (Sawahata and Neal 1983). Further evaluations against data not used in the calibration were not reported.

Target Tissues. The model simulates amounts and concentrations of benzene and phenol in bone marrow, a target tissue for benzene metabolites, as well as in blood, liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues. The model also simulates amounts of specific metabolites formed and urinary excretion of the major urinary metabolite, phenol. It does not simulate concentrations of metabolites, other than phenol, in these tissues.

Species Extrapolation. The model has been applied to simulations for rats. A human model has been developed that implements a scaled-down version of the rat metabolism model (see Bois et al. 1996).

High-low Dose Extrapolation. The model has been evaluated for simulating inhalation exposures in rats ranging from 13 to 870 ppm and gavage doses of 15–300 mg/kg.

Inter-route Extrapolation. The model simulates inhalation and oral exposures and has been applied to predicting internal dose metrics (e.g., amounts of metabolites formed) resulting from exposures by these routes.

Strengths and Limitations. Strengths of the model are that it simulates disposition of inhaled and ingested benzene (and phenol), including rates and amounts of most of the major metabolites formed in rats. Limitations include: (1) the model has not been evaluated for multiple exposures; (2) although the model simulates the fate of benzene and phenol, it does not simulate the fate of other metabolites formed and, therefore, cannot be used to predict concentrations of these metabolites in tissues; and (3) the model, as configured in Bois et al. (1991a), does not simulate benzene disposition in humans.

Bois et al. 1996

Description of the Model. The Bois et al. (1996) model simulates inhalation absorption and disposition of benzene in humans. Tissues simulated include the blood, bone marrow, fat, liver, lung, other slowly-perfused tissues, and other rapidly-perfused tissues. Absorption and excretion of benzene in the lung are assumed to be flow-limited as are exchanges of benzene between blood and tissues. The model simulates metabolic elimination of benzene as a single capacity-limited (i.e., Michaelis-Menten) reaction, occurring in bone marrow and liver. Endogenous formation of phenolic metabolites is also simulated as a zero-

order process occurring in liver. The model simulates first-order excretion of total metabolites and the phenol fraction (approximately 80% of total). Parameter values (physiological and chemical) were estimated by Bayesian optimization techniques (Markov Chain Monte Carlo analysis) using reference observations of benzene concentration in blood and urinary excretion of phenol in human subjects who were exposed to benzene in air (Pekari et al. 1992).

Risk Assessment. The model has been used to predict rates and amounts of benzene metabolized in human populations (Bois et al. 1996). The population model (population geometric means and SDs of parameter values) was derived using Markov Chain Monte Carlo analysis with observations from three human subjects serving as the reference data for inter-individual variability (from Pekari et al. 1992). The population model predicts probability distributions of model outputs (for example, rates or amounts of benzene metabolized for a given exposure). This approach could be used to evaluate uncertainty factors in risk assessments intended to account for uncertainties in our understanding of benzene pharmacokinetics variability.

Validation of the Model. The model was calibrated with observations of benzene concentrations in blood and urinary phenol levels, made in three human subjects who were exposed to 1.7 or 10 ppm benzene for 4 hours (Pekari et al. 1992). Further evaluations against data not used in the calibration have not been reported.

Target Tissues. The model simulates amounts and concentrations of benzene in bone marrow, a target of benzene toxicity, as well as blood, liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues. Amounts of total metabolites formed and excreted are simulated; however, the model does not simulate concentrations of metabolites in these tissues.

Species Extrapolation. The model has been applied to simulations for humans (Bois et al. 1996).

High-low Dose Extrapolation. The model has been evaluated for simulating inhalation exposures in humans ranging from 1.7 to 10 ppm (Bois et al. 1996).

Inter-route Extrapolation. The model simulates inhalation exposures. Extrapolation to other routes (e.g., oral, dermal) would require the extension of the model to include simulations of absorption from these routes.

Strengths and Limitations. Strengths of the model are that it simulates disposition of inhaled benzene and rates of total metabolism in humans. Limitations include that the model has not been evaluated for multiple exposures and that the model simulates total metabolism of benzene, and not the rates of formation of the major metabolites of benzene of toxicological interest.

Brown et al. 1998

Description of the Model. The Brown et al. (1998) model simulates inhalation absorption and disposition of benzene in humans. Tissues simulated include the blood, fat, liver, lung, other slowly-perfused tissues, and other rapidly-perfused tissues. Absorption and excretion of benzene in the lung and exchange of benzene between blood and tissues are assumed to be flow-limited. The model simulates capacity-limited (i.e., Michaelis-Menten) metabolic elimination of benzene as a function of the concentration of benzene in liver. Rates of formation of specific metabolites, or their disposition (e.g., excretion), are not simulated. For the purpose of comparing model predictions to observations, 80% of the total metabolites formed and excreted in urine (i.e., amount of benzene eliminated by metabolism) in 24 hours was assumed to be phenol. The K_m parameter for metabolism was derived from Travis et al. 1990; the V_{max} was estimated by optimization of the model to observations of blood concentrations of benzene and benzene in exhaled breath of female and male subjects who were exposed to 25 ppm benzene for 2 hours (Sato et al. 1975). Partition coefficients for males and females were derived from vial equilibrium studies conducted on blood and/or tissues from males and females (Fisher et al. 1997; Paterson and Mackay 1989).

Risk Assessment. This model has been used to predict the benzene concentrations in blood and amounts of benzene metabolized in females and males who experience the same inhalation exposure scenarios. Females were predicted to metabolize 23–26% more benzene than similarly-exposed males. This difference was attributed, in part, to a higher blood:air partition coefficient for benzene in females.

Validation of the Model. The model was calibrated by comparing predictions of blood concentrations of benzene and benzene in exhaled breath of female and male subjects who were exposed to 25 ppm benzene for 2 hours (Brown et al. 1998; Sato et al. 1975). Further evaluations against data not used in the calibration were not been reported.

Target Tissues. The model simulates concentrations of benzene in blood, liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues as well as rates of metabolic

elimination of benzene. It does not simulate concentrations of metabolites in these tissues and does not simulate metabolism in bone marrow, a major target of benzene metabolites.

Species Extrapolation. The model has been applied to simulations for humans.

High-low Dose Extrapolation. The model has been evaluated for simulating inhalation exposures in humans. Evaluations of predictions included observations made during inhalation exposures to 25 ppm (Brown et al. 1998; Sato et al. 1975).

Inter-route Extrapolation. The model simulates inhalation and has been applied to predicting internal dose metrics (e.g., benzene concentration in blood, amount benzene metabolized) resulting from exposures by this route (Brown et al. 1998). Extrapolation to other routes (e.g., oral, dermal) would require the extension of the model to include simulations of absorption from these routes.

Strengths and Limitations. Strengths of the model are that it simulates disposition of inhaled benzene in female and male humans as well as the concentrations of benzene and rates of elimination of benzene metabolites. Limitations of the model include: (1) the model does not simulate rates of formation of major benzene metabolites; (2) the model does not simulate fate of metabolites formed and, therefore, cannot be used to predict concentrations of metabolites in tissues; and (3) the model does not simulate kinetics of uptake or metabolism of benzene in bone marrow, a major target of benzene toxicity.

Cole et al. 2001

Description of the Model. The Cole et al. (2001) model simulates absorption and disposition of benzene in the mouse. Tissues simulated include the blood, fat, liver, lung, other slowly-perfused tissues, and other rapidly-perfused tissues. Gastrointestinal absorption of benzene is simulated as a first-order process. Absorption and excretion of benzene in the lung are assumed to be flow-limited as are exchanges of benzene between blood and tissues. The model simulates capacity-limited (i.e., Michaelis-Menten) and first-order metabolism of benzene and metabolites in liver (see Table 3-1). Capacity-limited reactions in bone marrow and liver include benzene to benzene oxide, phenol to hydroquinone, phenol to catechol, catechol to trihydroxybenzene, and conjugation of phenol and hydroquinone. First-order reactions in liver include conversion of benzene oxide to phenol, muconic acid, and phenylmercapturic acid. The model simulates rates of formation of metabolites, tissue distribution of benzene oxide, phenol, and hydroquinone; and first-order excretion of metabolites in urine. Capacity-limited metabolism

parameter values were estimated from *in vitro* studies of mouse liver (Lovern et al. 1999; Nedelcheva et al. 1999; Seaton et al. 1995); first-order parameters were estimated by optimization of model output to observations of metabolites formed in mice exposed by inhalation or to single gavage doses (Kenyon et al. 1995; Mathews et al. 1998; Sabourin et al. 1988). Blood:tissue partition coefficients for benzene and metabolites were derived from Medinsky et al. (1989a) or estimated based on the n-octanol-water partition coefficient (Poulin and Krishnan 1995).

Risk Assessment. This model has been used to predict amounts of benzene exhaled and amounts of benzene metabolites produced in mice during inhalation exposures or following gavage exposures to benzene (Cole et al. 2001).

Validation of the Model. The model was calibrated with observations made in mice exposed to single gavage doses of benzene, or to inhalation exposures (Cole et al. 2001; Kenyon et al. 1995; Mathews et al. 1998; Sabourin et al. 1988). Further evaluations against data not used in the calibration have not been reported.

Target Tissues. The model simulates amounts and concentrations of benzene in blood, liver, fat, and lumped compartments for other rapidly perfused and slowly perfused tissues; rates of formation of metabolites; tissue distribution of benzene oxide, phenol, and hydroquinone; and first-order excretion of metabolites in urine. It does not simulate concentrations of metabolites in bone marrow, a target tissue for benzene metabolites.

Species Extrapolation. The model has been applied to simulations for mice (Cole et al. 2001).

High-Low Dose Extrapolation. The model has been evaluated for simulating inhalation exposures in mice (50 ppm) and gavage doses of 0.1–100 mg/kg (Cole et al. 2001; Kenyon et al. 1995; Mathews et al. 1998; Sabourin et al. 1988).

Inter-route Extrapolation. The model simulates inhalation and oral exposures and has been applied to predicting internal dose metrics (e.g., amounts of metabolites formed) resulting from exposures by these routes (Cole et al. 2001).

Strengths and Limitations. Strengths of the model are that it simulates disposition of inhaled and ingested benzene, including rates and amounts of major metabolites. Most of the metabolism parameter

values were derived empirically from *in vitro* studies, rather than by model optimization. Limitations include: (1) the model has not been evaluated for multiple exposures; (2) the model does not simulate the metabolism of benzene in bone marrow, a major target of benzene toxicity; and (3) the model, as configured in Cole et al. (2001), does not simulate benzene disposition in humans.

Three studies have expanded or enhanced the Cole et al. (2001) mouse PBPK model (Knutsen et al. 2013a, 2013b; Manning et al. 2010; Yokley et al. 2006). Yokley et al. (2006) estimated parameter values for humans, including human population distributions for several metabolism parameters. Knutsen et al. (2013a, 2013b) expanded the Yokley et al. (2006) human model to include two additional compartments representing bone marrow and urinary bladder. This enabled dosimetry predictions for benzene and metabolites in bone marrow and provided a compartment for simulating background levels (e.g., pre-exposure) of benzene metabolite conjugates in urine. Manning et al. (2010) extended the Cole et al. (2001) mouse model to include a kidney compartment and subdivided the liver compartment into three zones to represent heterogeneous distribution of enzymes that participate in the production of benzene metabolites.

Yokley et al. (2006)

Yokley et al. (2006) estimated human population distributions of metabolism parameters for the Cole et al. (2001) model. Parameters evaluated included the specific activity of CYP2E1 in liver (V2E1), maximum rates of conjugation of phenol (VPH1, VPH2) and hydroquinone (VHQ), and first-order clearances for formation of phenylmercapturic acid (k3) and muconic acid (k4) from benzene oxide. Data from *in vitro* studies of human liver tissue were used to establish prior log-normal distributions for V2E1, VPH1, VPH2, and VHQ. Parameters k3 and k4 were assigned log-normal prior distributions with means equal to the mouse model values and SDs of 0.1 and 2, respectively. Posterior distributions were computed from Markov Chain Monte Carlo simulations with calibration data from human subjects. These data included a clinical study in which three subjects were exposed to 1.7 or 10 ppm benzene for 4 hours and benzene in blood and exhaled air were measured (Pekari et al. 1992), and an occupational study in which benzene metabolites in urine were monitored in 35 workers who were exposed to 25 ppm benzene during their work shifts (Rothman et al. 1998; Waidyanatha et al. 2004). The population model predicted the observed variability in blood benzene and exhaled benzene and urinary levels of muconic acid, phenylmercapturic acid, phenol, and hydroquinone; however, it underpredicted urinary catechol and benzenetriol levels.

Manning et al. (2010)

Manning et al. (2010) developed a PBPK model of benzene and its major metabolites benzene oxide, phenol, and hydroquinone. The model is an extension of the Cole et al. (2001) model, with the addition of a kidney compartment, and expansion of the liver compartment to include three sub-compartments. The three liver compartments were included in the model to simulate the heterogeneous distribution of CYP2E1 and sulfotransferases. CYP2E1 is more strongly expressed in the pericentral region of the liver and sulfotransferases are more strongly expressed in the periportal region of the liver (Ingelman-Sundberg et al. 1988; Tsutsumi et al. 1989). This heterogeneous, or zonal, distribution of enzymes is thought to give rise to different metabolic patterns following an external dose of benzene or phenol (Hoffmann et al. 1999; Koop et al. 1989). Following an external dose of phenol, sulfotransferases in the periportal region of the liver convert a large fraction of the absorbed dose to sulfate esters before it can be delivered to the pericentral region of the liver where it can be metabolized to benzene oxide through the CYP2E1 pathway and to downstream metabolites, including hydroquinone. Following an external dose of benzene, phenol is formed in the pericentral region of the liver and, as a result, a larger fraction of the benzene dose is converted to hydroquinone.

The three-compartment liver model has flow-limited transfer of chemical from blood to liver compartment 1, representing the periportal region, through an intermediate compartment 2, to compartment 3, representing the pericentral region. Each compartment is assumed to comprise one-third of the total volume of the liver. Activities of hepatic sulfotransferase and glucuronyltransferase are assigned to compartment 1, whereas CYP2E1, epoxide hydrolase, and GST activities are assigned to compartment 3. Non-enzymatic conversion of benzene oxide to phenol is assumed to occur in all three compartments. Sulfate and glucuronic acid conjugates of phenol and hydroquinone are formed in liver compartment 1. Metabolites formed in compartment 3 include the CYP2E1 metabolites benzene oxide (from phenol), hydroquinone and catechol (from phenol), and benzenetriol (from hydroquinone), the epoxide hydrolase metabolite of benzene oxide (muconic acid), and the GST metabolite of benzene oxide (phenyl-mercapturic acid). The kidney compartment is assigned 10% of the CYP2E1 activity relative to the liver. Formation of phenylmercapturic acid is assumed to occur in blood, kidney, fat, slowly perfused tissue, and rapidly perfused tissues.

Tissue/blood partition coefficients for benzene metabolites, phenol and hydroquinone, were estimated from physical-chemical properties (Poulin and Krishnan 1995). Phenol and hydroquinone were assumed to bind in all tissues. Binding was represented in the model with first-order clearance terms, which were

optimized. Information regarding tissue/blood partition coefficients for benzene was taken from the literature (Medinsky et al. 1989a). The partition coefficients for benzene were used for benzene oxide. Parameters governing CYP2E1 and conjugation rates (Km, Vmax) were scaled to the whole liver from estimates made in *in vitro* studies (Lovern et al. 1999; Seaton et al. 1995). First-order clearances for GST-mediated formation of phenylmercapturic acid and epoxide hydrolase-mediated formation of muconic acid were optimized.

Data used in optimizing the model were derived from benzene oral dosing studies (Henderson et al. 1989; Kenyon et al. 1995; Mathews et al. 1998; Sabourin et al. 1987) and inhalation studies (Sabourin et al. 1988) conducted in mice. The introduction of three liver compartments to account for zonal distribution of metabolism improved some aspects of performance of the model at predicting dose-dependent metabolism of benzene. For example, it improved agreement between observed and predicted benzene concentrations in liver and phenol concentrations in blood following inhalation of benzene, and predictions of formation of phenol and hydroquinone conjugates following oral dosing with benzene.

Knutsen et al. (2013a, 2013b)

Knutsen et al. (2013a, 2013b) expanded the Yokley et al. (2006) human model to include two additional compartments representing bone marrow and urinary bladder. Saturable metabolism was assumed for the formation of benzene oxide from benzene, hydroquinone and catechol from phenol, benzenetriol from catechol and hydroquinone, and conjugates. Bone marrow is assumed to oxidize to benzene oxide at approximately 4% of the hepatic maximal rate. Maximal rates for all other saturable conversions in liver and bone marrow (per mg tissue protein) were assumed to be proportional to tissue masses. First-order metabolic clearance was assumed for formation of muconic acid and phenylmercapturic acid from benzene oxide and phenol from benzene oxide, with first-order clearance rates identical in liver and bone marrow. The bladder compartment receives conjugated metabolites resulting from exposure to benzene and was assigned values for background levels of metabolites expected in the absence of exposure to benzene. Background levels of urinary metabolites were assigned values based on measurements made in humans who were not exposed to benzene (Waidyanatha et al. 2004).

Metabolism parameters for CYP2E1 were assigned initial values from quantitative studies of human liver CYP2E1 (Lipscomb et al. 2003a, 2003b) and all metabolism parameters were calibrated to achieve agreement with measurements of urinary benzene metabolites in workers exposed to benzene during the work shift (Waidyanatha et al. 2004). The calibrated model was validated by comparing observed levels

of benzene in blood and exhaled air in three subjects who inhaled benzene (1.9 and 9.4 ppm) for 4 hours (Pekari et al. 1992) and observed and predicted urinary metabolite levels measured in workers exposed to benzene (Kim et al. 2006a).

In comparison to the Yokley et al. (2006) human model, calibration of the Knutsen et al. (2013a, 2013b) model resulted in lower values for the maximal rate of metabolism of phenol and first-order clearance of benzene to benzene oxide and higher values for first-order clearance of phenol to catechol and hydroquinone to benzenetriol. Good agreement was achieved between observed and predicted levels of benzene in blood and exhaled air, and benzene metabolites in urine (Kim et al. 2006a; Pekari et al. 1992). Knutsen et al. (2013a, 2013b) did not report the sensitivity of these predictions (exhaled benzene or urinary metabolites) to model parameters that govern metabolite doses to marrow. Therefore, it is possible that the model could reliably simulate exhaled benzene and urinary metabolites while not reliably predicting metabolite doses to marrow. A contributor to this uncertainty is the relatively small contribution of marrow to benzene metabolism (marrow metabolism is assumed to be 4% of liver metabolism). The model was used to compare predicted blood and bone marrow metabolite exposures resulting from an 8-hour exposure to air concentrations of benzene of 5–100 ppm. The total metabolites formed (24-hour AUC) were higher in blood compared to bone marrow. Both compartments exhibited saturation kinetics, with saturation in bone marrow predicted at lower exposures. The model is configured to simulate kinetics following an inhalation exposure; there is no gastrointestinal tract compartment for simulating oral exposure.

Majumdar et al. (2016)

Majumdar et al. (2016) modified a PBPK model of tetrachloroethylene (Bernillon and Bois 2000) to create a human PBPK model for benzene. The compartment structure is identical to the Cole et al. (2001) model and includes lung, liver, adipose, and lumped compartments representing other slowly perfused tissue and rapidly perfused tissues. The model simulates capacity-limited metabolism of benzene (V_{max}, K_m) in liver. Excretion of total metabolites in urine is simulated as a fist-order function (minute^{-1}) of the concentration of total metabolites formed in liver. Urinary *trans*,*trans*-muconic acid is calculated as a fraction of total amount of metabolites excreted in urine. The sources for the benzene parameters (e.g., partition coefficients and metabolism) are reported in Table 1 of Majumdar et al. (2016). Comparisons of model predictions to observations were not reported. Majumdar et al. (2016) applied the model to predict the benzene body burden associated with measured levels of exposure and urinary *trans*,*trans*-muconic acid levels, and corresponding cancer risks, in a groups of petrol pump attendants and automobile drivers.

Pech et al. (2023a, 2023b) applied the Majumdrar et al. (2016) model to estimate benzene exposures from urinary *trans*,*trans*-muconic acid measurements, and corresponding cancer and noncancer risks, in children who resided in homes that also served as shoe-making workshops.

3.1.6 Animal-to-Human Extrapolations

Pathways of benzene metabolism are generally similar among various rodent and nonhuman primate species. However, species differences exist regarding capacity to metabolize benzene and relative proportions of various benzene metabolites formed.

Species differences exist in absorption and retention of benzene. For example, following 6-hour exposures to low concentrations (7–10 ppm) of benzene vapors, mice retained 20% of the inhaled benzene, whereas rats and monkeys retained only 3–4% (Sabourin et al. 1987, 1992). Mice exhibit a greater overall capacity to metabolize benzene, compared to rats. Inhalation exposure to 925 ppm resulted in an internal dose of 152 mg/kg in mice, approximately 15% of which was excreted as parent compound, and an internal dose of 116 mg/kg in rats, approximately 50% of which was excreted unchanged (Henderson et al. 1992; Sabourin et al. 1987).

The proportions of benzene metabolites produced depend on both species and exposure concentration. Hydroquinones and muconic acid (potential sources of benzene toxicity) were detected in much higher concentrations in the blood, liver, lung, and bone marrow of mice than rats, following a 6-hour inhalation exposure to benzene at a concentration of 50 ppm (Sabourin et al. 1988). It is generally understood that metabolic profiles of benzene in mice and humans are more similar than those of humans and rats. Sabourin et al. (1989a) noted increased production of detoxification metabolites (phenylglucuronide and prephenylmercapturic acid) and decreased production of potentially toxic metabolites (hydroquinones and muconic acid) in both mice and rats exposed to benzene at much higher concentrations (600 ppm in air or 200 mg/kg orally), which indicates that extrapolation of toxicological results from studies using high exposure concentrations to low exposure scenarios may result in an underestimation of risk.

PBPK models have been explored for applications of animal-to-human extrapolations of benzene dosimetry (Bois et al. 1991a, 1996; Cole et al. 2001; Medinsky 1995; Medinsky et al. 1989a, 1989b, 1989c; Travis et al. 1990). Each model simulates benzene metabolism in multiple compartments, including production of hydroquinone and muconaldehyde in the liver, with further metabolism in the

bone marrow. However, the models are not sufficiently refined to allow confident predictions of the kinetics of benzene metabolism in humans.

3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Children may be more or less susceptible than adults to health effects from exposure to hazardous substances and the relationship may change with developmental age.

This section also discusses unusually susceptible populations. A susceptible population may exhibit different or enhanced responses to certain chemicals than most persons exposed to the same level of these chemicals in the environment. Factors involved with increased susceptibility may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters can reduce detoxification or excretion or compromise organ function.

Populations at greater exposure risk to unusually high exposure levels to benzene are discussed in Section 5.7, Populations with Potentially High Exposures.

Several factors may contribute to alterations in the toxicity of benzene. These include age-related differences, genetic polymorphisms, and underlying conditions, as discussed below.

Age-Related Differences. The adverse health effects of benzene are due to reactive metabolites. At early stages of human development, metabolic pathways may not be fully functional, which might result in a lower level of susceptibility to benzene. In the elderly, metabolic pathways become less functional, which may lead to lower susceptibility. No clear evidence of age-related differences in susceptibility to benzene toxicity was located. Fetuses may be exposed as benzene crosses the placenta and is found in cord blood at concentrations that equal or exceed those of maternal blood (Dowty et al. 1976). In a study of rats exposed to 20 ppm benzene on GD 1–15, circulating erythroid precursors decreased and granulocytic precursor cells increased in neonates and 6-week-old pups (Keller and Snyder 1988). However, no information is available on effects in offspring of humans exposed to benzene *in utero*. In addition, nursing infants can be exposed to benzene in the breast milk (Fabietti et al. 2004).

Children could potentially be at increased risk for higher benzene exposure via the inhalation route based on higher activity levels and ventilation rates than adults. However, very limited information was located to indicate that children are at increased risk for benzene toxicity. Age-related differences in benzene metabolism could potentially affect susceptibility. Results of one human study indicate that CYP2E1, a major enzyme involved in benzene metabolism, is not present in the fetus, but appears in rapidly increasing concentrations during early postnatal development (Vieira et al. 1996). This suggests that fetuses and neonates may be at decreased risk of benzene toxicity due to a reduced metabolic capacity. No information was located regarding potential age-related differences in pharmacodynamic processes such as benzene-target interactions in the hematopoietic system.

As discussed in Section 2.17, studies in animals have identified several developmental effects following gestational exposure. These effects include decreased fetal weight, increased skeletal variations, alterations in hematological parameters, neurodevelopmental effects, and altered glucose homeostasis. Due to the very limited data in humans, human data are inadequate to verify or refute findings in animals. However, given that benzene is ubiquitous in the environment and cigarette smoke is a common and important source of benzene exposure, the potential for developmental effects in humans should be considered.

Sex-related Differences. Studies in humans found that the elimination of benzene is slower in women than in men, likely due to the higher percentage and distribution of body fat tissue (Sato et al. 1975). On the other hand, an association between urinary benzene metabolite levels and insulin resistance in elderly adults demonstrated a stronger relationship in men than in women (Choi et al. 2014). Furthermore, sex differences were observed upon occupational benzene exposure, particularly effects related to biotransformation of benzene to *trans*,*trans*-muconic acid and hematological parameters (Moro et al. 2017).

Genetic Polymorphisms. More recent studies of workers indicate that susceptibility to benzene-induced toxicity and genetic damage may be associated with polymorphisms in multiple genes. Information in workers was identified for polymorphisms in genes encoding for enzymes involved in benzene metabolism, DNA repair enzymes, and cytokines. The studies discussed below have evaluated associations between genetic polymorphisms and effects in workers. Results indicate that various subpopulations based on polymorphisms may be more susceptible to benzene-induced toxicity.

Polymorphisms of enzymes involved in benzene metabolism. Genetic polymorphisms exist for several genes encoding for enzymes involved in the metabolism of benzene. As discussed below, studies in workers have assessed the effects of polymorphisms on benzene toxicity for genes encoding for NQO1, GST, epoxide hydrolase, and MPO (De Palma and Manno, 2014).

The flavoenzyme, NQO1, catalyzes the reduction of 1,2- and 1,4-benzoquinone (reactive metabolites of benzene) to catechol and hydroquinone, respectively (Nebert et al. 2002), thus protecting cells from oxidative damage by preventing redox cycling. The NQO1*1 (wild-type) allele codes for normal NQO1 enzyme and activity. An NQO1*2 allele encodes a nonsynonymous mutation that has negligible NQO1 activity (NQO1 null). Approximately 5% of Caucasians and African Americans, 16% of Mexican-Americans, and 18–20% of Asians are homozygous for the NQO1*2 allele (Kelsey et al. 1997; Smith and Zhang 1998). Rothman et al. (1997) evaluated the relationship of NQO1 polymorphism on hematotoxicity in a case-control study of 50 benzene-exposed workers and 50 controls in China. Results indicate that workers with the NQO1*2 allele were at an increased risk of hematotoxicity. Similar results were observed in a cross-sectional study of 250 shoe factory workers in China, with lower leukocyte counts observed in NQO1 null workers compared to NQO1 wild-type workers (Lan et al. 2004a). In a study of a population of Bulgarian petrochemical workers (i.e., 158 and 50 controls; 208 total individuals), benzene exposure in workers with the NQO1-null allele showed an increased frequency of DNA single-strand breaks, compared to controls (Garte et al. 2008).

Glutathione-S-transferases (GSTs), which are involved in benzene oxide metabolism to the less toxic form PhMA, have several genotypes. In a cross-sectional study, Nourozi et al. (2018) assessed how positive (e.g., wild-type) and null polymorphisms of glutathione genotypes GSTP1, GSTT1, and GSTM1 may alter the hematological effect of benzene in a cross-sectional study of 124 workers and 184 controls at a petrochemical plant in Iran. Results showed that workers with GSTT1-null genotype and combined GSTT1-null and GSTM1-null genotypes had an increased risk of hematological effects. Garte et al. (2008) reported an increased frequency of DNA single-strand breaks in 158 Bulgarian petrochemical workers with GSTT1 and GSMT1 variants.

Epoxide hydrolase (EH) plays an important role in benzene metabolism by converting benzene derived epoxides to more water-soluble derivatives that are less toxic. Thus, EH-null phenotypes that result in decreased conversion of toxic epoxides have the potential to cause increased hematotoxicity. A case-control study of 268 workers with benzene hematotoxicity and 268 without hematotoxicity evaluated associations with epoxide hydrolase polymorphisms (Sun et al. 2007). EH-null haplotypes 2 (with a

AGAC substitution), 4 (with a GAGT substitution), and 6 (with a GGGT substitution) were associated with an increased risk of hematotoxicity.

In the benzene metabolism scheme, MPO catalyzes the oxidation of phenol to the reactive metabolites, catechol and hydroquinone. Polymorphisms of MPO that result in a decrease in this reaction could decrease the toxicity of benzene. In a study of 250 shoe factory workers in China, workers expressing the MPO-null gene had an increased risk of hematotoxicity (Lan et al. 2004a). Lower leukocyte counts were observed in MPO-null workers compared to MPO wild-type workers.

Cytokines. Several cytokines, chemokines, and cellular adhesion molecules are involved in hematopoiesis. Thus, polymorphisms for the genes encoding for these molecules have the potential to alter hematotoxicity of benzene. Lan et al. (2005) evaluated associations between several of these molecules, including several interleukins (IL) and vascular cell adhesion molecule 1 (VCAM1), and cell counts for granulocytes, lymphocytes, and $CD4^+$ T-cells in a cross-sectional study of 250 exposed shoe workers and 140 controls in China. The following inverse associations were observed between cell counts and polymorphisms: total lymphocyte counts and IL-4, IL-12A, and VCAM1; granulocyte counts and IL-1A, IL-4, IL-10, CSF3 $CD4^+$ and $CD8^+$, T-cells, and VCAM1.

DNA repair enzymes. Several enzymes are involved in the repair of oxidative damage to DNA, producing increased formation of the damaged DNA product, 7,8-dihydro-8-oxoguanine (8-oxoG). In humans, three enzymes are involved in the repair of this damage: hMTH1, hOGG1, and hMYH genes. Thus, genetic polymorphisms of these enzymes could alter susceptibility to the effects of benzene. A cross-sectional study evaluated polymorphisms of the genes for three repair enzymes, hMTH1, hOGG1, and hMYH in 152 chronic benzene poisoning patients and 152 healthy workers exposed to benzene (Wu et al. 2008). In the population, polymorphisms of hMTH1 and hMYH were associated with an increased risk of toxicity (depression of peripheral leukocyte counts).

Underlying Health Conditions. Individuals with medical conditions that include reduced bone marrow function, decreased blood factors, or low blood cell counts would be at increased risk for benzene toxicity. Treatments for certain medical conditions might result in decreases in blood cell counts, which could lead to increased susceptibility to benzene poisoning. Specific studies evaluating how benzene may affect patients with underlying hematopoietic diseases or conditions were not identified. However, it is hypothesized that individuals with underlying hematopoietic conditions or diseases would be at increased risk. For example, suppression of immune cells is a well-established effect of numerous cancer

treatments. Benzene exposure in immunosuppressed patients would likely increase susceptibility for benzene-induced hematotoxicity. In addition, individuals with anemia may be more susceptible to the hematopoietic effects of benzene.

3.3 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility (NAS/NRC 2006).

The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/exposurereport/). If available, biomonitoring data for benzene from this report are discussed in Section 5.6, General Population Exposure.

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 2006). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to benzene are discussed in Section 3.3.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that (depending on magnitude) can be recognized as an established or potential health impairment or disease (NAS/NRC 2006). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by benzene are discussed in Section 3.3.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the

biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.2, Children and Other Populations that are Unusually Susceptible.

3.3.1 Biomarkers of Exposure

Several biomarkers have been identified to demonstrate exposure to benzene. Unmetabolized benzene can be detected in the expired air and urine of humans exposed to benzene vapors (Farmer et al. 2005; Fustinoni et al. 2005; Sherwood 1988; Waidyanatha et al. 2001). In addition to unmetabolized benzene, urinary metabolites of benzene, including phenol, *trans,trans*-muconic acid (or urinary *trans,trans*-muconic acid, also reported as t,t-MA), and S-phenyl mercapturic acid (PhMA or urinary PhMA, also reported as SPMA), are commonly used as biomarkers of exposure (Boogard et al. 2022; Chaiklieng et al. 2021; Daugheri et al. 2022; Lovreglio et al. 2011). Urinary pre-PhMA has recently been assessed as a biomarker for benzene exposure (Bowman et al. 2023). Historically, urinary phenol was most often used to monitor benzene exposure; however, it is not specific for exposure to benzene and is a metabolic product of other chemicals (Astier 1992; Inoue et al. 1986, 1988; Karacic et al. 1987; Pekari et al. 1992). Urinary benzene and PhMA are specific biomarkers for benzene exposure. *trans,trans*-Muconic acid is not specific for benzene as it is also a metabolic product of preservative sorbic acid or sorbates found in food and beverages (IARC 2018). The Centers for Disease Control and Prevention (CDC) National Health and Nutrition Examination Survey (NHANES) reports benzene levels in blood (CDC 2022a).

According to IARC (2018), current practice is to use urinary markers that are more specific for benzene (unmetabolized benzene, *trans,trans*-muconic acid (t.t-MA), and PhMA) than phenol, although they are typically at lower urinary concentrations. The American Conference of Governmental Industrial Hygienists (ACGIH) recommends using urinary *trans,trans*-muconic acid and PhMA to monitor benzene exposure to workers (ACGIH 2019). Urinary levels of PhMA have been correlated with occupational exposure to benzene (Boogaard and van Sittert 1996; Farmer et al. 2005; Inoue et al. 2000; Qu et al. 2005). Significant exposure-response trends for urinary *trans,trans*-muconic acid and PhMA levels have been demonstrated in occupationally exposed subjects at exposure levels of ≤1 ppm (Qu et al. 2005).

3.3.2 Biomarkers of Effect

The most sensitive effects of benzene exposures are hematotoxicity, immunotoxicity, and leukemia. While these effects are considered hallmark effects of benzene poisoning and occupational exposure, they

are not unique to benzene, as exposure to other chemicals (e.g., toluene) and medical conditions can produce similar effects. Therefore, the occurrence of these effects should not be interpreted as confirmatory evidence for benzene exposure. However, these effects taken in conjunction with known benzene exposure may be considered as biomarkers of effect.

In addition to using levels of urinary benzene and benzene metabolites for monitoring purposes (discussed in Section 3.3.1), various biological indices might also be helpful in characterizing the effects of exposure to benzene. The monitoring for benzene exposure may best be accomplished by using a series of biomarkers of effect with correlation of the results. Decreases in leukocyte counts have been used as an indicator of occupational benzene exposures. DNA adducts with benzene metabolites, chromosomal aberrations in bone marrow and peripheral blood lymphocytes, and sister chromatid exchanges could be used to monitor for benzene effects (IARC 2018; McHale et al. 2012). However, other than the formation of DNA adducts with benzene metabolites, these biomarkers are not specific to benzene exposure.

3.4 INTERACTIONS WITH OTHER CHEMICALS

Studies have been conducted on the interaction of benzene with other chemicals, both *in vivo* and in the environment. Benzene metabolism is complex, and numerous xenobiotics can induce or inhibit specific routes of detoxification and/or activation in addition to altering the rate of benzene metabolism and clearance from the body. Phenol, hydroquinone, benzoquinone, and catechol have been shown to induce CYPs in human hematopoietic stem cells (Henschler and Glatt 1995). Therefore, exposure to chemicals that stimulate the activity of this enzyme system prior to exposure to benzene could increase the rate of benzene metabolism. As discussed below, toluene, Aroclor 1254, phenobarbital, acetone, and ethanol are known to alter the metabolism and toxicity of benzene. Interactions reported in *in vivo* studies occurred at benzene exposure levels higher than those likely be encountered near hazardous waste sites.

Benzene, toluene, ethylbenzene, and xylenes (BTEX) frequently occur together at hazardous waste sites; therefore, ATSDR (2004) evaluated potential interactions of this common mixture. Based on predictions from PBPK models and data from binary mixtures, joint neurotoxic action is expected for BTEX mixtures (or ternary or binary mixtures therein). Data were not adequate to predict interactions within this mixture for other health effects.

Pretreatment of mice with CYP inhibitors (toluene, propylene glycol, β-diethyl amino ethyl diphenyl propyl acetate hydrogen chloride [SKF-525A]) has been demonstrated to reduce both benzene metabolite

formation (Andrews et al. 1977; Gill et al. 1979; Ikeda et al. 1972; Tuo et al. 1996) and resulting genotoxicity in mice (Tuo et al. 1996). Pretreatment with CYP inducers (3-methylcholanthrene and β-naphthoflavone) increased both benzene metabolism and benzene clastogenicity (Gad-El-Karim et al. 1986).

Ethanol and benzene increase levels of the hepatic CYP isoenzyme, CYP2E1, in rabbits and rats (Gut et al. 1993; Johansson and Ingelman-Sundberg 1988). Benzene derivatives, such as toluene and xylene, can inhibit the enzymatic activity of the isozyme (Koop and Laethem 1992). Ethanol enhances both the metabolism (*in vitro*) and the toxicity (*in vivo*) of benzene in animals (Baarson et al. 1982; Nakajima et al. 1985). For 13 weeks, mice were administered ethanol at 5 or 15% in drinking water, 4 days/week and exposed to benzene vapors at 300 ppm, 6 hours/day, 5 days/week; this resulted in greater severity of benzene-induced hematological effects (anemia, lymphocytopenia, bone marrow aplasia, transient increases in normoblasts and peripheral blood atypia) relative to benzene-exposed mice not given ethanol (Baarson et al. 1982). The modulating effects of benzene were dose-dependent. The enhancement of the hematotoxic effects of benzene by ethanol may be of particular concern for benzene-exposed workers who consume alcohol (Nakajima et al. 1985), although the interactions demonstrated in the mice occurred at much higher benzene exposure concentrations than would likely be experienced in workplace air. Benzene can interfere with the disappearance of ethanol from the body. Accordingly, increased central nervous system disturbances (e.g., depression) may occur following concurrent exposure to high levels of benzene and ethanol.

Other chemicals that induce specific isoenzymes of CYP can increase the rate of benzene metabolism and may alter metabolism pathways favoring one over another. Ikeda and Ohtsuji (1971) presented evidence that benzene hydroxylation was stimulated when rats were pretreated with phenobarbital and then exposed to 1,000 ppm of benzene vapor for 8 hours/day, 6 days/week for 2 weeks. Rats exposed to phenobarbital showed no effects on the metabolism of micromolar amounts (35–112.8 μmol) of benzene *in vitro* (Nakajima et al. 1985). Phenobarbital pretreatment of the rats alleviated the suppressive effect of toluene on benzene hydroxylation by the induction of oxidative activities in the liver (Nakajima et al. 1985).

Co-administration of toluene inhibited the biotransformation of benzene to phenol in rats (Ikeda et al. 1972; Inoue et al. 1988). This was due to competitive inhibition of the oxidation mechanisms involved in the metabolism of benzene. Coexposure of mice to benzene and toluene resulted in higher frequency of

micronuclei in polychromatic erythrocytes compared to exposure to benzene or toluene alone (Bird et al. 2010; Wetmore et al. 2008).

Mathematical models of benzene and phenol metabolism suggest that the inhibition by benzene of phenol metabolism, and by phenol on benzene metabolism, occurs through competition for a common reaction site, which can also bind catechol and hydroquinone (Purcell et al. 1990; Schlosser et al. 1993). Flavonoids have been shown to inhibit phenol hydroxylase or increase phenol hydroxylase activity in a dose-dependent manner, dependent on the oxidation potential of the flavonoid (Hendrickson et al. 1994).

SKF-525A and carbon monoxide are classic inhibitors of CYPs. The binding between CYP and carbon monoxide or SKF-525A is coordinate covalent. Carbon monoxide inhibits all CYP isoenzymes since it binds to the heme component of CYP, whereas SKF-525A inhibits specific types. SKF-525A inhibited benzene metabolism in the rat (Ikeda et al. 1972). Injection of 80 mg/kg of SKF-525A in rats resulted in a depression of phenol excretion. It also prolonged phenol excretion and interfered in the conversion of benzene to glucuronides and free phenols. Carbon monoxide, aniline, aminopyrine, cytochrome C, and metyrapone inhibited benzene metabolism *in vitro* by mouse liver microsomes (Gonasun et al. 1973).

Li et al. (2009b) subjected groups of mice to intratracheal instillation of either benzene or carbon nanotubes or combined instillation of benzene and carbon nanotubes. Combined instillation resulted in considerably more severe histopathological pulmonary toxicity than that observed in mice exposed to benzene or carbon nanotubes alone.