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

## 3.1 TOXICOKINETICS

- Adverse effects in humans exposed by inhalation and oral routes show that naphthalene is readily absorbed across the respiratory and gastrointestinal tracts. In addition, dermal absorption has been proposed as an explanation for naphthalene toxicity in human infants exposed to diapers stored with naphthalene mothballs. In animals exposed by inhalation, uptake across the respiratory tract is dependent on concentration. Based on urinary excretion of radioactivity, at least 76% of a gavage dose was absorbed in rats.

- Systemic effects in animals exposed to 1-methylnaphthalene by inhalation or oral administration demonstrate absorption.

- 2-Methylnaphthalene is rapidly absorbed in rats exposed by inhalation. Oral absorption of 1-methylnaphthalene by guinea pigs was at least 80% of the administered dose based on urinary excretion of radioactivity.

- Naphthalene has been detected in adipose tissues and breast milk of humans and is known to cross the placenta at sufficient doses to cause toxicity in newborns.

- Naphthalene and 1-methylnaphthalene are widely distributed in animals after oral and dermal exposure.

- Naphthalene metabolism begins with cytochrome P450 (CYP)-mediated epoxidation to 1,2-naphthalene epoxide. This electrophilic intermediate may be further metabolized by one of three competing pathways: glutathione conjugation and excretion as mercapturic acids, spontaneous hydration to 1- or 2-naphthol and urinary excretion, or hydration by epoxide hydrolase, yielding naphthalene 1,2-dihydrodiol. Naphthalene 1,2-dihydrodiol may be transformed via dihydrodiol reductase to 1,2-dihydroxynaphthalene, which is subsequently oxidized to 1,2-naphthoquinone.

- *In vitro* and animal studies suggest that, unlike naphthalene, 1- and 2-methylnaphthalenes are preferentially metabolized via oxidation of the methyl group, yielding hydroxymethyl-naphthalenes. Ring oxidation occurs at lower rates and results in low quantities of dihydrodiol and naphthol metabolites.

- Naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene are primarily excreted in urine as metabolites; small quantities are excreted in exhaled air and feces.

### 3.1.1 Absorption

***Naphthalene.*** Based on the presence of adverse effects following exposure, humans and animals can absorb naphthalene by pulmonary, gastrointestinal, and cutaneous routes.

The rate and extent of naphthalene absorption in humans exposed by inhalation have not been determined, but there is evidence for uptake via inhalation from case reports and occupational health studies. Clinical reports suggest that prolonged exposure to naphthalene vapors can cause adverse health effects in humans (Harden and Baetjer 1978; Linick 1983; Valaes et al. 1963). In addition, naphthalene has been detected in the expired air of workers exposed to naphthalene-containing jet fuels, and naphthalene metabolites have also been detected in the urine of these workers (Egeghy et al. 2003; Rodrigues et al. 2014; Serdar et al. 2003, 2004). Presumably, naphthalene moves across the alveolar membrane by passive diffusion through the lipophilic matrix.

The absorption of naphthalene across the upper respiratory tract was studied in rats and mice exposed by nose-only inhalation (Morris 2013; Morris and Buckpitt 2009). In both studies, the upper respiratory tract was isolated by inserting an endotracheal tube in an incision below the larynx, and air was drawn from the exposure chamber, through the isolated upper respiratory tract, and into the endotracheal tube at two different flow rates. Uptake was calculated as the difference between the naphthalene concentration in the exposure chamber and the concentration in the endotracheal tube. In both male and female rats, uptake across the upper respiratory tract was dependent on concentration (when flow rate was held constant) (Morris and Buckpitt 2009). At exposure concentrations of 1, 4, 10, and 30 ppm, uptake efficiencies were 56–57, 40–49, 34–37, and 28–36%, respectively, at a flow rate of 150 mL/minute (Morris and Buckpitt 2009). At the higher flow rate (300 mL/minute), uptake rates were reduced by about half, but the concentration dependence remained. When rats were pretreated with a suicide CYP inhibitor (5-phenyl-1-pentene), the dependence on concentration was abolished, and uptake estimates were in the range of 25–29% at concentrations from 4 to 30 ppm (Morris and Buckpitt 2009). The higher uptake seen in rats that retained CYP metabolic capacity indicates that naphthalene is metabolized by CYP enzymes in the nasal cavity, leading to greater scrubbing of naphthalene from the air.

In mice, uptake from the upper respiratory tract followed a similar pattern of dependence on concentration when flow rate was held constant (Morris 2013). At concentrations of 0.5, 3, 10, and 30 ppm, uptake efficiencies of 89.3–91.4, 77.6–82.1, 61.6–64.8, and 51.6–58.3%, respectively, were observed in male and female mice at an inspiratory flow rate of 25 mL/minute (Morris 2013). Doubling the flow rate reduced uptake estimates, but not as much as it did in rats. As with rats, pretreatment of mice with 5-phenyl-1-pentene resulted in decreased uptake efficiencies and abolished the dependence on exposure concentration (Morris 2013).

The bioaccessibility of naphthalene adsorbed to $PM_{2.5}$ particles measured *in vitro* in simulated lung fluid was 68–76% (Luo et al. 2021). The $PM_{2.5}$ particles were collected from indoor microenvironments in China and had naphthalene concentrations ranging between 0.33 and 6.87 ng/m$^3$. The estimated bioaccessibility was higher in the system simulating an inflammatory lung condition (artificial lysosomal fluid) than in the system simulating a healthy lung (gamble's solution) (Luo et al. 2021).

Several case reports indicate that naphthalene ingested by humans can be absorbed in quantities sufficient to elicit toxicity (Ahmad et al. 2019; Bregman 1954; Chusid and Fried 1955; Dela Cruz et al. 2019; Ekambaram et al. 2017; Eskandarani and Alghamdi 2020; Gidron and Leurer 1956; Gupta et al. 1979; Haggerty 1956; Kurz 1987; Kuwada et al. 2022; MacGregor 1954; Mackell et al. 1951; Ojwang et al. 1985; Santhanakrishnan et al. 1973; Shannon and Buchanan 1982; Tang 2017; Tannor and Hutton-Mensah 2019; Uthuman et al. 2019; Zuelzer and Apt 1949). However, no studies have been located that report the rate or extent of absorption.

In one patient who died as a result of naphthalene ingestion, 25 mothballs were found in the stomach 5 days after her death (Kurz 1987). A single naphthalene mothball reportedly weighs between 0.5 and 5 g depending on its size (Ambre et al. 1986; Siegel and Wason 1986). The gastric contents of a person who mistakenly ingested naphthalene flakes still smelled strongly of naphthalene at least 2 days following ingestion (Ojwang et al. 1985). These findings suggest that dissolved naphthalene is transported slowly into the intestines. Uptake from the intestines is governed by the partition coefficient between the materials in the intestinal lumen and the membrane lipids. Ingestion of mothballs or other forms of particulate naphthalene will lead to continued absorption over a period of several days as the solid dissolves. Unfortunately, none of the human data permit a quantitative evaluation of absorption coefficients or rates.

Limited data in animals indicate that naphthalene is rapidly absorbed across the gastrointestinal tract. In rats administered $^{14}C$ naphthalene by gavage, ~76% of the administered radioactivity was recovered in urine within 24 hours (Bakke et al. 1985). When $^{14}C$ naphthalene was instilled in the lumen of isolated rat intestinal loops, 97% of the radioactivity was detected in portal blood 0.5 hours later (Bock et al. 1979).

Several cases of naphthalene toxicity in neonates have been reported in which the proposed route of exposure was dermal (Dawson et al. 1958; Schafer 1951). Each case involved the use of diapers that had been stored in contact with naphthalene (mothballs or naphthalene flakes). The study authors proposed that the naphthalene was absorbed through the skin, causing hemolytic anemia. It was suggested that this

absorption may have been enhanced by the presence of oils that had been applied to the babies' skin (Schafer 1951). Inhalation of vapors from the treated diapers probably contributed to the total exposure.

$^{14}$C-Naphthalene was rapidly absorbed when the neat material (43 μg) was applied for a 48-hour period under a sealed glass cap to shaved 13-cm$^2$ areas of rat skin. Half of the sample (3.3 μg/cm$^3$) was absorbed in 2.1 hours (Turkall et al. 1994). When the naphthalene was mixed with either a sandy soil or a clay soil prior to contact with the skin, the presence of the soil slowed the absorption (Turkall et al. 1994). The absorption half-times from the clay and sandy soil samples were 2.8 and 4.6 hours, respectively. The rate of absorption did not influence the total amount of naphthalene absorbed in 48 hours since the areas under the plasma concentration curve did not differ significantly with any of the three exposure scenarios (0.42–0.63%/mL hour). The study authors proposed that naphthalene was absorbed more slowly from the sandy soil than from the clay soil because the sandy soil had a higher organic carbon content (Turkall et al. 1994). The sandy soil contained 4.4% organic matter and the clay soil contained 1.6% organic matter.

***1-Methylnaphthalene.*** No information has been located that documented the absorption of 1-methyl-naphthalene in humans by any exposure route or in animals after oral or dermal exposure. Systemic effects observed after the ingestion of 1-methylnaphthalene demonstrate that intestinal absorption does occur in rats (Murata et al. 1993; NITE 2009). In rats exposed to 1-methylnaphthalene (50 or 200 mg/m$^3$) by inhalation (nose only) for 6 hours, 1-methylnaphthalene was detected in blood within minutes after the end of the exposure period (Świercz and Wąsowicz 2018).

***2-Methylnaphthalene.*** Data on the absorption of 2-methylnaphthalene in humans were not located, and information on absorption in animals is limited to one study of rats exposed by inhalation and one study of guinea pigs exposed orally. In rats exposed nose-only to concentrations of 200 or 400 mg/m$^3$, 2-methylnaphthalene was detected in blood after the first hour of a 6-hour exposure period, increased during hour 2, and then remained fairly constant through the remainder of the exposure (Świercz et al. 2010). Blood concentrations were roughly proportional to exposure levels. In guinea pigs, rapid absorption was seen after oral exposure. At least 80% of a 10 mg/kg oral dose of 2-methylnaphthalene was absorbed within 24 hours based on recovery of the radiolabel in the urine (Teshima et al. 1983).

### 3.1.2 Distribution

***Naphthalene.*** There are limited data concerning the distribution of naphthalene in human tissues. Naphthalene was present in 40% of the adipose tissue samples that were analyzed as part of the National

Human Adipose Tissue Survey (EPA 1986b). The maximum concentration observed was 63 ng/g. Naphthalene was also detected in human milk samples (concentration not reported) (Pellizzari et al. 1982). The sources of naphthalene in these milk and body fat samples are not known.

Naphthalene can cross the human placenta in concentrations high enough to cause red cell hemolysis and lead to anemia in newborn infants of mothers who consumed naphthalene during pregnancy (Anziulewicz et al. 1959; Sahni et al. 2019; Shafer et al. 2020; Zinkham and Childs 1957, 1958).

The distribution of naphthalene and its metabolites in young pigs given a single dose of 0.123 mg/kg (4.8 Ci/kg) $^{14}$C-labeled naphthalene was monitored at 24 and 72 hours (Eisele 1985). At 24 hours, the highest percentage of the label (3.48±2.16% dose/mg tissue) was in the adipose tissue. The kidneys had the next highest concentration of label (0.96% dose/mg tissue), followed by the liver (0.26±0.06% dose/mg tissue) and lungs (0.16% dose/mg tissue). The heart contained 0.09±00.04% dose/mg tissue and the spleen contained 0.07±0.01% dose/mg tissue. At 72 hours, the amount of label in the fat had fallen to 2.18±1.16% dose/mg tissue, the amount in the liver decreased to 0.34±0.24% dose/mg tissue, and the kidneys and lungs contained the same concentration (0.26% dose/mg tissue).

Pigs were also given oral doses of 0.006 mg/kg/day (0.22 Ci/kg/day) $^{14}$C-labeled naphthalene for 31 days (Eisele 1985). With repeated administration of the radiolabel, the tissue distribution differed considerably from that observed with a single dose of the compound. The highest concentration of label was in the lungs (0.15% dose/mg tissue), followed by the liver and heart (0.11% dose/mg tissue). There was very little label in the fat tissue (0.03% dose/mg tissue). The spleen had 0.09±0.05% dose/mg tissue and the kidney had 0.09% dose/mg tissue.

In one dairy cow, naphthalene distributed to milk with both single and repeated doses of $^{14}$C-labeled naphthalene. The label was distributed between the milk and the milk fat (Eisele 1985). When the cow was given naphthalene for a 31-day period, the amount of label found in the milk remained relatively constant throughout the exposure period. The amount in the milk fat was lower for the first 7 days than it was for the remainder of the exposure.

In rats, radiolabel from naphthalene distributed to the ileum, duodenum, and kidney (0.01–0.02% of initial dose) when tissues were analyzed 48 hours after naphthalene contact with the skin (Turkall et al. 1994). The largest concentration was found at the site of application (0.56% of initial dose). A total of 20 tissues were evaluated; the percentage of label in all other tissues was minimal.

***1-Methylnaphthalene.*** Information on the tissue distribution of 1-methylnaphthalene in humans was not located. Świercz and Wąsowicz (2018) measured the concentration of 1-methylnaphthalene in blood and tissues of rats after single or repeated (5 days) exposures to 50 or 200 mg/m$^3$ 1-methylnaphthalene for 6 hours via nose-only inhalation. In blood, 1-methylnaphthalene was detected minutes after the end of exposure at peak concentrations of ~1 and 0.1 mg/L at concentrations of 200 and 50 mg/m$^3$, respectively. Concentrations in blood declined rapidly after the end of exposure. After single or repeated exposures, the tissue concentrations were highest in kidney and fat, followed by lungs, spleen, liver, and brain. For example, after a single exposure to 50 mg/m$^3$, tissue concentrations were 1.88, 1.29, 0.41, 0.26, 0.21, and 0.16 µg/g in the kidney, fat, lungs, brain, spleen, and liver, respectively. Tissue concentrations were lower after five daily exposures to 1-methylnaphthalene than after a single day of exposure (Świercz and Wąsowicz 2018). When measured 24 hours after the end of exposure, 1-methylnaphthalene was not detected in any of these tissues from rats exposed to 50 mg/m$^3$ (single or repeated exposures) and was detected only in kidney and fat from rats exposed to 200 mg/m$^3$ (Świercz and Wąsowicz 2018).

***2-Methylnaphthalene.*** Data on the distribution of 2-methylnaphthalene in exposed humans were not located. The tissue distribution of 2-methylnaphthalene was measured in guinea pigs 3, 6, 24, and 48 hours after oral administration of tritium-labeled 2-methylnaphthalene (10 mg/kg; 59 µCi/kg) (Teshima et al. 1983). The highest concentration of label was present in the gallbladder, with 20.17 µg at 3 hours and 15.72 µg at 6 hours. (All concentrations are expressed in µg equivalents of $^3$H/g wet tissue.) The value fell to 0.43 µg at 24 hours and 0.04 µg at 48 hours. The presence of label in the gallbladder presumably reflects the excretion of hepatic metabolites in the bile. The values for the kidney were 5.64 µg at 3 hours, 7.62 µg at 6 hours, 0.29 µg at 24 hours, and 0.09 µg at 48 hours.

Radiolabeled compound was detected in the liver immediately after exposure (Teshima et al. 1983). When converted to units of mass, hepatic concentrations were 1.71 µg at 3 hours and 2.66 µg at 6 hours, falling to 0.18 µg at 24 hours. Lung concentrations were similar to those for blood at all time points. The amounts were 0.75 µg in blood and 0.69 µg in lungs at 3 hours; at 6 hours, concentrations were 0.71 µg in the blood and 0.76 µg in the lung. The half-life of 2-methylnaphthalene in the blood was 10.4 hours. The decay of 2-methylnaphthalene and metabolites in the other tissues examined was described as biphasic.

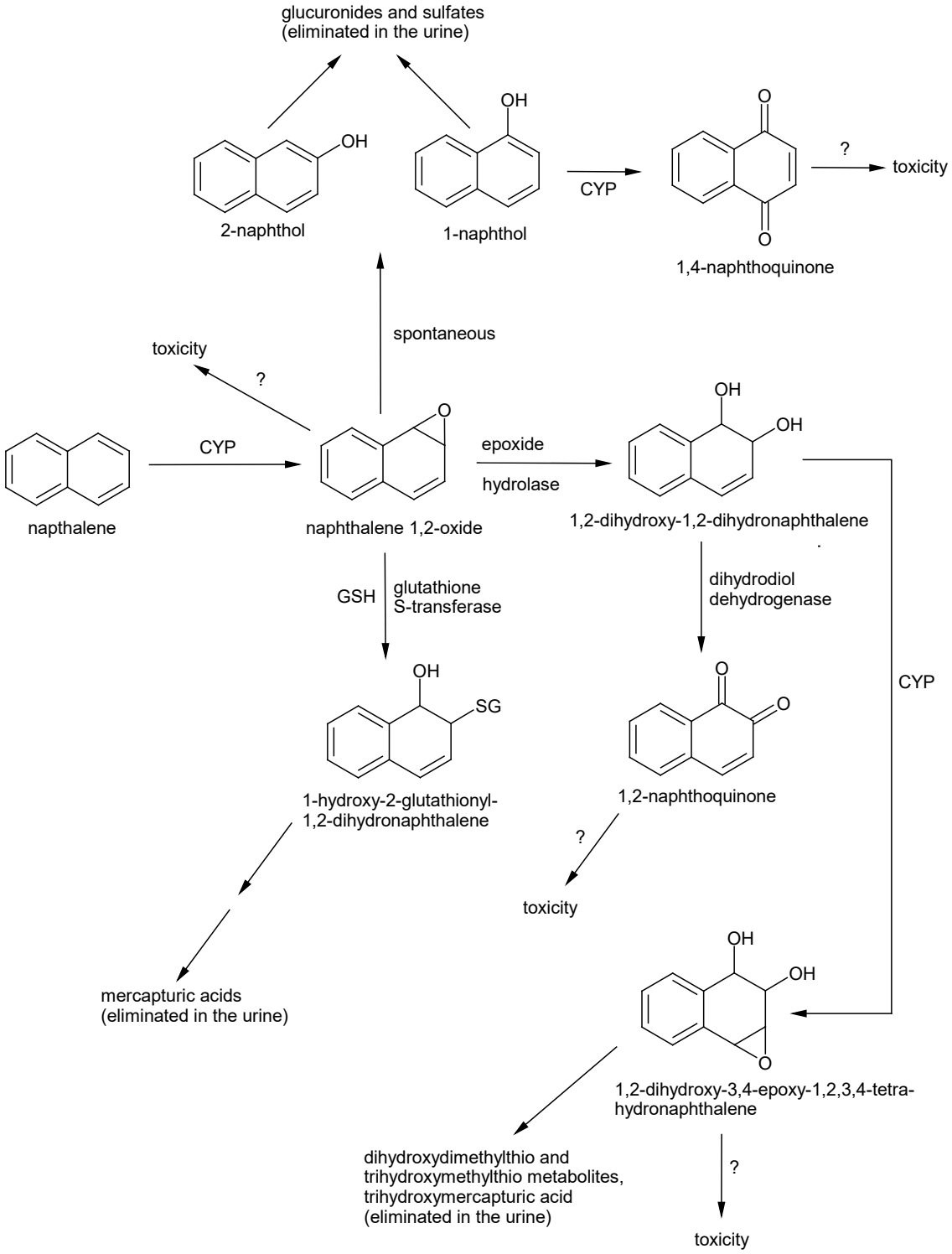
After i.p. administration in mice, $^{14}$C-labeled 2-methylnaphthalene distribution was measured in the fat, kidney, liver, and lung for 24 hours (Griffin et al. 1982). The amount of label in the fat peaked 3 hours after exposure and remained higher than the amount of label in other tissues at 8 hours. The liver, kidney, and lung followed the fat in order of decreasing concentration. The maximum concentration in the fat was 13 nmol equivalents/mg wet weight. The maximum value for the liver was 3.5 nmol equivalents/mg wet weight at 1 hour. Maximum values were about 1.75 nmol equivalents/mg wet weight for the kidneys at 2 hours and 0.8 nmol equivalents/mg wet weight for the lungs at 4 hours.

### 3.1.3 Metabolism

***Naphthalene.*** The metabolism of naphthalene in mammalian systems has been studied extensively and is depicted in Figure 3-1. The metabolic scheme in Figure 3-1 illustrates that there are multiple reactive metabolites formed from naphthalene: 1,2-naphthalene oxide, 1,2-naphthoquinone, 1,4-naphthoquinone, and 1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydronaphthalene. This section presents an overview of the metabolic scheme and the evidence for the involvement of the 1,2-epoxide and the naphthoquinones in naphthalene toxicity. The fourth metabolite listed above is expected to be reactive, but its potential role in naphthalene toxicity has not been investigated. A review of the metabolism and bioactivation of naphthalene has been published by Buckpitt et al. (2002).

The first step in naphthalene metabolism is catalyzed by CYP oxygenases and produces a reactive electrophilic arene epoxide intermediate, 1,2-naphthalene oxide. In mammalian systems, several CYP isozymes have been demonstrated to metabolize naphthalene, including 1A1, 1A2, 1B1, 3A7, 3A5 (Juchau et al. 1998), 2E1 (Wilson et al. 1996), 2F2 (Buckpitt et al. 1995; Shultz et al. 1999), and 2B4 (Van Winkle et al. 1996). The epoxide can spontaneously rearrange to form naphthols (predominantly 1-naphthol) and subsequently conjugate with glucuronic acid or sulfate to form conjugates, which are excreted in urine (Ayala et al. 2015; Bock et al. 1976).

**Figure 3-1. Scheme for Naphthalene Metabolism and Formation of Multiple Reactive Metabolites, that may be Involved in Naphthalene Toxicity**




CYP = cytochrome P450 enzyme(s); GSH = reduced glutathione; SG = glutathione

Sources: Buckpitt et al. (2002); Waidyanatha et al. (2002)

Alternatively, the 1,2-epoxide can react with tissue macromolecules. This reaction is thought to be involved in several aspects of naphthalene toxicity, especially injury to club cells (non-ciliated cells in the epithelium of proximal and distal airways of the lung) from acute exposure to naphthalene (Buckpitt et al. 2002; Zheng et al. 1997). In pH 7.4 buffer, the epoxide has been shown to have a half-life of approximately 2–3 minutes, which is extended by the presence of albumins to about 11 minutes (Buckpitt et al. 2002; Kanekal et al. 1991). Mice are markedly more susceptible than rats to acute naphthalene-induced club cell injury (Buckpitt et al. 1992; West et al. 2001). The susceptibility difference apparently extends to chronic exposure scenarios. Mice exposed by inhalation to 10 or 30 ppm naphthalene for 2 years showed lung inflammation, but rats exposed to concentrations up to 60 ppm showed no lung inflammation (Abdo et al. 2001; NTP 1992a, 2000). The species difference in lung susceptibility has been correlated with higher rates of formation of a specific enantiomeric epoxide (1*R*,2*S*-naphthalene oxide) in lung microsomes and isolated dissected airways of mice compared with rats (Buckpitt et al. 1992, 1995). Rat, hamster, and monkey lung microsomes preferentially formed the 1*S*,2*R*-naphthalene oxide enantiomer and showed lower rates of formation of epoxides than mouse lung microsomes (Buckpitt et al. 1992). Microsomes from human lymphoblastoid cells expressing recombinant human CYP2F1 also showed preferential formation of the 1*S*,2*R*-naphthalene oxide enantiomer, providing some evidence that human transformation of naphthalene to reactive epoxides in lung tissue may be more like rats than mice (Lanza et al. 1999).

In contrast to the lung, species differences in susceptibility at another sensitive target of naphthalene, the olfactory and respiratory epithelia of the nose, do not correlate with differences in rates of transformation to 1,2-epoxide derivatives in extracts of olfactory tissue (Buckpitt et al. 1992; Plopper et al. 1992). Metabolic rates (units of nmol naphthalene converted to epoxide derivatives/minute/mg protein) in olfactory tissue extracts showed the following order: mouse (87.1) > rat (43.5) > hamster (3.9). However, rats were more susceptible to naphthalene-induced cell injury than mice or hamsters. The lowest single i.p. doses producing necrosis and exfoliation in olfactory epithelium were 200 mg/kg in rats and 400 mg/kg in mice and hamsters. These observations suggest that the reasons for species differences in susceptibility to naphthalene toxicity are complex and do not solely involve the formation of the 1,2-epoxide metabolites. Although CYP monooxygenases, which are involved in naphthalene metabolism and bioactivation, have been demonstrated to exist in nasal respiratory epithelial and olfactory epithelial tissue from rodents and humans (Thornton-Manning and Dahl 1997), studies designed to specifically characterize metabolism of naphthalene in nasal tissue are restricted to those by Buckpitt et al. (1992) and Plopper et al. (1992).

In addition to being converted to the naphthols, the 1,2-epoxide can be conjugated with glutathione via glutathione-S-transferase catalysis. Figure 3-1 shows one such conjugate, 1-hydroxy-2-glutathionyl-1,2-dihydronaphthalene. The glutathionyl conjugates are converted in several steps to mercapturic acids, which are excreted in the urine. The conjugation of the epoxide is thought of as a detoxication mechanism, as evidenced by studies showing that glutathione depletion increased the degree of acute naphthalene-induced club cell injury in mice (Warren et al. 1982; West et al. 2000). In addition, elevated activities of γ-glutamylcysteine synthetase, the enzyme catalyzing the rate-limiting step in glutathione synthesis, were observed in dissected airways from mice that developed tolerance to acute naphthalene club cell cytotoxicity (West et al. 2000).

The 1,2-epoxide can also be enzymatically hydrated by epoxide hydrolase to form 1,2-dihydroxy-1,2-dihydronaphthalene (Figure 3-1). This 1,2-dihydrodiol derivative was the major stable metabolite of naphthalene produced by human liver microsomes, whereas the major stable metabolite formed by mouse liver microsomes was 1-naphthol (Tingle et al. 1993). In the presence of an inhibitor of epoxide hydrolase (trichloropropene oxide), the major stable metabolite with human liver microsomes was 1-naphthol. How this species difference in liver metabolism may relate to the human relevance of toxicity of inhaled naphthalene in sensitive target tissues in the nose and lungs of mice is unknown.

The 1,2-dihydrodiol can be catalytically transformed by dihydrodiol dehydrogenase to 1,2-naphthoquinone (also known as naphthalene-1,2-dione). 1,2-Naphthoquinone is both reactive itself and capable of producing reactive oxygen species through redox cycling (Flowers et al. 1997) and has been shown to be mutagenic in several strains of *S. typhimurium* (Flowers-Geary et al. 1996). In isolated club cells incubated with 0.5 mM naphthalene, 1,2-naphthoquinone was the major naphthalene derivative covalently bound to proteins, although covalent binding with the 1,2-epoxide was also observed (Zheng et al. 1997). The formation of the other naphthoquinone, 1,4-naphthoquinone, from 1-naphthol, presumably via a CYP monooxygenase, has been proposed based on the finding that, following incubations of liver microsomes with 1-naphthol, ethylene diamine (a compound that reacts readily with 1,2-naphthoquinone), did not trap reactive metabolites (Doherty et al. 1984). Cysteinyl adducts of both 1,2-naphthoquinone and 1,4-naphthoquinone (and of 1,2-naphthalene oxide) with hemoglobin and albumin have been detected in blood of rats given single oral doses of naphthalene ranging from 100 to 800 mg/kg (Troester et al. 2002; Waidyanatha et al. 2002). Levels of 1,2-naphthalene oxide adducts were greater than levels of 1,2-naphthoquinone adducts, which were greater than levels of 1,4-naphthoquinone adducts (Troester et al. 2002; Waidyanatha et al. 2002). In *in vitro* studies with whole human blood samples, 1,2- or 1,4-naphthoquinone induced increased frequencies of sister chromatid exchanges at concentrations

≥10 μM, whereas naphthalene 1,2-epoxide did not at concentrations up to 100 μM (Wilson et al. 1996). Similarly, incubation of human mononuclear leukocytes with 1,2-naphthoquinone or 1,4-naphthoquinone caused significant depletion of cellular glutathione levels and significant cytotoxicity at concentrations between 1 and 100 μM, whereas naphthalene 1,2-epoxide did not display these toxic actions in this concentration range (Wilson et al. 1996).

1,2-Naphthoquinone formed in lens tissue is thought to be involved in naphthalene-induced cataracts in rats and rabbits. The enzyme involved in the transformation of the 1,2-dihydrodiol to 1,2-naphthoquinone in lens tissue is thought to be aldose reductase (this enzyme, also known as aldehyde reductase, is not specified in Figure 3-1). Support for this hypothesis includes findings that aldose reductase inhibitors prevent cataract formation in naphthalene-fed rats (Tao et al. 1991; Xu et al. 1992a), dihydrodiol dehydrogenase is apparently absent in rat lens (Greene et al. 2000), and aldose reductase appears to be the only enzyme in rat lens that can transform 1,2-dihydroxy-1,2-dihydronaphthalene to 1,2-naphthoquinone (Sugiyama et al. 1999).

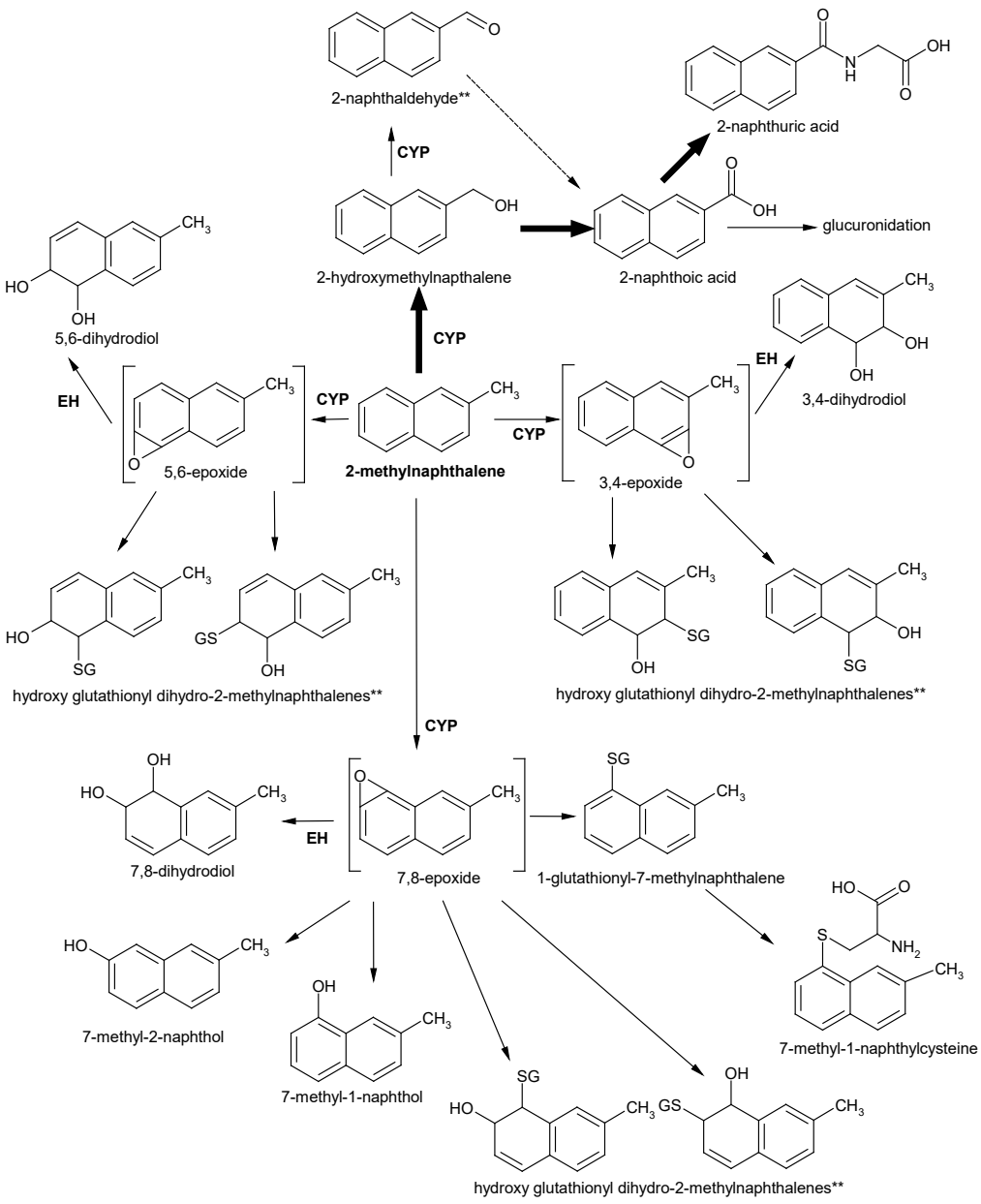
Support for the *in vivo* formation of another potentially reactive metabolite, 1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydronaphthalene, comes from the identification of several urinary metabolites, including a number of trihydroxytetrahydromethylthio derivatives (Horning et al. 1980) and a trihydroxytetrahydro-mercapturic acid (Pakenham et al. 2002). These urinary metabolites, however, are minor, and the importance of their common proposed precursor in naphthalene toxicity is unstudied to date. Figure 3-1 proposes an oxidative transformation of dihydrodiol derivative to the tetrahydrodiol epoxide derivative via CYP catalysis.

***1-Methylnaphthalene.*** Information on the metabolism of 1-methylnaphthalene in humans and animals was not located. In both human and rat liver microsomes incubated with 1-methylnaphthalene, the primary metabolite resulted from oxidation of the methyl group, yielding 1-(hydroxymethyl)naphthalene (Wang et al. 2020). Minor metabolites included dihydro-1-methyl-naphthalenediols and 1-methyl-naphthols (Wang et al. 2020). The rates of ring- and side-chain-oxidation differed between rat and human liver microsomes. In human liver microsomes, Wang et al. (2020) observed higher intrinsic clearance of 1-methylnaphthalene via aromatic ring oxidation compared with side chain oxidation, while the opposite was observed in rat liver microsomes.

***2-Methylnaphthalene.*** As with 1-methylnaphthalene, the methyl substituent of 2-methylnaphthalene presents the opportunity for side chain oxidation reactions in addition to the ring oxidation, which is the

sole initial step in naphthalene metabolism. A proposed metabolic scheme for 2-methylnaphthalene is shown in Figure 3-2. Oxidation at the methyl group (the predominant path), or at several competitive positions on the rings, is catalyzed by CYP monooxygenases (Figure 3-2).

## Figure 3-2. Metabolism of 2-Methylnaphthalene




[ ] = putative metabolite; CYP = cytochrome P450 enzyme(s); EH = epoxide hydrolase; GS = glutathione

**Metabolites identified *in vitro* only

Sources: Buckpitt and Franklin (1989); EPA (2003); Shultz et al. (2001); Teshima et al. (1983)

In rats and mice, about 50–80% of 2-methylnaphthalene is oxidized at the 2-methyl group to produce 2-hydroxymethylnaphthalene (Breger et al. 1983; Teshima et al. 1983). This 2-hydroxymethyl-naphthalene metabolite is further oxidized to 2-naphthoic acid (Grimes and Young 1956; Melancon et al. 1982; Teshima et al. 1983), and this step proceeds either directly or through the intermediate, 2-naphthaldehyde (Figure 3-2). Detection of 2-naphthaldehyde has only been reported following *in vitro* incubation of 2-methylnaphthalene with recombinant mouse CYP2F2 (Shultz et al. 2001). 2-Naphthoic acid may be conjugated with either glycine or glucuronic acid (Figure 3-2). The glycine conjugate of 2-naphthoic acid forms 2-naphthuric acid, which is the most prevalent urinary metabolite of 2-methyl-naphthalene detected in exposed animals (Grimes and Young 1956; Melancon et al. 1982; Teshima et al. 1983).

Ring epoxidation at the 7,8-, 3,4-, or 5,6- positions occurs in approximately 15–20% of 2-methyl-naphthalene (Breger et al. 1983; Melancon et al. 1985). These epoxidation reactions are catalyzed by CYP isozymes that include CYP1A and CYP1B. These epoxides are proposed intermediates based on experimentally observed metabolites but have not been individually isolated (Figure 3-2). These epoxides may be further oxidized by epoxide hydrolase to produce dihydrodiols (the 7,8-dihydrodiol, 3,4-dihydrodiol, or 5,6-dihydrodiol of 2-methylnaphthalene) or may be conjugated with glutathione (Griffin et al. 1982; Melancon et al. 1985) by glutathione S-transferase catalysis or can proceed spontaneously. The hydroxy glutathionyl dihydro-2-methylnaphthalenes (Figure 3-2) have been detected after incubation of 2-methylnaphthalene with hepatic microsomes from Swiss-Webster mice or with isolated recombinant mouse CYP2F2 enzyme and glutathione S-transferase (Shultz et al. 2001). Figure 3-2 indicates six hydroxy glutathionyl 2-methylnaphthalenes; two are formed for each of the epoxide intermediates (3,4-, 5,6-, and 7,8-epoxides), and each can exist in two enantiomeric forms not shown in Figure 3-2 (Shultz et al. 2001).

Three other minor metabolites formed via the 7,8-epoxide pathway are shown in Figure 3-2. Urinary 1-glutathionyl-7-methylnaphthalene was identified in guinea pigs and by *in vitro* experiments with guinea pig microsomes (Teshima et al. 1983). 7-Methyl-1-naphthol and 7-methyl-2-naphthol were identified in the urine of rats, mice, guinea pigs, and rabbits following oral exposure (Grimes and Young 1956).

In rats administered subcutaneous injections of 2-methylnaphthalene (0.3 mg/kg 2-methyl-[8-$^{14}$C]-naphthalene), 2-naphthoic acid and naphthoic acid conjugates were identified in the urine (Melancon et al. 1982). The naphthoic acid and various conjugates of the acid were estimated to account for 36–43% of the radiolabel in collected urine. Most of this (30–35% of radiolabel in urine) was found as a glycine

conjugate. The urine contained 3–5% unreacted 2-methylnaphthalene; free dihydrodiols accounted for 6–8% of the label. Unidentified highly polar metabolites comprised another 36–45% of the excreted label. At least three diol derivatives of 2-methylnaphthalene were produced by hepatic microsomes from mice (Griffin et al. 1982), suggesting that the ring oxidation reactions of 2-methylnaphthalene are similar to those for naphthalene. Rat liver microsomes also produced 2-hydroxymethylnaphthalene and three diols from 2-methylnaphthalene (Breger et al. 1981, 1983; Melancon et al. 1985; Wang et al. 2020). The three diols were identified as 3,4-dihydrodiol, 5,6-dihydrodiol, and 7,8-dihydrodiol (Breger et al. 1983; Wang et al. 2020). Both rat and human liver microsomes cleared 2-methylnaphthalene primarily via side-chain oxidation, with lower intrinsic clearance via ring oxidation (Wang et al. 2020).

Metabolites isolated in the urine of guinea pigs after oral dosing with tritium labeled 2-methylnaphthalene (10 mg/kg) were 2-naphthoic acid and its glycine and glucuronic acid conjugates (Teshima et al. 1983). These metabolites accounted for 76% of the label in collected urine. Glucuronic acid and sulfate conjugates of 7-methyl-1-naphthol, along with *S*-(7-methyl-1-naphthyl)cysteine, accounted for 18% of the excreted label. No diol metabolites were identified.

Glutathione conjugation appears to be an important detoxication pathway for 2-methylnaphthalene. Pretreatment of male C57BL/6J mice with 625 mg/kg of diethylmaleate (a depletor of glutathione) 1 hour prior to i.p. administration of 400 mg/kg of 2-methylnaphthalene resulted in mortality in four of five mice, whereas treatment without glutathione depletion was not fatal (Griffin et al. 1982). Bronchiolar necrosis was not observed in male ddY mice given single i.p. injections of 200 mg/kg of 2-methylnaphthalene; pretreatment with the glutathione depletor diethylmaleate (600 µL/kg) 1 hour prior to injections caused “extensive sloughing and exfoliation of bronchiolar epithelial cells” in all five animals (Honda et al. 1990). In contrast, pretreatment of male DBA/2J mice (5/group) with 625 mg/kg of diethylmaleate did not increase the severity of pulmonary necrosis induced by 400 mg/kg of 2-methylnaphthalene (Griffin et al. 1983). The observed differences among mouse strains in response to depletion of glutathione remain unexplained. Other experiments (without pretreatment) observed decreased tissue or intracellular levels of glutathione in response to exposure to high acute doses of 2-methylnaphthalene, demonstrative of glutathione conjugation (Griffin et al. 1982, 1983; Honda et al. 1990). Similarly, depletion of glutathione (by 35% compared to controls) was detected in primary cultures of female Sprague-Dawley rat hepatocytes treated with 1,000 µM of 2-methylnaphthalene (Zhao and Ramos 1998).

### 3.1.4 Excretion

***Naphthalene.*** Little information is available pertaining to the excretion of naphthalene in humans after inhalation exposure to naphthalene. Workers employed in the distillation of naphthalene oil and at a coke plant had peak levels of urinary 1-naphthol 1 hour after finishing a shift (Bieniek 1994). Of three workers and a nonoccupationally exposed group, naphthalene oil distribution plant workers had the highest concentrations of urinary 1-naphthol, with a mean excretion rate of 0.57% mg/hour. Investigators calculated the half-life for the urinary excretion of 1-naphthol as approximately 4 hours (Bieniek 1994). This urinary metabolite may indicate both exposure to naphthalene and low concentrations of 1-naphthol during naphthalene oil distillation (Bieniek 1994). Several other studies have shown urinary excretion of naphthalene metabolites in humans occupationally exposed to vapors or emissions from jet fuel, creosote, coke oven, asphalt, iron foundry, and aluminum production industries (Chao et al. 2006; Rappaport et al. 2004; Rodrigues et al. 2014; Serdar et al. 2003, 2004, 2016).

In rats and mice exposed by inhalation, naphthalene metabolites are primarily excreted as mercapturate conjugates (Ayala et al. 2015; Pakenham et al. 2002). In mice treated with seven daily 4-hour exposures to 15 ppm, ~60% of the urinary metabolites in each 24-hour sample consisted of mercapturates (from glutathione conjugation of naphthalene oxide), while glucuronide and sulfate conjugates (of 1-naphthol) made up nearly 20% each. A small fraction (≤ 3%) of the metabolites was excreted as N-acetyl glutathione conjugates (Ayala et al. 2015). Pakenham et al. (2002) compared the urinary mercapturate levels in mice and rats exposed by inhalation to concentrations of 0.8–100 ppm and observed concentration-dependent increases in mercapturate excretion in both species. Mice excreted more urinary mercapturates than rats at comparable exposure concentrations. At low concentrations (0.8 and 1 ppm in rats and mice, respectively), excreted mercapturates were similar (0.6 and 1 μmol/kg, respectively), but at the highest concentration tested (100 ppm), mice excreted 240 μmol/kg while rats excreted only 67 μmol/kg as urinary mercapturates (Pakenham et al. 2002). Species differences were also observed in the diastereomers excreted, with mice excreting more 1R,2S- than 1S,2R-epoxide derived mercapturates at all exposure levels (Pakenham et al. 2002). Ratios of mercapturates derived from 1R,2S- than 1S,2R-epoxides ranged from 1:1 to 0.5:1 from low to high exposure concentrations in rats, while the corresponding ratios ranged from 5:1 to 3:1 in mice (Pakenham et al. 2002).

Limited data were located on excretion of ingested naphthalene by humans. The urine of one patient was tested for naphthalene and its derivatives. Naphthol was found at the time of hospital admission (4 days post-ingestion). Smaller quantities were present 1 day later, but naphthalene was not detected in later

specimens (Zuelzer and Apt 1949). In another instance, the urine of an 18-month-old child was found to contain 1-naphthol, 2-naphthol, 1,2-naphthoquinone, and 1,4-naphthoquinone (but no naphthalene) 9 days after exposure (Mackell et al. 1951). With the exception of the 1,4-naphthoquinone, these metabolites were still detectable on day 13, but not on day 17. These data indicate that urinary excretion of metabolites may be prolonged following exposure. It is important to note, however, that delayed dissolution and absorption from the gastrointestinal tract may also be a contributing factor. Unabsorbed naphthalene was visible in the fecal matter after ingestion of naphthalene flakes or mothballs in several individuals (Zuelzer and Apt 1949).

In nonhuman primate studies, Rhesus monkeys given naphthalene at oral doses up to 200 mg/kg did not excrete naphthalene as thioethers in urine or feces (Rozman et al. 1982). In a similar study, chimpanzees orally administered naphthalene at 200 mg/kg did not excrete naphthalene as thioethers in urine (Summer et al. 1979). These data suggest that glutathione conjugation of naphthalene may not occur to any great extent in nonhuman primates. Data from two chimpanzees indicate that most of the naphthalene excreted in this species is excreted as glucuronic acid and sulfate conjugates (Summer et al. 1979).

In rats administered radiolabeled naphthalene, the amount of label recovered in 24 hours was 77–93% in urine and 6–7% in feces (Bakke et al. 1985). There was a dose-dependent increase in urinary thioether excretion following gavage doses of naphthalene at 30, 75, and 200 mg/kg within 24 hours (Summer et al. 1979). The levels of thioethers excreted accounted for approximately 39, 32, and 26% of the three dose levels tested.

The dermal exposure of rats to $^{14}C$-labeled naphthalene was evaluated over a 48-hour period (Turkall et al. 1994). Naphthalene (43 μg) samples were applied to shaved 13-$cm^2$ areas on the skin under a sealed plastic cap. Neat naphthalene or naphthalene adsorbed to the surface of sandy soil or clay soil was tested. In all three cases, excretion of the label was primarily through the urine (70–87%). With the pure naphthalene and naphthalene adsorbed to clay soil, the exhaled air accounted for 6–14% of the administered label. Exhaled air contained only 0.9% of the label in the sandy soil group. This finding was presumably related to the slower adsorption of naphthalene from the sandy soil and its more rapid metabolism to nonvolatile metabolites. Less than 0.02% of the label was exhaled as carbon dioxide in all groups. The feces contained 2–4% of the label.

The primary metabolites in the urine after dermal application of naphthalene were 2,7-dihydroxy-naphthalene, 1,2-dihydroxynaphthalene, and 1,2-naphthoquinone (Turkall et al. 1994). The ratio of these

metabolites for pure naphthalene and naphthalene adsorbed to clay soil were roughly 3:2:1. For the sandy soil, the corresponding ratio was 3:2:1.5. Small amounts of 1-naphthol and 2-naphthol were also excreted. In all cases, the amount of urinary free naphthalene was <0.4% of the administered label.

In mouse studies using the i.p. or subcutaneous exposure routes, several naphthalene metabolites were excreted in the urine. After i.p. administration of 100 mg/kg naphthalene, conjugates accounted for 80–95% of the urinary metabolites (Horning et al. 1980; Stillwell et al. 1982). Much of the conjugated material was present as thioethers (glutathione conjugates and their derivatives). The major oxidation products of naphthalene metabolism were 1-naphthol and trans-1,2-dihydro-1,2-naphthalenediol.

***1-Methylnaphthalene.*** Information on the excretion of 1-methylnaphthalene in humans exposed by any route or in animals exposed via oral or dermal administration was not located. In a study of rats exposed via nose-only inhalation to 50 or 200 mg/m$^3$ 1-methylnaphthalene for 6 hours/day on 1 day or 5 consecutive days, elimination of 1-methylnaphthalene from blood was rapid (Świercz and Wąsowicz 2018). The study authors calculated rates of elimination from blood using an open two-compartment model, yielding phase 1 and phase II half-lives of ~1 and ~40 minutes for 1 and 5 days of exposure to 50 mg/m$^3$, respectively, and ~2.5 and 90–100 minutes for 1 and 5 days of exposure to 200 mg/m$^3$, respectively. While the area under the curve (AUC) for blood concentration over time (first hour after exposure ended) was similar for single and repeated exposures to 50 mg/m$^3$, repeated exposures to 200 mg/m$^3$ resulted in a significantly lower AUC than a single exposure did (0.58 hours x mg/L compared with 2.65 hours x mg/L in the first 6 hours after exposure ended). The study authors suggested that 1-methylnaphthalene induced its own metabolism, resulting in faster elimination after repeated exposures (Świercz and Wąsowicz 2018).

In this study, urinary excretion of 1-methylnaphthalene was greatest during the first 24 hours after exposure ended (Świercz and Wąsowicz 2018). Urine concentrations during this time period were similar after single and repeated exposures to the same concentration. Concentrations of 0.069 and 0.051 µg/L were measured after 1 and 5 days of exposure to 50 mg/m$^3$, respectively and concentrations of 0.385 and 0.377 µg/L were measured after 1 and 5 days at 200 mg/m$^3$, respectively. Urine concentrations were much lower during subsequent 24-hour collection periods (Świercz and Wąsowicz 2018)

***2-Methylnaphthalene.*** In guinea pigs, 80% of a 10 mg/kg tritium-labeled 2-methylnaphthalene dose was excreted in the urine within 24 hours and about 10% was recovered in the feces (Teshima et al. 1983). Most of the excreted material (76%) was found as 2-naphthoic acid or its conjugates. About 18% of the

recovered label was found as conjugates of 7-methyl-1-naphthol. Following subcutaneous administration of 0.3 mg/kg $^{14}$C-labeled 2-methylnaphthalene, 55% was found in the urine of rats (Melancon et al. 1982). Naphthoic acid and its glycine conjugate were identified. Three other metabolites were tentatively identified as isomeric diols.

### 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).

Research on PBPK models of naphthalene have focused on simulating characteristics of the anatomy and physiology of the rodent and human that are thought to contribute to interspecies differences in dose-response relationships for nasal cavity lesions. Important features of naphthalene toxicity and kinetics that are relevant to interspecies extrapolation include: (1) necrotic lesions of the nasal cavity, most prominent in the olfactory epithelium (Dodd et al. 2010, 2012); (2) first-pass extraction of naphthalene by nasal cavity tissues, which decreases as the inhalation exposure concentration increases (Morris and Buckpitt 2009); and (3) production of reactive intermediates from CYP-mediated saturable metabolism of naphthalene in olfactory and nasal respiratory epithelia (Morris and Buckpitt 2009).

Several models have been developed to simulate the kinetics of naphthalene uptake and metabolism of inhaled naphthalene (Campbell et al. 2014; Kapraun et al. 2020; Willems et al. 2001). Two models are described in detail in the following discussion because they provide a means to simulate the nasal cavity

and systemic kinetics of naphthalene in rats and humans (Campbell et al. 2014; Kapraun et al. 2020); and the models have been used to predict nasal tissue and systemic doses for interspecies dosimetry extrapolation (Campbell et al. 2014; EPA 2022c; Kapraun et al. 2020).

### Campbell et al. 2014 Model

***Description.*** Campbell et al. (2014) developed a model to simulate the kinetics of inhaled naphthalene in rats and humans. The model consists of a nasal cavity and systemic model. The nasal cavity model simulates the kinetics of naphthalene uptake and metabolism in the nasal cavity and transfer of naphthalene to blood (absorption) and to distal regions of the respiratory tract. The systemic model simulates absorption, distribution, and elimination of naphthalene transferred from the nasal cavity to the lung. The structure of the nasal cavity model was based on models for vinyl acetate and acetaldehyde (Bogdanffy et al. 1999; Teeguarden et al. 2008). The systemic model was based on a model developed by Willems et al. (2001).

*Nasal cavity model.* The nasal cavity model divides the nasal tissues into compartments representing: (1) dorsal medial respiratory tissue; (2) dorsal/medial olfactory tissue; and (3) lateral/ventral respiratory tissue. The rat model includes two dorsal medial olfactory tissue compartments; the human model includes a single dorsal medial olfactory tissue compartment. Each tissue compartment is represented by layered subcompartments that provide a diffusion pathway for naphthalene between the surface mucus layer and deeper epithelial, basal cell, and submucosal layers. In the Campbell et al. (2014) model, the mucous and epithelial layers are represented by a single compartment. This simplification was justified on the basis that metabolism of naphthalene is not expected to occur in the mucous layer. Inhaled naphthalene deposits in the surface mucus layer of each tissue compartment and then diffuses to deeper subcompartments where it is cleared by metabolism and absorption to blood. The model simulates two air flow patterns in the nasal cavity. A dorsal/medial flow contacts the dorsal olfactory and respiratory compartments and a lateral/ventral flow that contacts the lateral ventral respiratory tissue. Naphthalene is assumed to be homogeneously distributed in the air flows and move through the nasal cavity by convection. Exchanges between naphthalene in air and the mucus surfaces are assumed to occur by diffusion, governed by the air-mucus concertation gradient, the mucus surface area, a mass transfer coefficient (cm/minute), and a tissue:air partition coefficient. Exchanges between nasal tissue subcompartments are governed by diffusion coefficients ($cm^2$/hour) and the concentrations gradient between subcompartments. Metabolism of naphthalene through the 1,2-epoxide pathway is simulated as a Michaelis-Menten processes ($V_{max}$, $K_M$), with parameter values assigned to the nasal respiratory and

olfactory epithelial cell compartments. The dispositions of metabolites are not simulated. Absorption of naphthalene from the submucosa is assumed to be flow-limited and governed by the mass of each chemical in the submucosa, blood flow to the submucosa, and a tissue:blood partition coefficient. Naphthalene that is not extracted into nasal cavity tissues is transferred to the lung, where flow-limited absorption occurs (see description of systemic model).

*Systemic model.* The systemic model includes compartments representing the upper respiratory tract (excluding the nasal cavity), lung, liver, fat, and two lumped compartments representing other richly perfused and poorly perfused tissues, respectively. Transfer of naphthalene from the lung to blood is assumed to be flow-limited, governed by the concentration in pulmonary air, ventilation rate, cardiac output, and the blood:air partition coefficient. Transfers between blood and other tissues are also assumed to be flow-limited, governed by tissue blood flow, the arterial blood concentration, and the tissue:blood partition coefficient. Metabolism of naphthalene through the 1,2-epoxide pathway is simulated as a Michaelis-Menten processes ($V_{max}$, $K_M$), with parameter values assigned to lung and liver. The dispositions of metabolites are not simulated.

***Parameter Estimates and Calibration.*** Values for nasal cavity physiological parameters (air flow, blood flow, subcompartment dimensions) were adopted from previously published nasal cavity models (Bogdanffy et al. 1999; Frederick et al. 1998; Morris 1999; Plowchalk et al. 1997). Values for nasal cavity blood flow in humans were based on estimates made in human clinical studies (Holmberg et al. 1989; Paulson et al. 1985). Physiological parameters for the systemic model were adopted from Brown et al. (1997). The value for the blood:air partition coefficient was reported in a naphthalene carcinogenicity assay (NTP 2000). Values for tissue:blood partition coefficients were cited to personal communications from A.R. Buckpitt.

Parameters ($V_{max}$, $K_M$) for metabolism of naphthalene in the nasal cavity and lung were from Buckpitt et al. (2013). The $K_M$ for liver was from Willems et al. (2001) and the liver $V_{max}$ was calibrated to the naphthalene blood-time profile following intravenous dosing of rats (Campbell et al. 2014). Human metabolism parameters for the nasal cavity and lung were based on estimates made in Rhesus monkeys (Buckpitt et al. 2013). The $V_{max}$ for the monkey nasal cavity (olfactory and respiratory tissues combined) from Buckpitt et al. (2013) was proportioned between olfactory and respiratory tissue in a 4:1 ratio based on estimates made in rats (Buckpitt et al. 2013). Nasal cavity olfactory and respiratory tissue $K_M$ values for humans were assigned values estimated for rats (Buckpitt et al. 2013). The $K_M$ for human lung was from (Buckpitt et al. 2013) and the $V_{max}$ from Buckpitt and Bahnson (1986). Values for $K_M$ and $V_{max}$ of

human liver were based on Cho et al. (2006). All *in vitro* estimates of $V_{max}$ were scaled to whole-tissue microsomal protein content.

After calibration of the liver $V_{max}$, the model predicted the overall dose-dependent time course for blood naphthalene concentrations in rats following a single intravenous dose of naphthalene (1, 3, or 10 mg/kg, Campbell et al. 2014). In general, the predicted concentrations were within a factor of 2 of observations. For the 1 mg/kg dose, most predictions were within 1 standard deviation (SD) of the observed means (shown in Figure 2 of Campbell et al. 2014).

A sensitivity analysis of the model showed that predictions of steady-state blood naphthalene concentrations and metabolism rates were most sensitive to values assigned to ventilation rate and cardiac output (reported in Tables 4 and 5 of Campbell et al. 2014). Within the nasal cavity, predicted tissue naphthalene concentrations and metabolism rates were most sensitive to values assigned to blood:air and tissue:blood partition coefficients and to the $K_M$ and $V_{max}$ for metabolism of naphthalene.

***Evaluation.*** The model was evaluated against observations of blood naphthalene concentrations in rats following 6-hour inhalation exposures to 10, 30 or 60 ppm naphthalene (NTP 2000). The model predicted the time course for the post-exposure decline in blood naphthalene levels, with all predictions within 1 SD of the observed means (shown in Figure 3 of Campbell et al. 2014). Model predictions of extraction of inhaled naphthalene in the nasal cavity were evaluated against observations made in rats following a 1-hour inhalation exposure to 3, 10, or 30 ppm naphthalene (Morris and Buckpitt 2009). Nasal cavity extraction was estimated based on the inhalation rate and naphthalene concentration in air sampled with a laryngeal cannula placed at the anterior end of the nasal cavity. The model predicted the observed dose-dependent decline in nasal cavity extraction predicted for saturable metabolism of naphthalene in the nasal cavity. Predictions were within 1 SD of the observed means (shown in Figure 4 of Campbell et al. 2014). The model also predicted a constant nasal cavity extraction that was independent of dose in rats pre-treated with 5-phenyl pentene to inhibit metabolism of naphthalene (Morris and Buckpitt 2009). The model was not evaluated against data from studies conducted in humans.

***Applications to Dosimetry.*** The model was used to predict naphthalene concentrations and rates of metabolism in rat and human dorsal olfactory epithelium, ventral nasal respiratory epithelium, lung, and liver for 6-hour inhalation exposures ranging from 0.1 to 60 ppm. Across this range of exposures, naphthalene concentrations in the dorsal olfactory epithelium were predicted to be 3–4 times higher in the

human compared to the rat. Rates of metabolism in the human dorsal olfactory epithelium were predicted to be approximately 20% of the rates predicted for rats (reported in Tables 6 and 7 of Campbell et al. 2014). Internal dose metrics were used to predict human equivalent exposure concentrations (HECs) corresponding to the same internal doses in rats and humans (reported in Table 9 of Campbell et al. 2014). The HEC based on dorsal olfactory epithelium naphthalene concentrations or rate of metabolism increased with dose, reflecting different assumptions about saturable metabolism of naphthalene in the rat and human nasal cavity. The HECs based on dorsal olfactory naphthalene concentration ranged from 0.0046 ppm (0.1 ppm rat exposure) to 4.47 ppm (60 ppm rat exposure). The HECs based on naphthalene metabolism in the dorsal olfactory epithelium ranged from 0.12 (0.1 ppm rat exposure) to 9.85 ppm (3 ppm rat exposure). These outcomes predict that HECs would be dependent on the exposure concentration and the internal dose metric selected.

### Kapraun et al. 2020 Model

***Description.*** Kapraun et al. (2020) developed a human model to simulate the kinetics of naphthalene absorbed from an application of JP-8 to the skin surface. The model is an extension of the Campbell et al. (2014) model with the addition of parameters describing the transfer of naphthalene from the exposure site ("exposure well") to blood. Two approaches to modeling skin penetration were explored, referred to in Kapraun et al. (2020) as a 2-compartment (2C) model and a partial differential equation (PDE) model. In the 2C model, transfer of naphthalene from the exposure well to the stratum corneum (SC) and from SC to viable epidermis (VE) are governed by the concentration gradient between compartments, a permeability coefficient (cm/minute) and tissue:well partition coefficient. Transfer of naphthalene from the VE to blood is assumed to be flow-limited and governed by tissue blood flow, the arterial blood concentration, and the tissue-blood partition coefficient. In the PDE model, transfer of naphthalene through the SC is simulated as diffusion through a series of 10 subcompartments representing progressively deeper layers of the SC and the outer layer of the VE. Each transfer is governed by the concentration gradient between layers and a diffusion coefficient ($cm^2$/minute). The PDE model predicts a SC depth profile for naphthalene concentrations in the SC, which is useful for simulating the loss of naphthalene from the SC resulting from tape stripping (Kim et al. 2006).

***Parameter Estimates and Calibration.*** Values for SC and VE thickness, SC:VE and BE:blood partition coefficients, and diffusion coefficients of naphthalene in the VE were from McCarley and Bunge (2001). Values for the SC:JP-8 and VE:JP-8 partition coefficients and the blood naphthalene dose rate from background sources of exposure were calibrated to blood naphthalene observations in humans. In these

studies, 10 adult subjects received 30-minute dermal applications of JP-8 to a 20-$cm^2$ area of the forearm (Kim et al. 2006). Parameters were calibrated against data from individual subjects and against the combined observations from all subjects. The two approaches yielded similar mean values for the three parameters (reported in Tables 5 and 6 of Kapraun et al. 2020). The calibrated 2C and PDE models predicted the time-course of blood naphthalene concentrations observed, with most predictions being less than a factor of 2 from observations (shown in Figure 3 of Kapraun et al. 2020)

A sensitivity analysis of the model evaluated predictions for average, peak, and steady-state blood naphthalene concentrations. In general, blood naphthalene concentrations were most sensitive to values assigned to parameters that determined the naphthalene dose to the skin surface such as the naphthalene concentration in JP-8, exposure surface area, and SC:JP-8 partition coefficient (shown in Figure 4 of Kapraun et al. 2020). The model was also sensitive to other systemic parameters such as cardiac output and blood flow to the liver and body weight. Predictions of blood naphthalene concentrations were relatively insensitive to values assigned to the nasal cavity parameters and to systemic metabolism parameters.

***Evaluation.*** Kapraun et al. (2020) did not report evaluations of performance of the model against observations that were not used for calibrating the model.

***Applications to Dosimetry.*** The model was used to predict naphthalene HECs to support derivation of an acute inhalation reference dose (EPA 2022c). The approach taken to deriving HECs was similar to the approach reported in Campbell et al. (2014). The study that formed the basis for the HECs exposed rats to air concentrations of naphthalene ranging from 0.1 to 30 ppm for a period of 6 hours. The cumulative amount of naphthalene metabolized in the dorsal olfactory epithelium during the 6-hour exposure period was selected as the internal dose metric for HEC calculations based on the rationale that lesions observed in the nasal cavity are likely to have resulted from reactive naphthalene metabolites formed in dorsal olfactory tissues. The point of departure (POD) based on benchmark dose (BMD) modeling of the rat internal doses was 24.2 µg metabolized/mL tissue. The Kapraun et al. (2020) human model was used to predict HECs corresponding to the rat internal doses. The corresponding HECs were 19.6 $\mu g/m^3$ (1-hour exposure), 1.41 $\mu g/m^3$ (8-hour exposure), and 0.451 $\mu g/m^3$ (24-hour exposure).

### 3.1.6 Animal-to-Human Extrapolations

***Naphthalene.*** Naphthalene-induced lesions in nasal epithelia of mice and rats appear to be the critical nonneoplastic effect (i.e., the effect occurring at the lowest exposure level) associated with inhalation exposure to naphthalene, while effects in the lung occur at higher exposures. Comparison of species susceptibility to naphthalene-induced nonneoplastic lung damage suggests that mice are much more sensitive than rats (e.g., nonneoplastic or neoplastic lung lesions were not found in chronically exposed rats in the NTP [2000] study) and that differences in rates and stereoselectivity of naphthalene metabolism to epoxide intermediates may be involved in this species difference (Buckpitt et al. 1992, 2002). Acute (4-hour) inhalation exposure of mice to naphthalene concentrations as low as 2–10 ppm induced lung injury, whereas rats exposed to naphthalene concentrations as high as 110 ppm showed no signs of lung injury (West et al. 2001). Some evidence has been reported that rates and stereoselectivity of naphthalene metabolism in primate lung tissue may be more like rats than mice (Buckpitt and Bahnson 1986; Buckpitt et al. 1992, 1995). In *in vitro* studies with microsomes from lymphoblastoid cells, which expressed recombinant human CYP2F1, metabolism of naphthalene to epoxide intermediates was demonstrated, but the predominant enantiomeric form produced (1*S*,2*R*-oxide) was different from the form (1*R*,2*S*-oxide) produced by mouse CYP2F2 (Lanza et al. 1999). Although these observations on epoxide formation may suggest that mice may be more sensitive than humans to acute naphthalene lung toxicity from epoxide intermediates, the possible role of other potentially reactive metabolites of naphthalene (e.g., the naphthoquinone metabolites) is unknown with chronic exposure scenarios.

In contrast to the lung, the olfactory epithelium and respiratory epithelium of the nose of rats and mice do not appear to differ in sensitivity to naphthalene nonneoplastic toxicity from inhalation exposure. Nonneoplastic nasal lesions were found in nearly all exposed animals of both species at the lowest exposure level, 10 ppm, in both chronic studies (NTP 1992a, 2000). *In vitro* and *ex vivo* data suggest that human nasal tissues may be susceptible to naphthalene toxicity. Kedderis et al. (2014) compared the cytotoxicity of naphthalene in freshly isolated cells from target tissues (lung and nasal epithelium) and nontarget tissues of F344 rats, B6C3F1 mice, and humans. Cytotoxicity in nasal respiratory epithelium, measured as cellular LDH, ATP, and reduced glutathione, was most severe in human nasal tissues (compared with rat and mouse), with dose-dependent decreases in all three measures, even though high-performance liquid chromatography (HPLC) analysis did not detect any naphthalene metabolites (Kedderis et al. 2014). The study authors suggested that human nasal respiratory epithelium may have a smaller pool of available reduced glutathione than rat or mouse. DeStefano-Shields et al. (2010) provided additional support for relevance of nasal lesions in rats to effects in humans. These investigators

compared the levels of adducted proteins in rat olfactory and septal turbinates with those in Rhesus monkey maxilloturbinates and ethmoturbinates incubated with $^{14}$C-naphthalene. No significant differences in the covalently bound radioactivity were detected between the rat and monkey tissues. Identification of the adducted proteins showed a wider variety in rat nasal tissue compared with monkey nasal tissue. In rats, adducted proteins included structural proteins (actin and tubulin), proteins involved in energy metabolism, and proteins involved in the unfolded protein response; in monkeys, the adducted proteins were primarily structural, but also included a multifunctional protease inhibitor (DeStefano-Shields et al. 2010).

Evidence for human susceptibility to naphthalene-induced nasal and pulmonary effects was provided in a study of transgenic mice expressing human CYP2A13 and CYP2F1 (Li et al. 2017). Mice expressing the humanized enzymes exhibited nasal and lung toxicity, while mice that lack CYP2 isozyme expression (Cyp2abfgs-null) did not. These findings support the human relevance of naphthalene toxicity seen in mice.

As described further in Section 2.20, Carratt et al. (2019a) observed greater quantities of radiolabeled naphthalene and 1,2-naphthoquinone DNA adducts in primate (Rhesus macaque) lung tissue explants compared with mouse lung tissue explants, and rat nasal tissue explants showed the smallest adduct levels. Carratt et al. (2019a) did not identify the specific adducts but did show that radiolabeled naphthalene and 1,2-naphthoquinone were covalently bound to DNA. The relevance of the DNA adducts to nasal and lung toxicity is uncertain.

Species differences in the effects of naphthalene may also be influenced by differing rates of hepatic metabolism, which contributes to circulating naphthalene and metabolites. Kovalchuk et al. (2017) compared airway histopathology changes in wild-type mice with those in liver-Cpr-null (LCN) mice after inhalation exposure to 5 or 10 ppm naphthalene for 4 hours. The LCN strain lacks hepatic microsomal P450 activity due to deletion of the *Cpr* gene. After inhalation exposure to naphthalene, the volume of damaged airway epithelial cells was significantly lower in LCN mice compared with wild-type mice, indicating that bioactivation of naphthalene in the liver contributes to lung tissue damage in mice.

***1- and 2-Methylnaphthalene.*** Pulmonary alveolar proteinosis induced in mice following chronic-duration oral exposure to 1- or 2-methylnaphthalene is assumed to be relevant to humans, in the absence of data to indicate otherwise. Pulmonary alveolar proteinosis is a condition that has been described in

humans, although reports noting associations with human exposure to 1- or 2-methylnaphthalene were not located.

*In vitro* studies comparing metabolism of naphthalene and alkylated naphthalenes by human and rat liver microsomes showed higher overall intrinsic clearance in human microsomes compared to rat microsomes (Wang et al. 2020). For naphthalene, metabolism by human microsomes yielded more 1,2-dihydro-1,2-naphthalenediol than 1-naphthol, while the opposite was true of rat microsomes (Wang et al. 2020).

## 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 naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene are discussed in Section 5.7, Populations with Potentially High Exposures.

***Naphthalene.*** Newborns and infants are thought to be more susceptible to adverse health effects from naphthalene (e.g., hemolytic anemia from acute exposure) because hepatic enzyme systems involved in conjugation and excretion of naphthalene metabolites are not well developed shortly after birth (EPA 1987). No studies were located, however, that specifically examined the influence of age on naphthalene toxicokinetic capabilities in humans.

Although the occurrence of hemolytic anemia in neonates of anemic, naphthalene-exposed mothers demonstrates that naphthalene and/or its metabolites can cross the placental barrier (Anziulewicz et al.

1959; Zinkham and Childs 1957, 1958), oral-exposure developmental toxicity studies in animals do not provide evidence that naphthalene was fetotoxic or impaired fetal development, even at maternally toxic dose levels as high as 450 mg/kg/day (NTP 1991; Plasterer et al. 1985; Texaco 1986).

Naphthalene has been detected in human milk samples (concentration not reported) (Pellizzari et al. 1982), but no studies were located that have specifically examined the rate or extent of naphthalene distribution to breast milk in exposed humans or animals.

The hemolytic response to naphthalene is enhanced by the presence of inherited erythrocyte glucose-6-phosphate dehydrogenase (G6PD) deficiency. Although any human may experience acute hemolysis if exposed to a sufficiently high dose of naphthalene, this enzyme deficiency may cause some persons to be unusually sensitive. The incidence of the deficiency among Caucasians of European origin is relatively low, while there is a higher incidence among certain groups of Asians and Middle Eastern populations. A study of hemolytic anemia in African-American children with G6PD deficiency by Shannon and Buchanan (1982) suggests that this is a population that may be susceptible to the hemolytic effects of naphthalene exposure. It was also reported that 16% of African-American males are G6PD-deficient (Calabrese 1986). According to Shannon and Buchanan (1982), a syndrome of acute severe hemolysis following exposure to oxidative stress is associated with the Mediterranean variant of the deficiency, whereas the hemolytic anemia seen in African Americans is generally mild.

Children with genetically determined G6PD deficiency are expected to be especially susceptible to the hemolytic action of naphthalene (Owa 1989; Owa et al. 1993; Santucci and Shah 2000; Valaes et al. 1963). In support of this hypothesis, in 21 cases of hemolytic anemia in Greek infants exposed to naphthalene, 10 of the children had a genetically determined deficiency in G6PD (Valaes et al. 1963). In a 10-year chart review of 24 African-American children hospitalized with acute hemolytic anemia, 14 were noted to have been exposed to naphthalene-containing moth repellants (Santucci and Shah 2000). Deficiency in G6PD makes red blood cells more susceptible to oxidative damage from a wide range of causes including naphthalene exposure. Relatively high rates of genetically determined G6PD deficiency have been reported in males of certain subpopulations of Asian, Arabic, Caucasian, African, and African-American ancestry (EPA 1987).

The limited mobility of infants when they are wearing naphthalene-treated clothing or when they are near other naphthalene-treated articles (e.g., blankets treated with naphthalene-containing moth repellants) may maximize exposure due to the development of a microenvironment with a high level of naphthalene

vapor in the space around the infant. The tendency for infants and small children to place small objects, such as mothballs, in their mouths also increases their risk.

Studies in animals have demonstrated greater susceptibility of young mice compared with adult mice to naphthalene-induced lung effects. Juvenile animals have been shown to be more susceptible to the lung toxicity of naphthalene after inhalation exposure than either adults or neonates. Carratt et al. (2019b) exposed 7-day old, 3-week-old, and adult mice to 5 or 10 ppm naphthalene in air for 4 hours and examined airway histology. Histopathology changes (club cell swelling and vacuolation) occurred in all of the examined airways and groups but were most severe in the proximal airways of juvenile females. The enhanced susceptibility of juvenile females was not explained by levels of glutathione-conjugated naphthalene in the lungs, which were higher in neonatal females than juveniles. Gene expression analysis showed that juvenile female mice were predisposed to upregulation of DNA damage and cancer pathways, suggesting a possible mechanism for the susceptibility (Carratt et al. 2019b).

In contrast, neonatal mice were more susceptible than juvenile or adult mice to lung injury from single i.p. doses of 25, 50, or 100 mg/kg naphthalene (Fanucchi et al. 1997). Epithelial damage in terminal bronchioles (principally in the club cells) was observed in 7-day-old mice exposed to 25 mg/kg but was absent in adult mice at the same dose level. In adult mice exposed to 50 mg/kg, injury was only mild and variable (from mouse to mouse) and only became consistent with exposure to 100 mg/kg. Epithelial damage in 14-day-old mice was less severe than the damage in 7-day-old mice. Activities of CYP-mediated naphthalene metabolism in bronchiolar tissues were 2.5 times lower in neonatal mice than in adult mice, suggesting that the difference in susceptibility is not explained by differences in ability to form reactive metabolites alone (e.g., 1,2-naphthalene oxide). Differences between neonates and adults in the balance between formation of reactive naphthalene metabolites and downstream transformations could potentially explain the difference in susceptibility to naphthalene toxicity, but the possibilities for specific, age-related differences in downstream enzyme activities for naphthalene (e.g., epoxide hydrolase, dihydrodiol dehydrogenase) have not been studied to date. Alternatively, toxicodynamic differences may exist between neonatal and adult mice (e.g., different target macromolecules). Based on findings that *in utero* exposure to other chemicals (which are bioactivated by CYP), caused club cell tumors in adult offspring, Fanucchi et al. (1997) postulated that naphthalene exposure during the neonatal period (when increased susceptibility to naphthalene-induced cytotoxicity occurs) may lead to loss of regulatory mechanisms resulting in club cell proliferation and tumor formation in adult animals. However, direct evidence for naphthalene in support of this hypothesis is not available (e.g.,

demonstration that *in utero* or neonatal naphthalene exposure will cause increased incidence of lung tumors in adult mice).

Female mice, but not male mice, developed lung tumors after chronic exposure to naphthalene (NTP 1992a). Several studies have suggested that female mice are more susceptible to lung injury than male mice after exposure to naphthalene. In the study of age-dependent lung toxicity in mice described above, Carratt et al. (2019b) observed the most severe damage to airway epithelium in juvenile females. These investigators also conducted studies examining DNA adducts in mouse and primate lung explants exposed to naphthalene, and in both species, the adduct levels were significantly (2–5-fold) higher in females than in males. In male and female Swiss-Webster mice given i.p. injections of 0 or 200 mg/kg naphthalene in corn oil, club cell injury in terminal bronchioles occurred earlier, affected cells farther up the airway tree, and showed a different temporal pattern of changes in female mice compared with male mice (Van Winkle et al. 2002). Twenty-four hours after injection, club cell injury in the lobar bronchus of female mice was evidenced by numerous vacuolated cells, whereas normal bronchiolar epithelium containing club and ciliated cells was found in vehicle-control males and females, as well as in exposed male mice. Assessment of *in vitro* naphthalene metabolism in micro-dissected regions of airways from male and female mice indicated that the rate of formation of a dihydrodiol metabolite (1,2-dihydroxy-1,2-dihydronaphthalene) was greater in female tissue than in male tissue (Van Winkle et al. 2002). Sutherland et al. (2012) showed that the higher susceptibility of female mice persisted when both sexes were exposed to a naphthalene regimen designed to induce tolerance. After seven daily i.p. injections of 10 μL/g body weight naphthalene followed by a challenge i.p. injection of 300 mg/kg, both males and females showed less damage than control animals given the challenge injection, but histopathology findings in female airways remained more extensive than in airways of males (Sutherland et al. 2012). Measurement of CYP2F2 messenger ribonucleic acid (mRNA) and protein levels showed that expression of this enzyme was lower in airways of tolerant females than tolerant males. However, protein expression of glutamate-cysteine ligase, a critical enzyme in glutathione synthesis that is believed to be upregulated in tolerant males, was also lower in females (Sutherland et al. 2012).

Sex differences in susceptibility to nasal tumors in rats were also observed; male rats developed respiratory adenomas, while female rats developed olfactory neuroblastomas (Abdo et al. 2001; NTP 2000). Cichocki et al. (2014) observed no difference in cytotoxicity in the nasal respiratory or olfactory epithelium in F344 rats exposed nose-only to naphthalene vapor concentrations up to 30 ppm for 4 or 6 hours. However, the study authors also observed greater induction of glutamate-cysteine ligase catalytic subunit gene expression in the olfactory epithelium of males compared to females (~2.5–4-fold

higher), and higher induction of heme oxygenase 1 gene expression. The study authors proposed that the reduced ability of female rat olfactory epithelium to induce antioxidant-responsive genes may play a role in the susceptibility of this tissue to tumor formation (Cichocki et al. 2014).

Other factors that could influence the susceptibility to naphthalene toxicity may include nutritional status and genetic differences in the form or expression of enzymes involved in the bioactivation and/or detoxification of naphthalene. Nutritional status can influence detoxification pathways such as glutathione conjugation and antioxidant capacity; however, no studies examining the role of nutrition in naphthalene toxicity were located. Some studies have shown that polymorphisms in genes encoding CYP2E1 and glutathione-S-transferase can modify the excretion of urinary naphthols in humans exposed to naphthalene (Rodrigues et al. 2014; Yang et al. 1999), but the relationship to naphthalene toxicity is uncertain.

***1- and 2-Methylnaphthalene.*** No direct information was located on the relative susceptibility of children or young animals to 1- or 2-methylnaphthalene toxicity, compared with adults. However, clinical experience with humans displaying pulmonary alveolar proteinosis of unknown etiology has indicated that children with this condition experience more severe symptoms and a worse prognosis for survival than adults (EPA 2003; Mazzone et al. 2001).

## 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 naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene 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 naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene 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 naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene 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. Biomarkers of susceptibility are discussed in Section 3.2, Children and Other Populations that are Unusually Susceptible.

### 3.3.1 Biomarkers of Exposure

***Naphthalene.*** In cases where humans have swallowed one or more mothballs, it is possible to identify the undissolved naphthalene in the stomach or duodenum by radioluminescence (Woolf et al. 1993). Thus, radiography of the abdominal area is of value in determining if exposure has occurred, especially in children who are often unreliable sources of exposure information. Of the 2,400 cases on naphthalene ingestion reported to 72 Poison Control Centers in the United States, 2,100 involve children <6 years old. Radioluminescence has the advantage of differentiating naphthalene-containing solids in the gastrointestinal tract from paradichlorobenzene or other materials used in moth repellants and deodorizers.

Methods are available for the determination of naphthalene in human adipose tissue (EPA 1986b; Liao et al. 1988). In the National Human Adipose Tissue Survey, 40% of the subjects surveyed had measurable levels of naphthalene with concentrations of up to 63 ng/g. Naphthalene and its metabolites can be detected in human and animal urine (Horning et al. 1980; Mackell et al. 1951; Stillwell et al. 1982). Investigators have reported strong correlations between 1-naphthol concentrations in the urine of exposed

workers and naphthalene concentrations in the breathing zone air (Bieniek 1994). Peak naphthalene concentrations in the urine occurred immediately after the end of the exposure period and declined thereafter. In some instances, 1-naphthol concentrations had returned to baseline 8 hours later. Few current data are available relating naphthalene levels in adipose tissue or urine with the human exposure concentrations.

In swine, a good correlation existed between 1-naphthol levels in hydrolyzed urine samples collected in the first and second 24 hours after dosing with as little as 7 µg/kg/day naphthalene (Keimig and Morgan 1986). Thus, 1-naphthol may be an appropriate biomarker for monitoring naphthalene exposures in the occupational setting. Some caution must be exercised in using 1-naphthol as a biomarker of naphthalene exposure in the general population since this metabolite is also excreted after exposure to the common insecticide, carbaryl (Benson and Dorough 1984).

Early work to develop biomarkers of exposure, such as naphthalene mercapturic acid derivatives in urine (Marco et al. 1993) and naphthalene hemoglobin adducts in blood (Cho et al. 1994b), has been extended to develop techniques to measure cysteinyl adducts formed from reactions of hemoglobin and albumin with reactive metabolites of naphthalene (Troester et al. 2002; Waidyanatha et al. 2002). One of the reasons for developing these techniques is that it is difficult to measure reactive metabolites of naphthalene *in vivo*. Using these techniques, hemoglobin and albumin adducts of 1,2-naphthalene oxide, 1,2-naphthoquinone, and 1,4-naphthoquinone were shown to increase with increasing dose in F344 rats given single oral doses of 0, 100, 200, 400, or 800 mg/kg naphthalene (Waidyanatha et al. 2002). The stabilities of the adducts were measured in rats following exposure to naphthalene (Troester et al. 2002). Some were found to be stable and others unstable, although they all were more stable than the reactive metabolites themselves. As such, the adducts are expected to be useful in estimating internal doses of these metabolites.

***1- and 2-Methylnaphthalene.*** An analytical method is available to determine levels of 2-methyl-naphthalene and its derivatives in rat urine (Melancon et al. 1982). This method would probably also be useful in measuring 2-methylnaphthalene levels in human urine. Because of the lack of information for 1-methylnaphthalene, it is not possible to identify a biomarker of exposure for this substance.

### 3.3.2 Biomarkers of Effect

***Naphthalene.*** Hemolytic anemia has been frequently reported to be a consequence of exposure to naphthalene. However, this effect can also occur without exposure to naphthalene, and may not be useful as a specific biomarker of effect. Club cell damage may be identified by the presence of naphthalene/ protein adducts in lung lavage fluids (Cho et al. 1994a). Additional research is needed to improve the specificity of this technique as a biomarker of effect. Similarly, naphthalene DNA adducts may serve as biomarkers of naphthalene effects (Carratt et al. 2019a), but data on adduct identity, abundance, and fate in various animal and human tissues are needed to ascertain specificity.

***1- and 2-Methylnaphthalene.*** Because of the lack of information for 1- or 2-methylnaphthalene, it is not possible to identify a biomarker of effects for these chemicals.

## 3.4 INTERACTIONS WITH OTHER CHEMICALS

Roberts et al. (2018) showed that concurrent exposure to naphthalene and carbon particles increased the deposition of naphthalene in the respiratory tract of male rats compared with naphthalene vapor alone. Significantly higher tissue concentrations of radioactivity were found in the bronchioles and lung of rats co-exposed to 20 ppm $^{3}$H-naphthalene and 5 mg/m$^{3}$ carbon particles compared with naphthalene alone. No effect of co-treatment with particles was seen in the radioactivity levels in the nasopharynx or trachea (Roberts et al. 2018).

When naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene was applied dermally in combination with benzo[a]pyrene (BaP), there was an inhibitory effect on the induction of skin tumors in female mice (Schmeltz et al. 1978). These investigators also reported that a mixture containing naphthalene (0.02%), 2-methylnaphthalene (0.02%), and 10 other methylated and ethylated naphthalenes (each at 0.02%) also appeared to inhibit the development of BaP-induced skin tumors. The study authors suggested that it is likely that certain naphthalenes compete with BaP for the same enzyme sites, resulting in alteration of the BaP metabolic pathway and decreased production of the active BaP metabolite. This hypothesis is consistent with the observation that benzo(a)pyrene hydroxylase is inhibited by naphthalene (Shopp et al. 1984). Dermal application of the naphthalene mixture did not induce tumors in the absence of BaP. The results of these studies were not analyzed statistically.

Several studies have been conducted to assess factors that influence the toxicity of naphthalene. For the most part, these studies have evaluated the effects of mixed function oxidase activity (MFO) and alterations in glutathione levels on pulmonary and ocular toxicities. The effects of cyclooxygenase activity, antioxidants, and epoxide hydrolase inhibitors on the cataractogenic effect of naphthalene have also been evaluated. The administration of MFO inhibitors (SKF-525A, metyrapone) and antioxidants (caffeic acid and vitamin E) decreased ocular toxicity in mice (Wells et al. 1989). Use of ALO1576, an inhibitor of the enzyme aldose reductase (also known as aldehyde reductase), prevented cataract formation in both *in vivo* and *in vitro* studies (Xu et al. 1992a, 1992b). On the other hand, naphthalene-induced cataracts were enhanced by pretreatment with a MFO inducer (phenobarbital) and a glutathione depletor (diethyl maleate) (Wells et al. 1989). Pulmonary damage was decreased by prior treatment with a MFO inhibitor (piperonyl butoxide), but enhanced by prior treatment with a glutathione depletor (diethyl maleate) (Warren et al. 1982). For the most part, these studies support the role for MFO activity and glutathione conjugation in naphthalene-induced pulmonary and ocular lesions.

MFO inducers also affect the metabolism of 2-methylnaphthalene. Inducers that influence CYP increase the oxidation of the side chain and the concentration of one dihydrodiol. Induction of CYP increased the production of two other dihydrodiols (Melancon et al. 1985). The production of naphthoic acid in preference to the diols may explain why acute exposure to 2-methylnaphthalene is less toxic to club cells than acute exposure to naphthalene.

In general, interactions with environmental contaminants, such as other polycyclic aromatic hydrocarbons (PAHs), heavy metals, and organic solvents, may be expected at hazardous waste sites. For example, many such sites contain arsenic, which is well known to increase oxidative stress and deplete GSH levels, and thus may influence naphthalene toxicity.