3. HEALTH EFFECTS

3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure (inhalation, oral, and dermal) and then by health effect (death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects). These data are discussed in terms of three exposure periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between

"less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of naphthalene, are indicated in Tables 3-1 and 3-2 and Figures 3-1 and 3-2.

A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

3.2.1 Inhalation Exposure

3.2.1.1 Death

Two Greek infants died as a consequence of acute hemolysis that resulted from exposure to naphthalene-treated materials (clothing, diapers, blankets, rugs, etc.). Both infants exhibited a severe form of jaundice (kernicterus), which often causes brain damage (Valaes et al. 1963). Exposure levels experienced by these children are unknown. One infant suffered from a glucose-6-phosphate dehydrogenase (G6PD) deficiency. The other infant was apparently heterozygous for this trait. Individuals with a G6PD genetic defect are prone to hemolysis after exposure to a variety of chemical oxidizing agents including nitrates, nitrites, aniline, phenols (Dean et al. 1992), and naphthalene.

No studies were located that documented lethal effects in humans after inhalation exposure to 1-methyl-naphthalene or 2-methylnaphthalene.

Exposure to 78 ppm naphthalene for 4 hours did not cause any deaths in rats. In addition, no definitive adverse clinical signs were observed during the 14 days after exposure, and no gross pathologic lesions were observed at necropsy (Fait and Nachreiner 1985). A high background mortality in the male control group precluded drawing conclusions regarding the effects of lifetime exposures to 10 and 30 ppm naphthalene (6 hours/day, 5 days/week) on lifetime mortality; no apparent effects on mortality occurred in the females (NTP 1992a). Similarly, exposure of male and female rats to 10, 30, or 60 ppm naphthalene (6 hours/day, 5 days/week) for 2 years did not affect survival, compared to controls (Abdo et al. 2001; NTP 2000).

No studies were located that documented lethal effects in animals after inhalation exposure to 1-methylnaphthalene or 2-methylnaphthalene.

3.2.1.2 Systemic Effects

No studies were located that documented dermal effects in humans or animals after inhalation exposure to naphthalene. Most of the human data come from occupational and domestic settings where mothballs were the source of the naphthalene vapors. The highest NOAEL values and all LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1. No studies were located that documented systemic effects in humans after inhalation exposure to 1-methylnaphthalene or 2-methylnaphthalene. In animals, one study evaluated hematological end points in dogs following acute inhalation exposure to undetermined air concentrations of 1-methylnaphthalene or 2-methylnaphthalene (Lorber 1972). This study, however, did not identify reliable NOAEL or LOAEL values, and the results are not included in Table 3-1 or Figure 3-1.

Respiratory Effects. No studies were located that documented respiratory effects in humans after inhalation exposure to naphthalene.

The nose is the most sensitive toxicity target in rats and mice following chronic inhalation exposure to naphthalene. Chronic inhalation exposure resulted in increased incidences of nonneoplastic and neoplastic lesions in the nose of rats (Abdo et al. 2001; Long et al. 2003; NTP 2000), nonneoplastic lesions in the nose of mice (NTP 1992a), and neoplastic and nonneoplastic lesions in the lungs of mice (NTP 1992a). No exposure-related lesions were found in other tissues or organs in these studies, which included comprehensive histopathological examinations of major tissues and organs. Nearly all mice of

Table 3-1 Levels of Significant Exposure to Naphthalene (Nap) Or Methylnaphthalene (1-Mn Or 2-Mn) - Inhalation

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
	ACUTE EXPOSURE						
	Systemic						
1	Rat (Sprague-Dawley)	4 h	Resp	100			West et al. 2001 NAP
2	Mouse B6C3F1	14 d 5 d/wk 6 hr/d	Hemato	30			NTP 1992a NAP

Table 3-1 Levels of Significant Exposure to Naphthalene (Nap) Or Methylnaphthalene (1-Mn Or 2-Mn) - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)		LOAEL Serious (ppm)		Reference Chemical Form
3	Mouse (Swiss-Webster)	4 h	Resp	2	10	(Clara cell necrosis and decreased Clara cell mass [volume/surface area] in proximal airways)	75	(Proximal and terminal epithelium devoid of Clara cells)	West et al. 2001 NAP
	Neurological								
4	Rat (Wistar)	4 h		26	44	(increased latency of paw lick response to being placed on a hot surface [decreased pain sensitivity]; no change in rotarod performance)			Korsak et al. 1998 1-MN
5	Rat (Wistar)	4 h		39	61	(increased latency of paw lick response to being placed on a hot surface [decreased pain sensitivity]; no change in rotarod performance)			Korsak et al. 1998 2-MN

Table 3-1 Levels of Significant Exposure to Naphthalene (Nap) Or Methylnaphthalene (1-Mn Or 2-Mn) - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
	CHRONIC EXPOSURE						
	Systemic						
6	Rat (Fischer- 344)	105 wk 5 d/wk 6 hr/d vapor	Resp		10[b] (inflammation of the nose; olfactory epithelium: atypical hyperplasia, atrophy, degeneration; nasal respiratory epithelium: hyperplasia, squamous metaplasia, degeneration; Bowman's glands: hyperplasia)		NTP 2000 (Abdo et al. 2001) NAP
			Cardio	10			
			Gastro	10			
			Musc/skel	10			
			Hepatic	10			
			Renal	10			
			Endocr	10			
			Ocular	10			
			Bd Wt	10			

Table 3-1 Levels of Significant Exposure to Naphthalene (Nap) Or Methylnaphthalene (1-Mn Or 2-Mn) - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
7	Mouse B6C3F1	104 wk 5 d/wk 6 hr/d	Resp		10 (inflammation of the nose and lung, metaplasia of the olfactory epithelium, and hyperplasia of the respiratory epithelium)		NTP 1992a NAP

Table 3-1 Levels of Significant Exposure to Naphthalene (Nap) Or Methylnaphthalene (1-Mn Or 2-Mn) - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
8	Mouse B6C3F1	104 wk 5 d/wk 6 hr/d	Cardio	30			NTP 1992a NAP
			Gastro	30			
			Hepatic	30			
			Renal	30			
			Dermal	30			
	Neurological						
9	Rat (Fischer- 344)	105 wk 5 d/wk 6 hr/d vapor		60			NTP 2000 (Abdo et al. 2001) NAP

Table 3-1 Levels of Significant Exposure to Naphthalene (Nap) Or Methylnaphthalene (1-Mn Or 2-Mn) - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
10	Mouse B6C3F1	104 wk 5 d/wk 6 hr/d		30			NTP 1992a NAP
	Reproductive						
11	Rat (Fischer- 344)	105 wk 5 d/wk 6 hr/d vapor		60			NTP 2000 (Abdo et al. 2001) NAP

Table 3-1 Levels of Significant Exposure to Naphthalene (Nap) Or Methylnaphthalene (1-Mn Or 2-Mn) - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)		Reference Chemical Form
12	Mouse B6C3F1	104 wk 5d/wk 6 hr/d		30				NTP 1992a NAP
	Cancer							
13	Rat (Fischer- 344)	105 wk 5d/wk 6hr/d vapor				10	(CEL: nasal respiratory epithelial adenomas in males & in females at higher concentrations; olfactory epithelial neuroblastomas in both sexes at higher concentrations)	NTP 2000 (Abdo et al. 2001) NAP
14	Mouse B6C3F1	104 wk 5 d/wk 6 hr/d				30	(CEL: pulmonary alveolar adenomas in females)	NTP 1992a NAP

a The number corresponds to the entries in Figure 3-1.

b Used to derive a chronic-duration Minimal Risk Level (MRL) of 0.0007 ppm; based on a human equivalent concentration LOAEL of 0.2 ppm which was divided by an uncertainty factor of 300 (10 for the use of LOAEL, 3 for extrapolating from rodents to humans with interspecies dosimetric adjustment, and 10 for human variability).

Cardio = cardiovascular; d = day(s); Gastro = gastrointestinal; Hemato = hematological; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s)

Figure 3-1. Levels of Significant Exposure to Naphthalene (NAP) Or Methylnaphthalene (1-MN Or 2-MN)- Inhalation

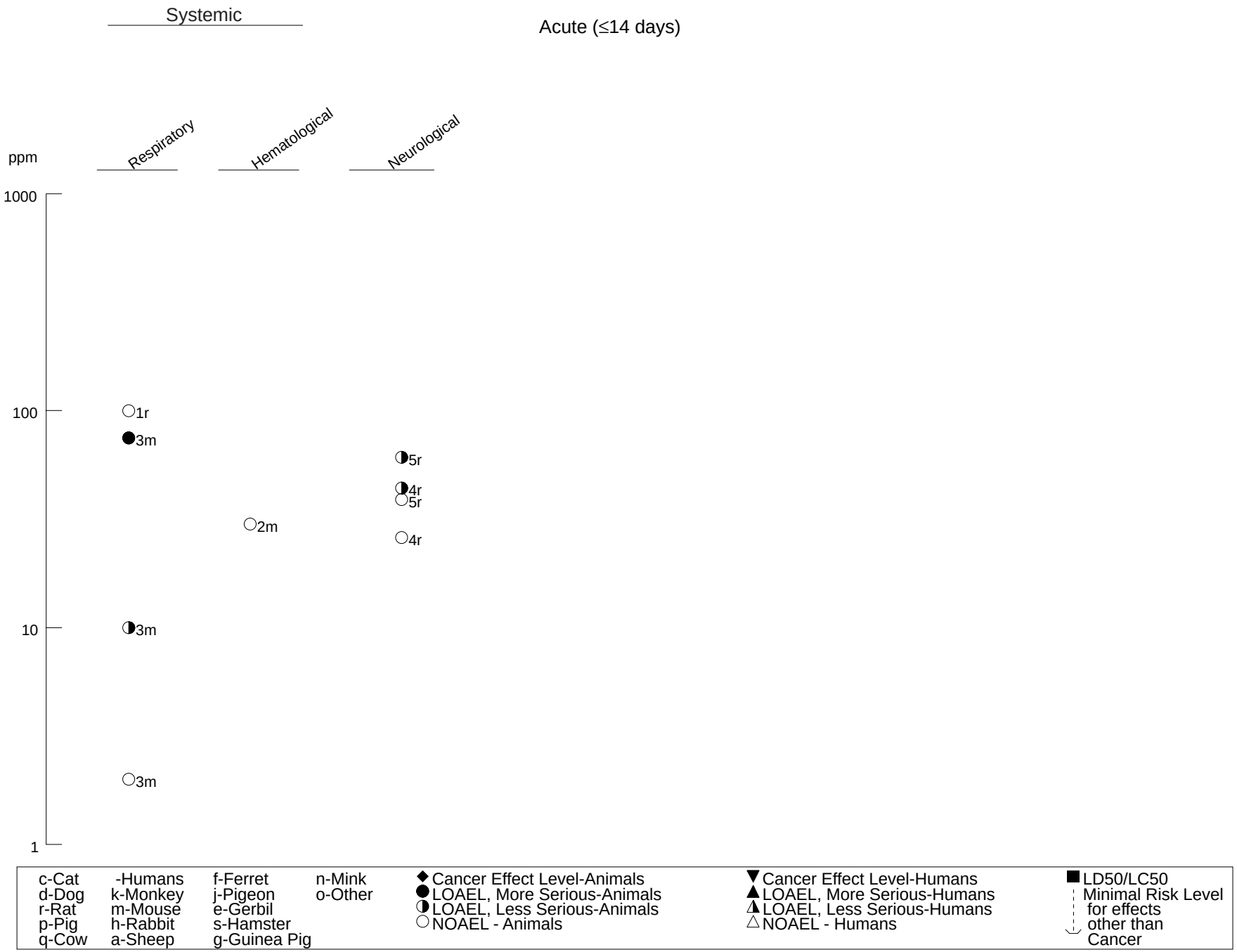
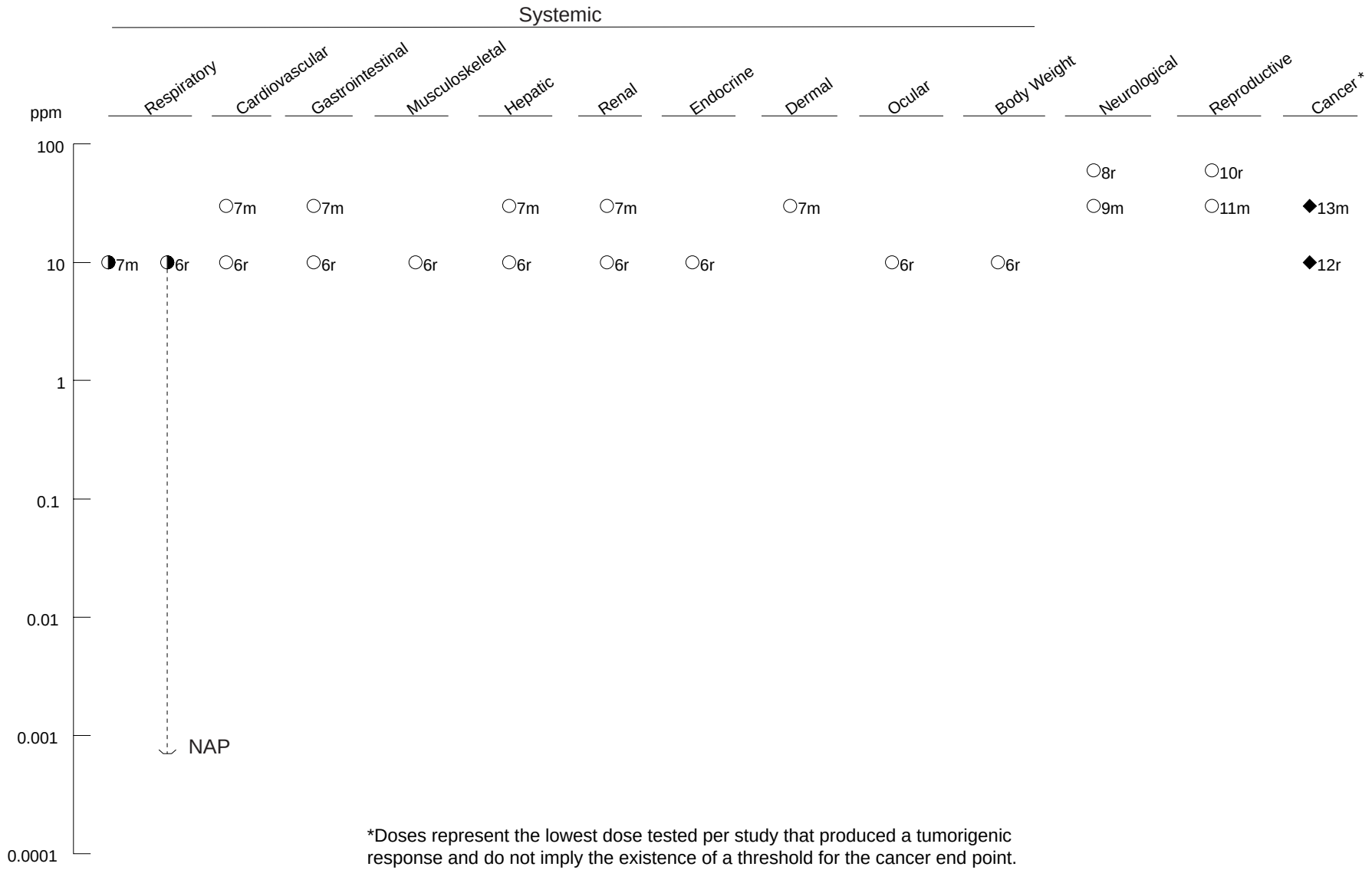


Figure 3-1. Levels of Significant Exposure to Naphthalene (NAP) Or Methylnaphthalene (1-MN Or 2-MN) - Inhalation (*Continued*)

Chronic (≥365 days)



both sexes (>95%) exposed to naphthalene vapors for 2 years (10 or 30 ppm) showed chronic inflammation and metaplasia of the olfactory epithelium and hyperplasia of the nasal respiratory epithelium (NTP 1992a). Chronic lung inflammation was also observed in exposed mice, but at lower incidences than incidences for nasal lesions. Incidences for chronic lung inflammation were 0/70, 21/69, and 56/135 for male mice and 3/69, 13/65, and 52/135 for female mice exposed to 0, 10, or 30 ppm. In addition, exposure to 30 ppm (but not 10 ppm) increased the incidence of benign lung tumors (alveolar/bronchiolar adenomas) in female mice, compared with controls. One other female mouse exposed to 30 ppm showed a malignant lung tumor (alveolar/bronchiolar carcinoma). In rats of both sexes, inhalation of 10, 30, or 60 ppm naphthalene induced nonneoplastic and neoplastic lesions only in the nasal cavity (Abdo et al. 2001; NTP 2000). Nearly all rats in each exposure group (>95%) showed nonneoplastic nasal lesions. Nonneoplastic nasal lesions in exposed rats included (1) hyperplasia, atrophy, chronic inflammation, and hyaline degeneration of the olfactory epithelium and (2) hyperplasia, metaplasia, or degeneration of the respiratory epithelium or glands. Neoplastic lesions associated with naphthalene exposure in rats were olfactory epithelial neuroblastoma (a rare malignant tumor) and respiratory epithelial adenoma.

The chronic inhalation MRL for naphthalene is based on the LOAEL of 10 ppm for nonneoplastic lesions in the olfactory epithelium and respiratory epithelium of the nose of rats (NTP 2000; see Table 3-1, Figure 3-1, Appendix A, and Section 2.3). To derive the chronic MRL, the rat LOAEL was converted to a human equivalent concentration of 0.2 ppm for continuous exposure using EPA (1994b) equations for a category 1 gas producing nasal effects and divided by an uncertainty factor of 300 (10 for the use of a LOAEL, 3 for extrapolation from animals to humans using dosimetric adjustment, and 10 for human variability). Naphthalene-induced damage to the nasal tissue is thought to be due to reactive metabolites formed in the nasal tissues (Buckpitt et al. 2002). Sections 3.4.3 and 3.5 discuss current mechanistic hypotheses in more detail.

Acute (4-hour) inhalation exposure to naphthalene induced necrosis of Clara cells in the epithelium of the proximal airways of the lungs of mice at exposure levels as low as 10 ppm, but did not affect lung tissue in rats at concentrations as high as 100 ppm (West et al. 2001). These results, and those from the chronic inhalation studies, show that mice are more susceptible than rats to lung damage from inhaled naphthalene. However, there are no studies that have examined nasal tissues for the development of lesions following acute inhalation exposure. No acute inhalation MRL was derived for naphthalene, due to the lack of such data and the results of the chronic studies indicating that nasal tissues are the critical toxicity targets of inhaled naphthalene in both rats and mice.

A change to mouth breathing occurred in rats during exposure to 78 ppm naphthalene, but no other effects on respiration were noted (Fait and Nachreiner 1985).

Cardiovascular Effects. No studies were located that documented cardiovascular effects in humans after inhalation exposure to naphthalene.

No histological changes were seen in the hearts of mice (30 ppm) or rats (60 ppm) that were exposed to naphthalene for 2 years (Abdo et al. 2001; NTP 1992a, 2000).

Gastrointestinal Effects. Nausea, vomiting, and abdominal pain were reported in eight adults and one child exposed to naphthalene vapors from large numbers of mothballs (300–500) scattered throughout their homes for odor and pest control (Linick 1983). Air samples collected in one home contained naphthalene at 20 ppb; concentrations could have been higher when the mothballs were fresh. Gastrointestinal symptoms disappeared after the mothballs were removed. Few location-specific background data to support this air concentration were reported.

There were no histopathological changes in the stomach or intestines of mice (30 ppm) or rats (60 ppm) exposed to naphthalene for 2 years (Abdo et al. 2001; NTP 1992a, 2000).

Hematological Effects. Hemolytic anemia is the most frequently reported manifestation of naphthalene exposure in humans. Acute hemolytic anemia was observed in 21 infants exposed to naphthalene via mothball-treated blankets, woolen clothes, or materials in the infants' rooms (Valaes et al. 1963). Ten of these children had a G6PD genetic defect that increased their sensitivity to hemolysis from a variety of chemicals, including naphthalene. Clinical observations included high serum bilirubin values, methemoglobin, Heinz bodies, and fragmented red blood cells. Inhalation appeared to be the primary route of exposure because in all children but two, the naphthalene-treated material was not worn next to the skin. One of the exceptions was an infant who wore diapers that had been stored in naphthalene.

Anemia was reported in nine individuals exposed to large numbers of mothballs distributed throughout their homes (Linick 1983). The nature of the anemia and specific levels of naphthalene exposure were not identified. In one home, the naphthalene concentration was determined to be 20 ppb at the time of testing, but could have been higher when the mothballs were first distributed.

In another study, a woman who was exposed to reportedly high (but unmeasured) concentrations of a combination of naphthalene and paradichlorobenzene for several weeks in a hot, poorly ventilated work area developed aplastic anemia (Harden and Baetjer 1978). It is difficult to determine the contribution of naphthalene to the aplastic anemia since there was simultaneous exposure to paradichlorobenzene.

In animals, no treatment-related effects on hematologic parameters (hematocrit, hemoglobin concentration, erythrocyte counts, mean cell volume, reticulocytes, and leucocytes) were observed among mice exposed to 10 and 30 ppm naphthalene for 14 days (NTP 1992a). Due to high mortality in the control males, hematology measurements were not continued beyond 14 days.

The effects of 1-methylnaphthalene (pure and practical grade) and 2-methylnaphthalene (pure and practical grade) on the hematocrit values, total and differential white blood cell counts, and reticulocyte counts were determined in intact and splenectomized dogs. Each compound was dispersed in the atmosphere in a refined kerosene base using a fogger. Exposures occurred on four consecutive mornings (Lorber 1972). Based on the information presented, it was not possible to determine the exposure concentration.

Pure 1-methylnaphthalene increased the reticulocyte counts in the splenectomized dogs but not the intact dogs. Reticulocyte values remained elevated for 10 days after the fogging ceased. Practical grade 1-methylnaphthalene increased leukocyte counts in intact and splenectomized dogs and neutrophil counts in intact dogs, but pure 1-methylnaphthalene had no effect on these parameters. 2-Methylnaphthalene had no effect on any of the parameters monitored (Lorber 1972).

Neither 1-methylnaphthalene nor 2-methylnaphthalene had an effect on hematocrit values, suggesting that these compounds do not cause hemolysis under the conditions of the study. Since the increased reticulocyte counts were seen only in splenectomized dogs, it is difficult to interpret whether or not this change signifies increased hematopoiesis in response to 1-methylnaphthalene exposure (Lorber 1972).

Musculoskeletal Effects. No studies were located that documented musculoskeletal effects in humans after inhalation exposure to naphthalene.

Histological examination of the femur did not reveal compound-related effects in mice (NTP 1992a) or rats (Abdo et al. 2001; NTP 2000) exposed for 2 years to naphthalene concentrations as high as 30 or 60 ppm, respectively.

Hepatic Effects. Jaundice has been reported in infants and adults after exposure to naphthalene (Linick 1983; Valaes et al. 1963). However, the jaundice is a consequence of hemolysis rather than a direct effect of naphthalene on the liver. Infant exposures lasted 1–7 days (Valaes et al. 1963); adult exposure durations were not provided (Linick 1983). Dose was not determined in either instance, although a concentration of 20 ppb was measured in the home of one affected individual (Linick 1983).

In animals, no treatment-related gross or histopathological lesions of the liver were reported in mice (NTP 1992a) or rats (Abdo et al. 2001; NTP 2000) exposed for 2 years to naphthalene concentrations as high as 30 or 60 ppm, respectively.

Renal Effects. Renal disease was reported in nine individuals (details not specified) exposed to large numbers of mothballs in their homes, but symptoms were not described and dose could not be determined (Linick 1983).

In animals, no treatment-related gross or histopathological lesions of the kidneys were observed in mice (NTP 1992a) or rats (Abdo et al. 2001; NTP 2000) exposed for 2 years to naphthalene concentrations as high as 30 or 60 ppm, respectively.

Ocular Effects. Twenty-one workers exposed to naphthalene for up to 5 years in a plant that manufactured dye intermediates were examined for eye problems (Ghetti and Mariani 1956). During the period of exposure, plant conditions were primitive, involving heating of naphthalene in open vats and considerable worker contact with the naphthalene. Eight of the 21 workers developed multiple pin-point lens opacities that had no correlation with the age of the workers. These effects were not overtly noticeable and apparently had no effect on vision. They were judged to be a consequence of naphthalene exposure on the basis of their location in the crystalline lens and the fact that occurrence did not correlate with age. Exposure involved long-term inhalation of vapors and direct contact of vapors with the eyes and skin.

Retinal bleeding and the beginnings of a cataract were identified in a worker from a naphthalene storage area who was most likely exposed to naphthalene through inhalation and dermal/ocular contact (van der Hoeve 1906). The duration of exposure prior to seeking medical attention for eye irritation and problems with vision was not identified.

In animals, no treatment-related gross or histopathological lesions of the eyes were observed in mice (NTP 1992a) or rats (Abdo et al. 2001; NTP 2000) exposed for 2 years to naphthalene concentrations as high as 30 or 60 ppm, respectively. However, during a 4-hour exposure of rats to a concentration of 78 ppm, irritation to the eyes was evidenced through lacrimation (Fait and Nachreiner 1985).

3.2.1.3 Immunological and Lymphoreticular Effects

No studies were located that examined immunological or lymphoreticular end points in humans or animals after inhalation exposure to naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene.

3.2.1.4 Neurological Effects

Infants are prone to permanent neurological damage (kernicterus) as a consequence of the jaundice that results from naphthalene-induced hemolysis. Bilirubin is absorbed by vulnerable brain cells and this leads to convulsions and sometimes death. Survivors often suffer from motor disturbances and mental retardation (McMurray 1977). Kernicterus was diagnosed in 8 of 21 Greek infants that experienced hemolysis as a result of naphthalene exposure (Valaes et al. 1963). Two of the eight died. One of the infants that died had no G6PD enzyme activity and the other had intermediate activity. Two of the infants were normal with regard to the G6PD trait. Of the remaining infants, three had no G6PD activity and the fourth had intermediate activity. Brain damage seldom occurs in adults as a consequence of jaundice (McMurray 1977).

Nausea, headache, malaise, and confusion were reported in several individuals (children and adults) exposed to large numbers of mothballs in their homes (Linick 1983). Actual levels and duration of exposure were unknown, although a concentration of 20 ppb was measured in one of the affected residences.

In animals, no treatment-related gross or histopathological lesions of the brain were observed in mice (NTP 1992a) or rats (Abdo et al. 2001; NTP 2000) exposed for 2 years to naphthalene concentrations as high as 30 or 60 ppm, respectively. Clinical observations (made twice daily in these studies) revealed no gross behavioral changes except that exposed mice tended to huddle together in cage corners during exposure periods.

No studies were located that documented neurological effects in humans after inhalation exposure to 1-methylnaphthalene or 2-methylnaphthalene.

In male Wistar rats, decreased sensitivity to pain occurred after 4-hour inhalation exposures to 253 or 407 mg/m^3 1-methylnaphthalene (44 or 70 ppm), or 352 or 525 mg/m^3 2-methylnaphthalene (61 or 90 ppm), but not after exposure to 152 mg/m^3 (26 ppm) 1-methylnaphthalene or 229 mg/m^3 (39 ppm) 2-methylnaphthalene (Korsak et al. 1998). Decreased sensitivity to pain was measured as a decreased time to begin licking of the paws after being placed on a hot plate at 54.5 °C. The ability of exposed rats to balance on a rotating rod (rotarod performance), however, was not affected by any of these exposure conditions (Korsak et al. 1998). NOAEL and LOAEL values for decreased pain sensitivity from this study are included in Table 3-1 and Figure 3-1.

3.2.1.5 Reproductive Effects

No studies were located that documented reproductive effects in humans after inhalation exposure to naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene.

In animals, histological examination did not reveal damage to male or female reproductive organs in mice (NTP 1992a) or rats (Abdo et al. 2001; NTP 2000) exposed for 2 years to 30 or 60 ppm, respectively.

No studies were located that documented reproductive effects in animals after inhalation exposure to 1-methylnaphthalene or 2-methylnaphthalene.

3.2.1.6 Developmental Effects

No studies were located that examined developmental end points in humans or animals after inhalation exposure to naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene.

3.2.1.7 Cancer

No studies were located that documented carcinogenic effects in humans after inhalation exposure to naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene.

In animals, inhalation exposure to naphthalene (6 hours/day) has been associated with: (1) increased incidences of F344/N rats of both sexes with nasal tumors following 2 years of exposure (Abdo et al. 2001; NTP 2000); (2) increased incidences of female B6C3F1 mice, but not male mice, with lung tumors following 2 years of exposure (NTP 1992a); and (3) increased number of tumors per tumor-bearing A/J strain mice following 6 months of exposure (Adkins et al. 1986).

In F344/N rats, incidences of nasal respiratory epithelial adenomas were statistically significantly elevated, compared with controls, in males exposed to 0, 10, 30, or 60 ppm naphthalene (0/49, 6/49, 8/48, or 15/48), but not in females (0/49, 0/49, 4/49, 2/49) (Abdo et al. 2001; NTP 2000). Incidences for olfactory epithelial neuroblastoma were 0/49, 0/49, 4/48, and 3/48 in male rats, and 0/49, 2/49, 4/48, and 12/49 in female rats. Both tumor types are rare in NTP control F344/N rats (NTP 2000). For example, neither tumor type was observed in 299 control male rats given NTP-2000 feed or 1,048 control male rats given NIH-07 feed. NTP (2000) concluded that there was clear evidence of carcinogenic activity of naphthalene in male and female F344/N rats based on increased incidences of respiratory epithelial adenoma and olfactory epithelial neuroblastoma of the nose. Nearly all rats in all exposure groups showed nonneoplastic nasal lesions in both olfactory and respiratory epithelia, including atypical hyperplasia in olfactory epithelium, hyaline degeneration in olfactory and respiratory epithelia, and Bowman’s gland hyperplasia.

In B6C3F1 mice, statistically significant increased incidence of alveolar/bronchiolar adenomas and carcinoma was found in 30-ppm females, but not in 10-ppm females or in males (females: 5/69, 2/65, 29/135; males: 7/70, 17/69, and 31/135) (NTP 1992a). Although Fisher Exact tests indicated that incidences in both exposed male groups and the high-dose female group were significantly increased compared with control groups, logistic regression analysis, which modeled tumor incidence as a function of dose and exposure time, indicated that only the incidence in the 30-ppm female group was elevated compared with controls. The response was predominantly benign; only one female mouse in the 30-ppm group developed a carcinoma. Exposed mice of both sexes also showed increased incidences of chronic lung inflammation (males: 0/70, 21/69, 56/135; females: 3/69, 13/65, 52/135). Nonneoplastic nasal lesions were found in nearly all exposed mice, but no nasal tumors developed. On the basis of this analysis, NTP (1992a) determined that there was some evidence of naphthalene carcinogenicity in female mice, but no evidence of carcinogenicity in male mice in this study.

In a 6-month study, there was a statistically significant increase in the number of tumors per tumor-bearing mouse, but not in the number of mice with pulmonary adenomas after exposure to 10 or 30 ppm

naphthalene vapors (Adkins et al. 1986). However, the incidence of adenomas in the control group for this experiment was significantly lower than the pooled incidence observed in the control groups of eight concurrently conducted 6-month studies, and the difference in tumor incidence was not significantly greater than that of the historic controls.

No studies were located that documented carcinogenic effects in animals after inhalation exposure to 1-methylnaphthalene or 2-methylnaphthalene.

3.2.2 Oral Exposure

3.2.2.1 Death

Death has been documented in humans who intentionally ingested naphthalene. A 17-year-old male died 5 days after the ingestion of an unknown quantity of naphthalene mothballs. Death was preceded by vomiting, evidence of gastrointestinal bleeding, blood-tinged urine, and coma (Gupta et al. 1979). A 30-year-old female died following similar sequelae 5 days after reportedly swallowing 40 mothballs (25 were recovered intact from the stomach upon autopsy) (Kurz 1987). No studies were located that documented lethal effects in humans after oral exposure to 1-methylnaphthalene or 2-methylnaphthalene.

Several animal studies have been conducted to estimate lethal doses of naphthalene. Mice appear to be more sensitive than rats or rabbits. The LD_{50} values in male and female mice were 533 and 710 mg/kg, respectively (Shopp et al. 1984). An LD_{50} of 354 mg/kg was estimated in female mice treated with naphthalene once daily by gavage for 8 consecutive days (Plasterer et al. 1985). The dose response curve appeared to be very steep because no deaths occurred at 250 mg/kg/day, but all animals died with a dose of 500 mg/kg/day. At the 300 mg/kg/day dose, mortality was approximately 15%. In a different study with a 14-day dosing period, 10% of the males and 5% of the females died at a dose of 267 mg/kg/day, but none were affected by doses of 27 and 53 mg/kg/day (Shopp et al. 1984).

The oral LD_{50} values in male and female rats were 2,200 and 2,400 mg/kg, respectively, in one study (Gaines 1969), and 2,600 in a second study that did not differentiate by sex (Papciak and Mallory 1990). Male rats tolerated daily doses of 1,000 mg/kg without lethality, even after 18 days of administration (Yamauchi et al. 1986). In an increasing dose study, Germansky and Jamall (1988) treated male rats with naphthalene at doses beginning at 100 mg/kg/day and raised the dose weekly to a final level of 750 mg/kg/day over 6 weeks. Doses were then kept constant for an additional 3 weeks. The animals

tolerated 750 mg/kg/day with no mortalities. No increase in mortality was observed in rats administered naphthalene at 41 mg/kg/day in a 2-year feeding study (Schmahl 1955).

Although few data are available, rabbits appear to tolerate naphthalene in doses similar to those administered to rats. Two different rabbit strains were administered 1,000 mg/kg twice per week for 12 weeks without lethality (Rossa and Pau 1988).

Male and female mice survived oral exposure to doses of 71.6–143.7 mg/kg/day 1-methylnaphthalene for 81 weeks (Murata et al. 1993). No studies were located that documented lethal effects in animals after ingestion of 1-methylnaphthalene.

All LOAEL values for lethality in each species after acute exposure to naphthalene are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.2 Systemic Effects

No studies were located that documented musculoskeletal or dermal effects in humans or animals after oral exposure to naphthalene; data were available for all other systems. The highest NOAEL values and all LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

No studies were located that documented systemic effects in humans after oral exposure to 1-methylnaphthalene or 2-methylnaphthalene. In animals, data are restricted to two studies with B6C3F1 mice exposed to 1-methylnaphthalene (Murata et al. 1993) or 2-methylnaphthalene (Murata et al. 1997) in the diet for 81 weeks. The highest chronic NOAEL values and the lowest LOAEL value for systemic effects in mice are recorded in Table 3-2 and plotted in Figure 3-2.

Respiratory Effects. No reports have been located to indicate that there are direct effects of oral exposure to naphthalene on the respiratory system in humans. In situations where respiratory effects such as hypoxia or pulmonary edema were noted, the respiratory effects appear to be secondary to hemolysis and the events leading to general multiple organ failure (Gupta et al. 1979; Kurz 1987). On hospital admission, one male infant was described as experiencing labored breathing after presumably chewing a

Table 3-2 Levels of Significant Exposure to Naphthalene (nap) Or Methylnaphthalene (1-mn Or 2-mn) - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
ACUTE EXPOSURE							
Death							
1	Rat Sherman	once (GO)				2200 (LD50 - male) 2400 (LD50 - female)	Gaines 1969 NAP
2	Rat Sprague-Dawley	once (GO)				2600 LD50	Papciak and Mallory 1990 NAP
3	Mouse CD-1	8 d 1x/d (GO)				300 (5/33 died)	Plasterer et al. 1985 NAP
4	Mouse CD-1	once (GO)				710 (LD50) 533 (LD50)	Shopp et al. 1984 NAP
5	Mouse CD-1	14 d 1x/d (GO)				267 (10/96 male, 3/60 female)	Shopp et al. 1984 NAP
Systemic							
6	Human	once	Gastro		109 (adbominal pain)		Gidron and Leurer 1956 NAP
			Hemato			109 (hemolytic anemia)	
			Other			109 (106 degree F fever)	
7	Rat Sprague-Dawley	9 d Gd 6-15 (GO)	Bd Wt	50		150 (31% decrease in maternal body weight gain)	NTP 1991a NAP

Table 3-2 Levels of Significant Exposure to Naphthalene (nap) Or Methylnaphthalene (1-mn Or 2-mn) - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg)	LOAEL Less Serious (mg/kg)	LOAEL Serious (mg/kg)	Reference Chemical Form
8	Rat Sprague-Dawley	once (GO)	Resp		1000 lung lesions		Papciak and Mallory 1990 NAP
9	Rat Sprague-Dawley	once (GO)	Gastro		1000 stomach lesions		Papciak and Mallory 1990 NAP
10	Rat NS	10 d 1x/d (G)	Hepatic		1000 (39% increase in liver weight; increased lipid peroxidation, aniline hydroxylase activity)		Rao and Pandya 1981 NAP
			Renal	1000			
			Ocular	1000			
11	Mouse CD-1	14 d 1x/d (GO)	Resp	267 M 53 F	267 F (increase in lung weight)		Shopp et al. 1984 NAP
			Hemato	267			
			Hepatic	267			
			Renal	267			
			Bd Wt	53	267 (6% (female) or 13% (male) decreased final body weight)		
12	Dog NS	once (F)	Hemato			1525 (hemolysis)	Zuelzer and Apt 1949 NAP

Table 3-2 Levels of Significant Exposure to Naphthalene (nap) Or Methylnaphthalene (1-mn Or 2-mn) - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
13	Rabbit NS	5 d (F)	Hepatic	2000					Srivastava and Nath 1969 NAP
			Ocular				2000	(cataracts)	
14	Rabbit NS	10 d 1x/d (GO)	Ocular				1000	(lens opacities, decreased ascorbic acid in aqueous humor)	van Heyningen and Pirie 1967 NAP
Immuno/ Lymphoret									
15	Mouse CD-1	14 d 1x/d (GO)		53	267	(30% decrease in thymus weight in males; 18% decrease in spleen weight in females)			Shopp et al. 1984 NAP
Neurological									
16	Rat Sprague-Dawley	9d Gd 6-15 (GO)			50[b]	(transient clinical signs of toxicity in dams; at higher exposure levels, signs were more persistent and accompanied by decreases in body weight gain)			NTP 1991a NAP
17	Mouse CD-1	14 d (GO)		267					Shopp et al. 1984 NAP
Reproductive									
18	Rat Sprague-Dawley	9 d Gd 6-19 (GO)		450					NTP 1991a NAP

Table 3-2 Levels of Significant Exposure to Naphthalene (nap) Or Methylnaphthalene (1-mn Or 2-mn) - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
19	Mouse CD-1	8d Gd 7-14 (GO)					300	(>10% maternal mortality)	Plasterer et al. 1985 NAP
20	Rabbit New Zealand white	14 d Gd 6-19 (GO)		120					NTP 1992b NAP
Developmental									
21	Rat Sprague-Dawley	9 d Gd 6-15 (GO)					150	(decreased maternal weight gain >20%; no fetotoxic or teratogenic effects at 150 or 450 mg/kg/day)	NTP 1991a NAP
				50					
22	Mouse CD-1	8d Gd 7-14 (GO)		300					Plasterer et al. 1985 NAP
23	Rabbit New Zealand white	14 d Gd 6-19 (GO)		120					NTP 1992b NAP
24	Rabbit New Zealand white	13 d 1x/d Gd 6-18 (G)		40	200	(maternal dyspnea, cyanosis, body drop, hypoactivity with no pathological aberrations)			PRI 1985, 1986 NAP

Table 3-2 Levels of Significant Exposure to Naphthalene (nap) Or Methylnaphthalene (1-mn Or 2-mn) - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
INTERMEDIATE EXPOSURE							
Systemic							
25	Rat blue spruce	9 wk 3.5d/wk (GO)	Resp	169			Germansky and Jamall 1988 NAP
			Hepatic		169 (elevated lipid peroxides)		
			Bd Wt			169 (20% decreased body weight gain)	
26	Rat Brown-Norway	4 wk 3.5d/wk (GO)	Ocular			500 (lens opacity)	Kojima 1992 NAP
27	Rat Sprague-Dawley Brown-Norway	6 wk	Ocular			500 (cataract formation)	Murano et al. 1993 NAP

Table 3-2 Levels of Significant Exposure to Naphthalene (nap) Or Methylnaphthalene (1-mn Or 2-mn) - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
28	Rat Fischer 344	13 wk 5x/wk (GO)	Resp	400					NTP 1980b NAP
			Cardio	400					
			Gastro		400	(intermittent diarrhea)			
			Hemato	400					
			Hepatic	400					
			Renal	200 M 400 F	400 M	(10% had cortical tubular degeneration)			
			Ocular	400					
			Bd Wt	100	200	(decreased terminal body weight: 12% male & 6% female)	400		
29	Rat black-hooded	79 d (GO)	Ocular				5000	(lens opacity)	Rathbun et al. 1990 NAP
30	Rat Brown-Norway	102 d NS (GO)	Ocular				700	(lens opacity)	Tao et al. 1991 NAP
31	Rat 5 strains	4-6 wk (GO)	Ocular				1000	(lens opacity)	Xu et al. 1992b NAP

Table 3-2 Levels of Significant Exposure to Naphthalene (nap) Or Methylnaphthalene (1-mn Or 2-mn) - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
32	Rat Wistar	18 d 1x/d (G)	Hepatic		1000 (elevated lipid peroxides)		Yamauchi et al. 1986 NAP
			Ocular			1000 (cataracts)	
33	Mouse B6C3F1	13 wk 5x/wk 1x/d (GO)	Resp	200			NTP 1980a NAP
			Cardio	200			
			Gastro	200			
			Hemato	200			
			Hepatic	200			
			Renal	200			
			Ocular	200			
			Bd Wt	200			

Table 3-2 Levels of Significant Exposure to Naphthalene (nap) Or Methylnaphthalene (1-mn Or 2-mn) - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
34	Mouse CD-1	90 d 7d/wk 1x/d (GO)	Resp	133					Shopp et al. 1984 NAP
			Hemato	133					
			Hepatic	133					
			Renal	133					
			Bd Wt	133					
			Other	53	133	(decreases in absolute weights of brain (9%), liver (18%), and spleen (28%) and relative weight of spleen (24%) in females only)			
35	Rabbit NS	5 wk	Ocular				500	(destruction of retinal photoreceptors and vascularization of the retinal area)	Orzalesi et al. 1994 NAP
36	Rabbit Chinchilla Bastard New Zealand white	12 wk 2d/wk 1x/d (GO)	Ocular				1000	(cataracts)	Rossa and Pau 1988 NAP
37	Rabbit NS	4 wk 1x/d (GO)	Ocular		1000	(increased ascorbic acid in lens)			van Heyningen 1970 NAP

Table 3-2 Levels of Significant Exposure to Naphthalene (nap) Or Methylnaphthalene (1-mn Or 2-mn) - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
38	Rabbit NS	4 wk 1x/d (GO)	Ocular			1000 (lens opacities, retinal damage)	van Heyningen and Pirie 1967 NAP
Immuno/ Lymphoret							
39	Rat Fischer 344	13 wk 5d/wk 1x/d (GO)			400 (lymphoid depletion of thymus in 2/10 females)		NTP 1980b NAP
				200			
40	Mouse CD-1	90 d (GO)		133			Shopp et al. 1984 NAP
Neurological							
41	Rat Fischer 344	13 wk 5x/wk (GO)			400 (hunched posture and lethargy)		NTP 1980b NAP
42	Mouse B6C3F1	13 wk 5d/wk 1x/d (GO)		200			NTP 1980a NAP
43	Mouse CD-1	90 d (GO)		133			Shopp et al. 1984 NAP
Reproductive							
44	Rat Fischer 344	13 wk 5x/wk (GO)		400			NTP 1980b NAP

Table 3-2 Levels of Significant Exposure to Naphthalene (nap) Or Methylnaphthalene (1-mn Or 2-mn) - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
45	Mouse B6C3F1	13 wk 5d/wk 1x/d (GO)		200			NTP 1980a NAP
CHRONIC EXPOSURE							
Systemic							
46	Mouse B6C3F1	81 wk (F)	Resp		71.6[c] (increased incidence of pulmonary alveolar proteinosis in males and females)		Murata et al. 1993 1-MN
			Cardio	143.7			
			Gastro	143.7			
			Hemato	143.7			
			Hepatic	143.7			
			Renal	143.7			
			Endocr	143.7			
			Bd Wt	143.7			

Table 3-2 Levels of Significant Exposure to Naphthalene (nap) Or Methylnaphthalene (1-mn Or 2-mn) - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
47	Mouse (B6C3F1)	81 wk (F)	Resp		50.3[d] (increased incidence of pulmonary alveolar proteinosis in males and females)		Murata et al. 1997 2-MN
			Cardio	113.8			
			Gastro	113.8			
			Hemato	113.8			
			Musc/skel	113.8			
			Hepatic	113.8			
			Renal	113.8			
			Dermal	113.8			
			Ocular	113.8			
			Bd Wt	113.8			
Immuno/ Lymphoret							
48	Mouse B6C3F1	81 wk 1x/d		143.7			Murata et al. 1993 1-MN
		(F)					
49	Mouse (B6C3F1)	81 wk (F)		113.8			Murata et al. 1997 2-MN

Table 3-2 Levels of Significant Exposure to Naphthalene (nap) Or Methylnaphthalene (1-mn Or 2-mn) - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
Neurological							
50	Mouse B6C3F1	81 wk 1x/d (F)		143.7			Murata et al. 1993 1-MN
51	Mouse (B6C3F1)	81 wk (F)		113.8			Murata et al. 1997 2-MN
Reproductive							
52	Mouse B6C3F1	81 wk 1x/d (F)		143.7 F			Murata et al. 1993 1-MN
53	Mouse (B6C3F1)	81 wk (F)		113.8			Murata et al. 1997 2-MN

Table 3-2 Levels of Significant Exposure to Naphthalene (nap) Or Methylnaphthalene (1-mn Or 2-mn) - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference Chemical Form
Cancer								
54	Mouse B6C3F1	81 wk 1x/d (F)				71.6	(CEL: increased incidence of lung adenomas in males only)	Murata et al. 1993 1-MN
55	Mouse (B6C3F1)	81 wk (F)				54.3	(CEL: increased incidence of lung adenomas in males only; not at higher exposure level in males or in females at either exposure level)	Murata et al. 1997 2-MN

a The number corresponds to the entries in Figure 3-2

b Used to derive an acute-duration Minimal Risk Level (MRL) of 0.6mg/kg/day; based on a minimal LOAEL of 50 mg/kg/day for transient clinical signs of toxicity in pregnant rats, which was divided by an uncertainty factor of 90 (3 for the use of a minimal LOAEL, 10 for extrapolation from animals to humans, and 3 for human variability). Based on an analysis of results from the three available intermediate-duration oral toxicity studies in animals (NTP 1980a,b; Shopp et al. 1984), the acute-duration MRL is expected to be applicable to and protective for intermediate-duration exposure scenarios (see Section 2.3 and Appendix A).

c Used to derive a chronic-duration Minimal Risk Level (MRL) of 0.07 mg/kg/day for 1-MN; based on a LOAEL of 71.6 mg/kg/day which was divided by an uncertainty factor of 1000 (10 for use the use of a LOAEL, 10 for extrapolation from animals to humans; and 10 for human variability)

d Used to derive a chronic-duration Minimal Risk Level (MRL) of 0.04 mg/kg/day for 2-MN; based on a BMDL (LED05) of 4 mg/kg/day which was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans, and 10 for human variability).

Bd Wt = body weight; BMDL (LED05) = lower 95% confidence limit on a dose associated with 5% extra risk; BUN = blood urea nitrogen; Cardio = cardiovascular; d = day(s); Endocr = endocrine; F = females; (F) = feed; (G) = gavage; Gastro = gastrointestinal; Gd = gestation day(s); (G) = gavage in oil; Hemato = hematological; hr = hour(s); Immuno = immunological; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = males; NOAEL = no-observed-adverse-effect level; NS = not specified; Resp = respiratory; wk = week(s); x = time(s); 1-Mn = 1-methylnaphthalene; 2-Mn = 2-methylnaphthalene.

Figure 3-2. Levels of Significant Exposure to Naphthalene (NAP) or Methylnaphthalene (1-MN or 2-MN) - Oral

Acute (≤14 days)

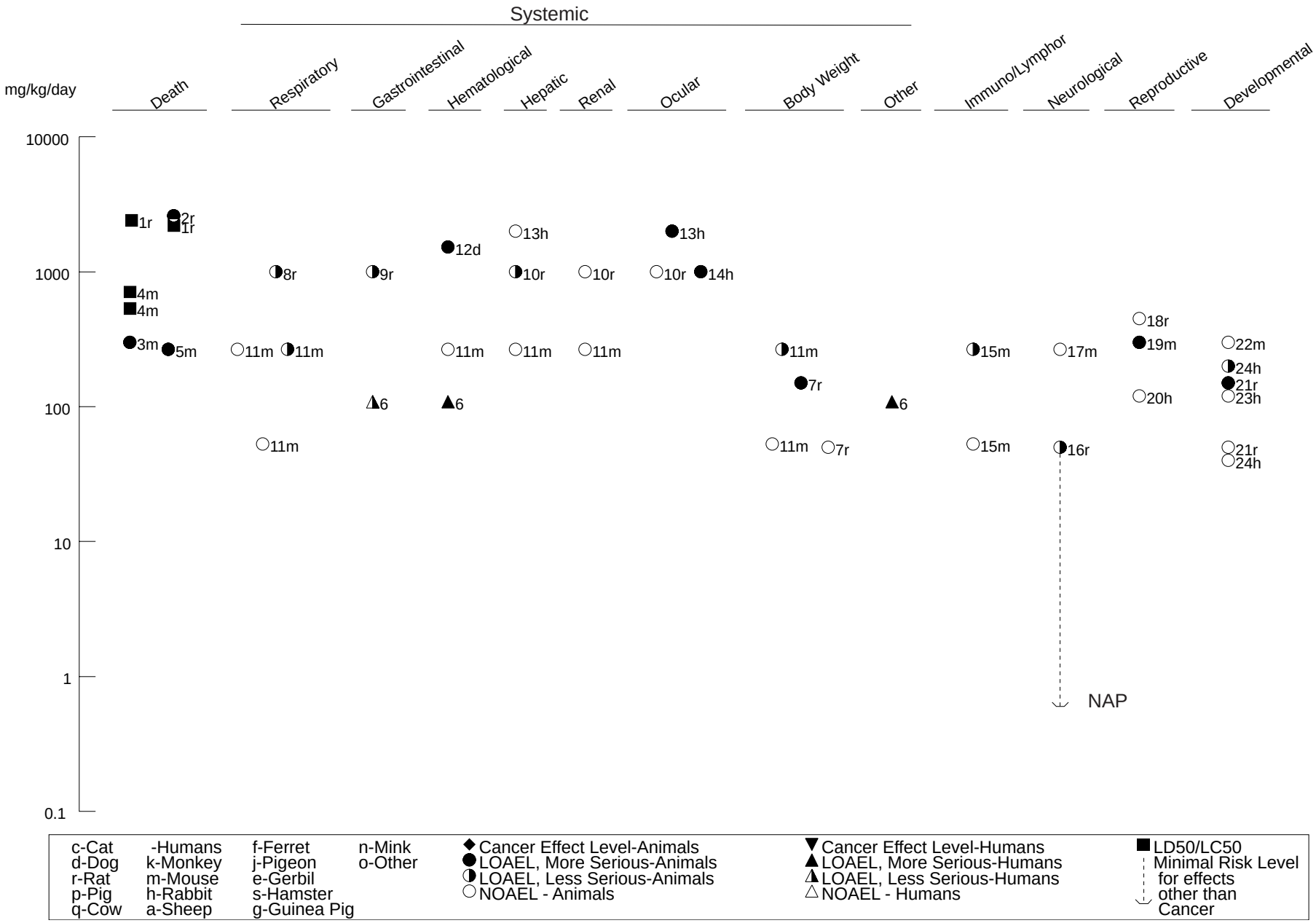


Figure 3-2. Levels of Significant Exposure to Naphthalene (NAP) or Methylnaphthalene (1-MN or 2-MN) - Oral (*Continued*)

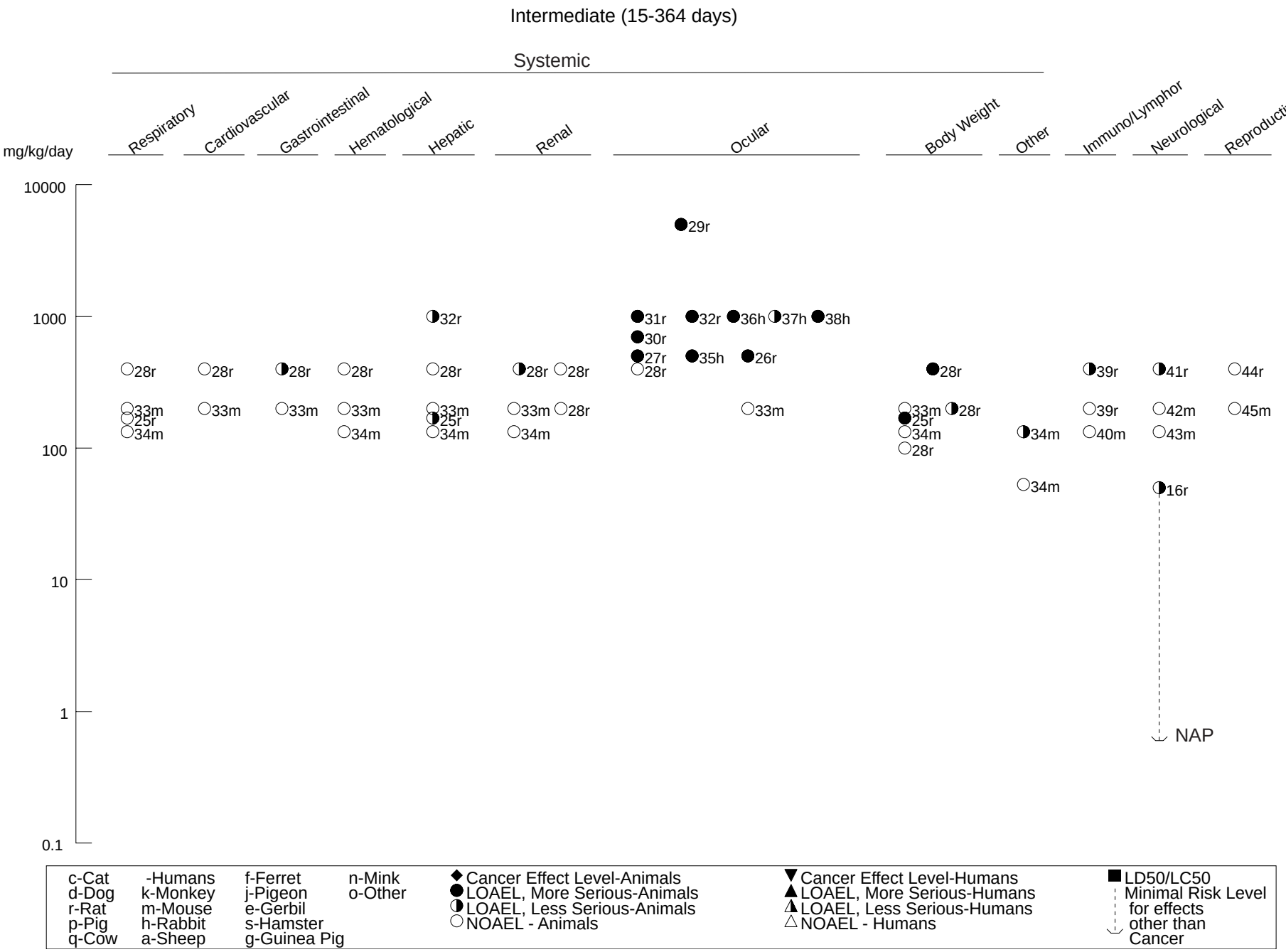
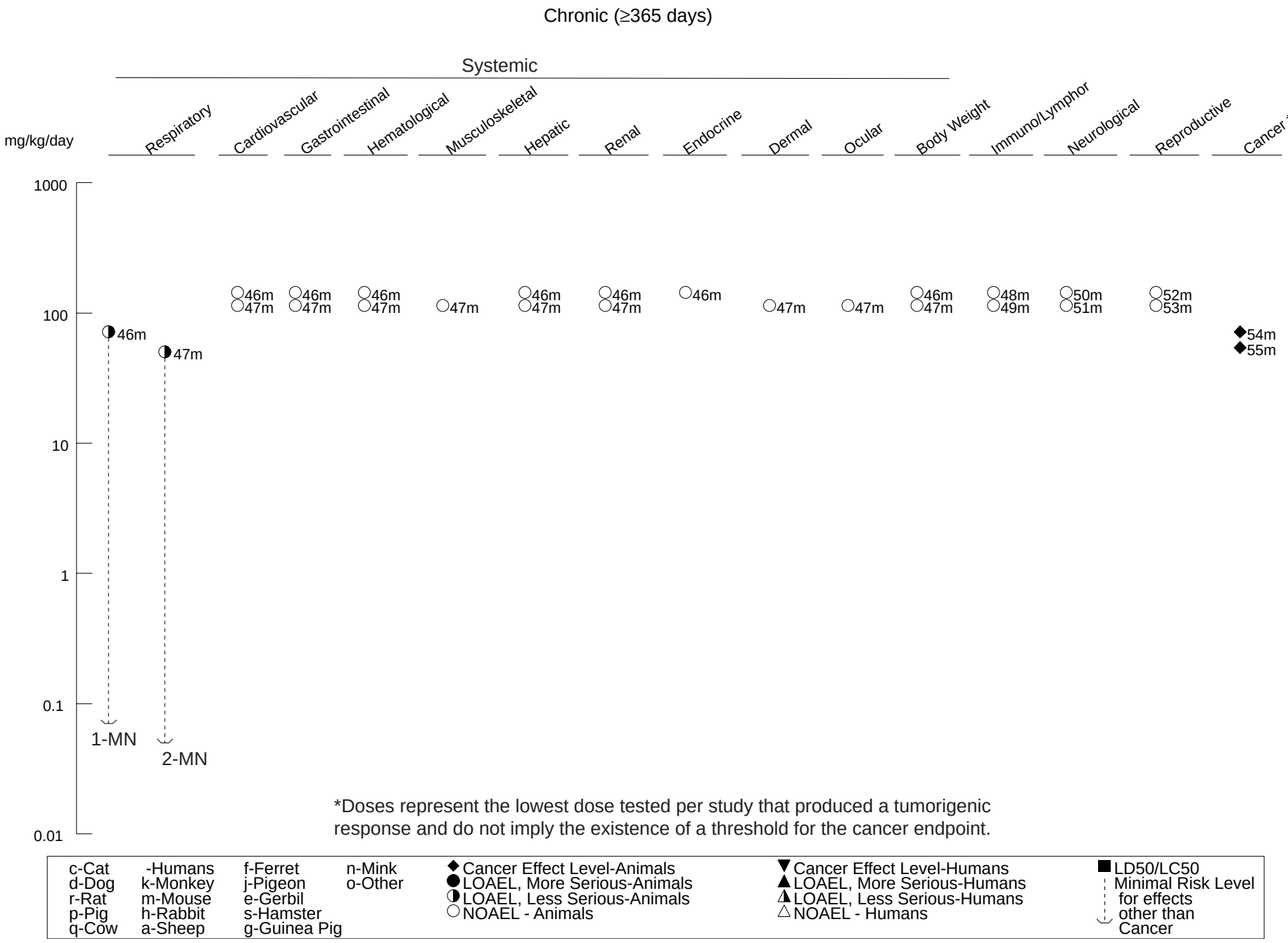


Figure 3-2. Levels of Significant Exposure to Naphthalene (NAP) or Methylnaphthalene (1-MN or 2-MN) - Oral (*Continued*)



naphthalene-containing diaper pail deodorant block (Haggerty 1956). This may have been a reflection of the reduced oxygen carrying capacity of the blood due to hemolysis.

Lesions of the lungs were seen in rats that died after being given a single large dose of naphthalene (1,000–4,000 mg/kg) during an LD_{50} study (Papciak and Mallory 1990). On the other hand, no significant respiratory toxicity was seen in rats following oral administration of naphthalene at time-weighted average doses of 169 mg/kg/day for 9 weeks (Germansky and Jamall 1988). Dosages were increased from 100 to 750 mg/kg/day over a 6-week period and held constant at 750 mg/kg/day for the last 3 weeks of the 9-week exposure period.

Lung weights were increased in female mice administered naphthalene at 267 mg/kg/day for 14 days; however, these effects were not seen in either sex at 133 mg/kg/day for 90 days (Shopp et al. 1984). No gross or histopathological lesions of the lungs were noted in mice at doses up to 200 mg/kg/day (NTP 1980a) or in rats at doses of 400 mg/kg/day (NTP 1980b) after 13 weeks of exposure.

There was a significantly increased incidence of pulmonary alveolar proteinosis in male and female B6C3F1 mice fed diets containing 1-methylnaphthalene for 81 weeks (Murata et al. 1993). The lesions contained acidophilic amorphous material, foam cells, and cholesterol crystals. There was no apparent inflammation, edema, or fibrosis of the tissues. Average administered doses were 0, 71.6, or 140.2 mg/kg/day for males and 0, 75.1, or 143.7 mg/kg/day for females. Respective incidences for pulmonary alveolar proteinosis in the control, low-, and high-dose groups were 4/49, 23/50, and 19/49 for males and 5/50, 23/50, and 17/49 for females. Histopathological examination of major organs and tissues only found exposure-related lesions in the lung. This effect was used as the basis of the chronic-duration oral MRL for 1-methylnaphthalene.

Pulmonary alveolar proteinosis is characterized by the accumulation of surfactant material in the alveolar lumen, and has been hypothesized to be caused by either excessive secretion of surfactant by type II pneumocytes, or disruption of surfactant clearance by macrophages (Lee et al. 1997; Mazzone et al. 2001; Wang et al. 1997). Electron microscopic examination of lungs of mice exposed dermally to a mixture of 1-methylnaphthalene and 2-methylnaphthalene showed that alveolar spaces were filled with numerous myelinoid structures resembling lamellar bodies of type II pneumocytes (Murata et al. 1992).

In a companion study, pulmonary alveolar proteinosis was the only exposure-related lesion found in B6C3F1 mice of both sexes exposed to 2-methylnaphthalene in the diet at doses as low as 50.3 mg/kg/day

(Murata et al. 1997). Average administered doses were 0, 54.3, or 113.8 mg/kg/day for males and 0, 50.3, or 107.6 mg/kg/day for females. Respective incidences for pulmonary alveolar proteinosis in the control, low-, and high-dose groups were 4/49, 21/49, and 23/49 for males and 5/50, 27/49, and 22/49 for females. This effect was used as the basis of the chronic-duration oral MRL for 2-methylnaphthalene.

Cardiovascular Effects. No studies were located that demonstrate any direct effects of naphthalene ingestion on the cardiovascular system. In those reports where cardiovascular effects such as increased heart rate and decreased blood pressure were noted in humans, the cardiovascular effects appeared to be secondary to the hemolytic effects and the events leading to general multiple organ failure (Gupta et al. 1979; Kurz 1987).

No gross or histopathological lesions of the heart were noted in mice at doses up to 200 mg/kg/day (NTP 1980a) or in rats at doses of 400 mg/kg/day (NTP 1980b) after 13 weeks of exposure.

Heart weights were significantly decreased (6–7%) in male and female mice that were fed 1-methyl-naphthalene for 81 weeks in their diet. However, the changes in heart weight were not dose-related and there were no accompanying tissue abnormalities (Murata et al. 1993). Histopathological examination revealed no lesions in the hearts of mice fed 1-methylnaphthalene at doses as high as 143.7 mg/kg/day (Murata et al. 1993) or 2-methylnaphthalene at doses as high as 113.8 mg/kg/day (Murata et al. 1997).

Gastrointestinal Effects. Gastrointestinal disorders are common following naphthalene ingestion by humans. These effects have been attributed to the irritant properties of naphthalene (Kurz 1987). Nausea, vomiting, abdominal pain, and diarrhea (occasionally containing blood) have been reported (Bregman 1954; Gidron and Leurer 1956; Gupta et al. 1979; Haggerty 1956; Kurz 1987; MacGregor 1954; Ojwang et al. 1985). While the presence of blood in the stool is indicative of intestinal bleeding, only a few areas of mucosal hemorrhage were noted in postmortem examination of the intestines (Kurz 1987). These areas were restricted to the small bowel and colon. No frank erosions or perforations were noted anywhere in the gastrointestinal tract.

A single dose of 1,000–4,000 mg/kg was associated with stomach lesions and discoloration of the intestines in rats that died during an LD_{50} study. The survivors were not affected (Papciak and Mallory 1990). No gross or histopathological lesions of the stomach, small intestine, and colon were noted in mice at doses of up to 200 mg/kg/day (NTP 1980a) or in rats at doses of up to 400 mg/kg/day after

13 weeks of exposure (NTP 1980b). There was some intermittent diarrhea in the rats, but this may not have been treatment related.

No histopathological lesions were seen in the stomach or intestines of mice fed 71.6–143.7 mg/kg/day 1-methylnaphthalene for 81 weeks (Murata et al. 1993) or 50.3–113.8 mg/kg/day 2-methylnaphthalene for 81 weeks (Murata et al. 1997).

Hematological Effects. The most commonly reported hematologic effect in humans following the ingestion of naphthalene is hemolytic anemia (Dawson et al. 1958; Gidron and Leurer 1956; Gupta et al. 1979; Haggerty 1956; Kurz 1987; MacGregor 1954; Mackell et al. 1951; Melzer-Lange and Walsh-Kelly 1989; Ojwang et al. 1985; Shannon and Buchanan 1982). Changes observed in hematology and blood chemistry are consistent with this effect: hemolysis, decreased hemoglobin and hematocrit values, increased reticulocyte counts, serum bilirubin levels, and Heinz bodies. This was caused by hemolysis. Most of the reported case studies provide no information on dose. However, in one case report, a 16-year-old girl swallowed 6 g of naphthalene before exhibiting hemolytic anemia (Gidron and Leurer 1956). This is a dose of 109 mg/kg (assuming a 55-kg body weight). The hematological condition of this individual, who was an immigrant from Kurdistan, was not provided.

As mentioned previously, there is an association between G6PD deficiency and the hemolytic effects of naphthalene (Dawson et al. 1958; Melzer-Lange and Walsh-Kelly 1989; Shannon and Buchanan 1982). Individuals with a genetic defect for this enzyme show an increased susceptibility to hemolysis from naphthalene exposure.

Few hematologic changes have been reported in animals. Standard laboratory animals do not appear to be sensitive to the hemolytic effects of naphthalene. In CD-1 mice, naphthalene at doses up to 267 mg/kg/day for 14 days or up to 133 mg/kg/day for 90 days did not result in hemolytic anemia (Shopp et al. 1984). However there was an increase in eosinophils in the 14- and 90-day studies. There was an increase in prothrombin time at 14 days. The clinical significance of these observations is not clear; the effects are not considered to be adverse.

There were no pronounced changes in red cell related hematological parameters in mice following 13-week exposures to doses of up to 200 mg/kg/day (NTP 1980a) and up to 400 mg/kg/day in rats (NTP 1980b). In male mice exposed to 200 mg/kg/day for 13 weeks, there was a decrease in segmented neutrophils and an increase in lymphocytes, but in male rats given 400 mg/kg/day, there were increased

neutrophils and decreased lymphocytes. These effects are not considered to be biologically significant or adverse.

Hemolytic anemia was reported by Zuelzer and Apt (1949) in a dog receiving a single 1,525 mg/kg dose of naphthalene in food and in another dog receiving approximately 263 mg/kg/day for 7 days in food. Dogs are more susceptible to chemically induced hemolysis than are rats and mice.

Exposure to 75.1 or 143.7 mg/kg/day 1-methylnaphthalene for 81 weeks was associated with a slight but statistically significant increase in the hemoglobin concentration, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration in female mice (Murata et al. 1993). Corresponding changes were not observed in male mice given comparable doses of 1-methylnaphthalene, or in male or female mice exposed to 2-methylnaphthalene doses as high as 113.8 mg/kg/day (Murata et al. 1997). Consistent exposure-related changes were not found in differential white blood cell counts or several serum biochemical parameters in male and female mice exposed to 1-methylnaphthalene or 2-methyl-naphthalene in these studies. The results from these studies do not provide consistent evidence that hematological parameters are consistent toxicity targets of chronic oral exposure to 1-methylnaphthalene or 2-methylnaphthalene.

Hepatic Effects. Evidence of hepatotoxicity following oral exposure to naphthalene has been reported in humans, based on elevated plasma levels of hepatic enzymes (such as aspartate aminotransferase and lactic acid dehydrogenase) (Kurz 1987; Ojwang et al. 1985) and liver enlargement (Gupta et al. 1979; MacGregor 1954). The relationship between liver enlargement and potential naphthalene-induced hemolysis is unknown.

There is limited evidence of hepatic effects in laboratory animals, but the liver does not appear to be a critical toxicity target of orally administered naphthalene. A 39% increase in liver weight, a modest elevation in activity of aniline hydroxylase, and evidence of lipid peroxidation were observed in male rats treated with naphthalene at 1,000 mg/kg/day for 10 days (Rao and Pandya 1981). Male rats demonstrated an elevation in hepatic lipid peroxides at naphthalene doses of 1,000 mg/kg/day for 18 days (Yamauchi et al. 1986). In rats administered increasing doses of naphthalene up to 750 mg/kg/day (time-weighted average of 169 mg/kg/day), hepatic lipid peroxides were doubled at the end of 9 weeks of treatment (Germansky and Jamall 1988).

No effects on liver weight were observed in male or female mice receiving naphthalene at doses up to 267 mg/kg/day for 14 days or male mice receiving 133 mg/kg/day for 90 days (Shopp et al. 1984). Absolute liver weight was statistically significantly decreased, compared with the control value (by about 18%), in female mice receiving 133 mg/kg/day naphthalene for 90 days, but the biological significance of this change is unclear. Relative liver weight in exposed females was not changed to a statistically significant degree, and several serum biochemical end points indicative of liver damage (e.g., lactate dehydrogenase, SGPT, SGOT, and alkaline phosphatase) were unaffected in male and female mice exposed to doses up to 133 mg/kg/day for 90 days (Shopp et al. 1984). No other consistent biologically relevant exposure-related changes in serum chemistry end points were found. Activities of two hepatic microsomal mixed function oxidases (aniline hydroxylase, aminopyrine N-demethylase) were unchanged in exposed mice, although hepatic activities of benzo[a]pyrene hydroxylase were statistically significantly decreased in exposed mice (Shopp et al. 1984). The biological significance of this change is unclear. Supporting the concept that the liver is not a critical toxicity target of oral exposure to naphthalene, no gross or histopathological lesions of the liver were noted in mice at doses of up to 200 mg/kg/day (NTP 1980a) or in rats at doses of up to 400 mg/kg/day after 13 weeks of exposure (NTP 1980b).

There were no changes in liver weights or tissue histopathology in male or female mice that consumed 71.6–143.7 mg/kg/day 1-methylnaphthalene in the diet for 81 weeks (Murata et al. 1993) or 50.3–113.8 mg/kg/day 2-methylnaphthalene in the diet for 81 weeks (Murata et al. 1997).

Renal Effects. Renal toxicity has been reported in case studies of humans who ingested naphthalene. Frequent findings include the elevation of creatinine and blood urea nitrogen and the presence of proteinuria and hemoglobinuria (Gupta et al. 1979; Haggerty 1956; Kurz 1987; MacGregor 1954; Ojwang et al. 1985; Zuelzer and Apt 1949). The presence of blood in the urine and increased concentrations of urobilinogen are a consequence of acute hemolysis and do not reflect any direct action of naphthalene on the kidney. Oliguria (Kurz 1987) and anuria (Gupta et al. 1979) were noted in two case reports, although urine output was normal in a third (Ojwang et al. 1985). Painful urination with swelling of the urethral orifice was also associated with medicinal naphthalene ingestion (Lezenius 1902). Proximal tubule damage and general tubular necrosis were found in postmortem examinations of two individuals who died following naphthalene ingestion (Gupta et al. 1979; Kurz 1987).

Renal effects were not consistently observed in animals exposed orally to naphthalene. Following 10 days of exposure of rats to naphthalene at 1,000 mg/kg/day, no changes were noted in kidney weight, lipid peroxidation, or in the activity of alkaline phosphatase and aniline hydroxylase (Rao and Pandya

1981). No changes were observed in the kidney weights of mice administered naphthalene at doses up to 267 mg/kg/day for 14 days or 133 mg/kg/day for 90 days (Shopp et al. 1984). No gross or histopathological lesions of the kidney were noted in mice at doses of up to 200 mg/kg/day (NTP 1980a) or in rats at doses of up to 200 mg/kg/day after 13 weeks of exposure (NTP 1980b). In the male rats, 10% showed cortical tubular degeneration that may have been compound-related at a dose of 400 mg/kg/day (NTP 1980b).

Relative kidney weights were increased slightly in male mice fed diets containing 71.6 or 140.2 mg/kg/day 1-methylnaphthalene for 81 weeks (Murata et al. 1993). The females were not affected, and there were no histopathological lesions in the males or females. There were no changes in kidney weights or tissue histopathology in male or female mice consuming 50.3–113.8 mg/kg/day 2-methylnaphthalene in the diet for 81 weeks (Murata et al. 1997).

Ocular Effects. In an early report of naphthalene toxicity, a 36-year-old pharmacist who ingested an unspecified amount of unpurified naphthalene in a castor oil emulsion over a 13-hour period as treatment of an intestinal disorder became nearly blind 8 or 9 hours later (Lezenius 1902). A medical examination the following month revealed constricted visual fields associated with optic atrophy and bilateral zonular cataracts. At 1.5 meters, the patient's vision was limited to finger counting.

Several animal studies have demonstrated ocular changes following oral naphthalene exposure. Within 1 week following exposure to naphthalene (500 or 1,000 mg/kg/day), lens densities were increased in rats and cataracts developed within 4 weeks (Kojima 1992; Murano et al. 1993; Yamauchi et al. 1986). Eight rabbits (strain not identified) developed cataracts during oral administration of naphthalene at 2,000 mg/kg/day for 5 days (Srivastava and Nath 1969). Cataracts began to develop by the first day after a single 1,000 mg/kg naphthalene dose in three Chinchilla Bastard rabbits (Rossa and Pau 1988). In the solitary New Zealand white rabbit tested, cataracts began to develop after administration of four 1,000 mg/kg doses (dosing 2 times/week) and maximized after 12 weeks (Rossa and Pau 1988).

When naphthalene was administered orally at 1,000 mg/kg/day for up to 28 days, cataracts developed in 10 of 16 Dutch (pigmented) rabbits and in 11 of 12 albino rabbits (Van Heyningen and Pirie 1976). Lens changes were seen as early as day 2 of exposure. The authors noted that albino strains were more likely to develop cataracts over a 4-week course of treatment at 1,000 mg/kg/day than pigmented strains such as the Dutch rabbit.

In contrast, administration of a time-weighted-average 500-mg/kg/day dose of naphthalene in corn oil by gavage for 6 weeks resulted in more rapid development of cataracts in pigmented Brown-Norway rats than in nonpigmented Sprague-Dawley rats (Murano et al. 1993). Cataracts developed in three distinct phases. In the first phase, water clefts formed in the anterior subcapsular region of the eye. The second stage was the development of a semicircular opaque area in the lens, and the last stage was the appearance of a wedge-shaped opacity that could be seen with retroillumination and a wide, zonular-ring opacity that was seen with slit imaging. Each stage occurred about 1 week earlier in the Brown-Norway rats than in the Sprague Dawley rats. The first stage began 1 week after treatment was initiated in the Brown-Norway rats, and stage three cataracts were seen in all animals by the end of the 6 weeks. Progressive development of lens opacities was also reported in rats that were exposed to 700 or 5,000 mg/kg/day naphthalene by gavage for 79–102 days (Rathburn et al. 1990; Tao et al. 1991).

Damage to the eyes with continued exposure to naphthalene is not limited to lens opacification (Orzalesi et al. 1994). Retinal damage was noted in pigmented rabbits given time-weighted-average doses of 500 mg/kg/day naphthalene in corn oil by gavage for 5 weeks. The first changes to the retina occurred at about 3 weeks with degeneration of the photoreceptors. There was a subsequent increase in the retinal pigment epithelium as these cells phagocytized the debris from the photoreceptors. By the end of 6 weeks, the photoreceptor layer had almost entirely disappeared and was replaced with fibroglial tissue. As damage progressed, there was dense subretinal neovascularization of the area.

A number of biochemical changes were seen in the eyes after acute- and intermediate-duration naphthalene exposures. After 1 week of treatment with 1,000 mg/kg/day, glutathione levels in the lens were decreased in rats (Xu et al. 1992b; Yamauchi et al. 1986). After 30 days of treatment with doses of 5,000 mg/kg/day, total glutathione levels were reduced by 20% (Rathbun et al. 1990), and there was a 22% reduction at 60 days with a dose of 700 mg/kg/day (Tao et al. 1991). At 60 days, glutathione peroxidase activity in the lens was decreased by up to 45% and there was a 20–30% decrease in glutathione reductase activity (Rathbun et al. 1990). Comparable decreases in the activities of both enzymes were seen at 102 days with lower naphthalene doses (Tao et al. 1991). No changes were observed in the activity of glutathione synthetase or gamma-glutamyl cysteine synthetase (Rathbun et al. 1990). After 4 weeks of compound treatment (500 mg/kg/day), the activities of aldose reductase, sorbitol dehydrogenase, lactic dehydrogenase, and glutathione reductase were lower than in controls (Kojima 1992). No changes in ocular lipid peroxides were reported when male Blue Spruce pigmented rats were administered incremental doses of naphthalene that peaked at 750 mg/kg/day for 9 weeks (Germansky

and Jamall 1988). Lens and capsule LDH activities were greatly reduced in rabbits while o-diphenyl oxidase activity was elevated with a dose of 2,000 mg/kg/day for 5 days (Srivastava and Nath 1969).

In 13-week studies, histopathologic examination revealed no ocular lesions in F344/N rats or B6C3F1 mice exposed to doses as high as 400 or 200 mg/kg/day, respectively (NTP 1980a, 1980b). In a 2-year rat feeding study, no eye damage was seen at a naphthalene dosage of 41 mg/kg/day (Schmahl 1955). The details of the eye examination were not provided.

There were no changes in eye tissue histopathology in male or female mice that consumed 71.6–143.7 mg/kg/day 1-methylnaphthalene in the diet for 81 weeks (Murata et al. 1993) or 50.3–113.8 mg/kg/day 2-methylnaphthalene in the diet for 81 weeks (Murata et al. 1997).

Body Weight Effects. No studies were located that documented effects on body weight in humans after oral exposure to naphthalene.

In pregnant Sprague-Dawley rats exposed to 50, 150, or 450 mg/kg/day on gestation days 6–15, body weight gains were depressed by 31 and 53% at 150 and 450 mg/kg/day, respectively, but were unaffected at 50 mg/kg/day. The decreased body weight gains were accompanied by persistent clinical signs of toxicity (slow respiration, lethargy, or prone position) at the 150 and 450 mg/kg/day dose levels, but these signs were only apparent at the 50-mg/kg/day level during the first 2 days of dosing. The minimal LOAEL of 50 mg/kg/day for transient clinical signs and the LOAEL of 150 mg/kg/day for clinical signs associated with decreased body weight gains in pregnant rats are the basis of the acute oral MRL for naphthalene (see Section 2.3 and Appendix A).

In animals, body weight effects appear to be the critical effect associated with intermediate-duration oral exposure to naphthalene. After 13 weeks of exposure to naphthalene, mean terminal body weights in F344/N rats exposed to gavage doses ≥200 mg/kg/day were decreased by more than 10% relative to control values (NTP 1980b). Body weights were decreased by 12 and 28% in 200- and 400-mg/kg/day male rats, and by 23% in 400-mg/kg/day female rats. Food consumption was not affected by exposure. In B6C3F1 mice exposed to naphthalene doses up to 200 mg/kg/day for 13 weeks, exposed males gained more weight than controls during exposure, whereas exposed females gained less weight than controls (NTP 1980a). However, terminal body weights in exposed female mice were within 95% of control values, indicating that the naphthalene-induced changes were not biologically significant. In male and female CD-1 mice exposed to doses as high as 133 mg/kg/day for 90 days, average terminal body weight

in exposed groups were within 90% of control values (Shopp et al. 1984). Mice exposed to 267 mg/kg/day naphthalene for 14 days showed a decreased body weight gain; terminal body weights were decreased by 6% in females and 13% in males compared with control values (Shopp et al. 1984).

As discussed in Section 2.3 and Appendix A, the NOAEL of 100 mg/kg/day and the LOAEL of 200 mg/kg/day for decreased body weights in rats exposed by gavage to naphthalene 5 days/week for 13 weeks (NTP 1980b) provide the best available basis for MRL derivation among the findings from the studies in animals orally exposed to naphthalene for intermediate-durations. However, because an intermediate-duration oral MRL based on these data is slightly larger than the acute-duration oral MRL for naphthalene, the acute MRL was adopted as the intermediate-duration oral MRL for naphthalene (as indicated in Figure 3-2 and discussed in Section 2.3).

There was no significant difference between body weights of mice that were given up to 143.7 mg/kg/day 1-methylnaphthalene in their diets and those of the control animals throughout an 81-week exposure period (Murata et al. 1993). In mice exposed to 2-methylnaphthalene doses as high as 113.8 mg/kg/day in the diet for up to 81 weeks, average body weights were within 10% of control values (Murata et al. 1997).

Other Systemic Effects. Several humans who consumed naphthalene experienced elevated body temperatures which may have been related to their hemolytic crisis (Chusid and Fried 1955; Gidron and Leurer 1956; Haggerty 1956; Kurz 1987; MacGregor 1954; Ojwang et al. 1985). However, in some situations, bacterial infections rather than hemolysis may have been the cause of the fever (Kurz 1987; Melzer-Lange and Walsh-Kelly 1989; Ojwang et al. 1985; Zuelzer and Apt 1949).

No studies were located that documented other systemic effects in animals after oral exposure to naphthalene.

3.2.2.3 Immunological and Lymphoreticular Effects

No studies were located that documented immunological or lymphoreticular effects in humans after oral exposure to naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene. However, an enlarged spleen is a frequent consequence of hemolysis and was noted in the postmortem examination of one human subject who died after ingesting a large quantity of naphthalene (Kurz 1987).

Mice treated with naphthalene at oral doses as high as 267 mg/kg/day for 14 days showed no effects on humoral immune responses, delayed hypersensitivity responses, bone marrow stem cell number, or bone marrow DNA synthesis (Shopp et al. 1984). Mitogenic responses to concanavalin A (but not to lipopolysaccharide) were reduced in high dose females only. None of these effects were noted at doses of 27 or 53 mg/kg/day. At naphthalene doses of 133 mg/kg/day for 13 weeks, naphthalene had no effect on immune function (Shopp et al. 1984). After 14 days, thymus weights were reduced approximately 30% in male mice, but no differences were seen with a dose of 133 mg/kg/day at 13 weeks (Shopp et al. 1984). There was lymphoid depletion of the thymus in 2 of 10 female rats exposed to 400 mg/kg/day naphthalene for 13 weeks (NTP 1980b).

Spleen weights were reduced approximately 20% in female mice exposed to 267 mg/kg/day naphthalene for 14 days and 25% in females exposed to 133 mg/kg/day for 13 weeks (Shopp et al. 1984).

Monocyte concentrations were significantly elevated in male and female mice exposed to 71.6–143.7 mg/kg/day 1-methylnaphthalene for 81 weeks (Murata et al. 1993). The increase in monocyte counts appeared to be dose related. The authors hypothesized that these changes may have been a physiological response to the pulmonary alveolar proteinosis seen in the exposed animals. There were no changes in spleen or thymus weights and the histopathology of these tissues was normal. With 81 weeks of exposure of male and female B6C3F1 mice to 2-methylnaphthalene, neutrophils were reported to be decreased, and lymphocytes increased, compared with control values, but neither the magnitude of these changes, or the dose groups in which they occurred, were specified in the study report (Murata et al. 1997). As with 1-methylnaphthalene, histologic examination revealed no exposure-related lesions in the spleen or thymus.

The highest NOAEL values and all LOAEL values from each reliable naphthalene study for immunological/lymphoreticular effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2. The highest NOAEL values from the 1-methylnaphthalene and 2-methyl-naphthalene studies for immunological/lymphoreticular effects are also recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.4 Neurological Effects

The neurologic symptoms of naphthalene ingestion reported in human case studies include confusion (Ojwang et al. 1985), altered sensorium (Gupta et al. 1979), listlessness and lethargy (Bregman 1954; Chusid and Fried 1955; Kurz 1987; MacGregor 1954; Zuelzer and Apt 1949), and vertigo (Gidron and Leurer 1956). Muscle twitching, convulsions (Kurz 1987; Zuelzer and Apt 1949), decreased responses to painful stimuli, and coma occurred prior to death in individuals who ingested naphthalene (Gupta et al. 1979; Kurz 1987). At autopsy, the brain has appeared edematous (Gupta et al. 1979; Kurz 1987), with separation of neural fibers and swelling of myelin sheaths being noted histologically (Gupta et al. 1979). The neurologic symptomatology could result from the cerebral edema, which was probably secondary to acute hemolysis.

No studies were located that documented neurological effects in humans after oral exposure to 1-methylnaphthalene or 2-methylnaphthalene.

Dose-related clinical signs of toxicity were apparent in female Sprague-Dawley rats exposed to doses of 50, 150, or 450 mg/kg/day naphthalene for 10 days during organogenesis. Slow respiration and lethargy were observed in a large percentage of the exposed animals. Some rats were dazed, had periods of apnea, or were unable to move after exposure. In the lowest dose group, 73% of the animals were affected on the first day of dosing. In the two higher dose groups, over 90% of the rats were affected (NTP 1991a).

The animals in the 50-mg/kg/day group acclimatized quickly. Symptoms were only apparent during the first 2 days of dosing. Clinical signs of toxicity persisted for longer periods in the higher dose groups, and were accompanied by decreased body weight gains (31 and 53% decreased at 150 and 450 mg/kg/day, respectively compared with control). It is not known if the observed clinical signs were due to treatment-related effects on the nervous system or were the indirect consequence of severe systemic toxicity, as indicated by the dramatic decreases in body weight gain. Comparable effects were not observed in F344/N rats exposed to doses of up to 400 mg/kg/day for 13 weeks or in B6C3F1 mice at doses of up to 200 mg/kg/day (NTP 1980a, 1980b). These results suggest that pregnant animals may be more susceptible to the effects of naphthalene than non-pregnant animals. The minimal LOAEL of 50 mg/kg/day for transient clinical signs of toxicity and the LOAEL of 150 mg/kg/day for more persistent signs of toxicity accompanied with depressed weight gain in pregnant rats exposed on gestation days 6–15 are the basis of the acute oral MRL for naphthalene (see Section 2.3 and Appendix A).

There were no changes in the brain weights in mice exposed to naphthalene at doses up to 267 mg/kg/day for 14 days or 133 mg/kg/day for 90 days (Shopp et al. 1984). No gross or histopathological lesions of the brain were noted in mice at doses of up to 200 mg/kg/day (NTP 1980a) or in rats at doses of up to 400 mg/kg/day after 13 weeks of exposure (NTP 1980b). Transient clinical signs of neurotoxicity were observed in rats following daily gavage administration of 400 mg/kg, but not 200 mg/kg, doses (NTP 1980b). In mice, transient lethargy was observed following dose administration only between weeks 3 and 5 in the highest dose group, 200 mg/kg/day (NTP 1980a).

Absolute brain weight was significantly increased in male mice fed diets containing 71.6 or 140.2 mg/kg/day 1-methylnaphthalene for 81 weeks (Murata et al. 1993), or 54.3 or 113.8 mg/kg/day 2-methylnaphthalene for 81 weeks (Murata et al. 1997). The increases in brain weights were not dose related and there were no histopathological abnormalities of the brain. There were no differences in brain weights or histopathology in the female mice given comparable doses (Murata et al. 1993, 1997).

No studies were located that documented neurological effects in animals after oral exposure to 2-methylnaphthalene.

The highest NOAEL values and all LOAEL values from each reliable study for neurological effects for naphthalene exposure in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2. The highest NOAEL values for neurological effects in the intermediate-duration 1-methylnaphthalene and 2-methylnaphthalene mouse studies are also recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.5 Reproductive Effects

No studies were located that documented reproductive effects in humans after oral exposure to naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene.

Oral exposures of pregnant rabbits to naphthalene at dosages up to 400 mg/kg/day (gestational days 6–18), using methylcellulose as the vehicle, resulted in no apparent adverse reproductive effects (PRI 1986). When administered in corn oil to pregnant mice, however, a dosage of 300 mg/kg/day (gestational days 7–14) resulted in a decrease in the number of live pups per litter (Plasterer et al. 1985). It is not clear whether the observed differences in response are attributable to species differences or a possible increase

in the absorption of naphthalene when it is administered in corn oil compared with administration as a suspension in methyl cellulose.

Transient signs of toxicity were present in female rats exposed to doses of 50, 150, or 450 mg/kg/day on gestational days 6–15 (NTP 1991a). Effects on maternal weight gain were noted in the mid- and high-dose groups but not in the lowest dose group. The mid-dose group had a 31% decrease in weight gain while the high-dose group had a 53% weight gain decrease.

No treatment-related effects were reported on testicular weights of mice administered naphthalene at doses up to 267 mg/kg/day for 14 days or 133 mg/kg/day for 90 days (Shopp et al. 1984). No gross or histopathological lesions of the testes were noted in mice at doses of up to 200 mg/kg/day (NTP 1980a) or in rats at doses of up to 400 mg/kg/day after 13 weeks of exposure (NTP 1980b).

No gross or histopathological lesions of the testis, seminal vesicles, ovaries, uterus, or vagina were observed in mice exposed to 1-methylnaphthalene doses as high as 143.7 mg/kg/day (Murata et al. 1993) or 2-methylnaphthalene doses as high as 113.8 mg/kg/day (Murata et al. 1997).

The highest NOAEL values and all LOAEL values from each reliable study for reproductive effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.6 Developmental Effects

In humans, transplacental exposure of the fetus to naphthalene that had been ingested by the mother resulted in neonatal (and presumably fetal) hemolytic anemia (Anziulewicz et al. 1959; Zinkham and Childs 1957, 1958). No estimates of dose or duration were available, although in one case naphthalene consumption was described as being most pronounced during the last trimester (Zinkham and Childs 1958).

No studies were located that documented developmental effects in humans after oral exposure to 1-methylnaphthalene or 2-methylnaphthalene.

No congenital abnormalities were observed after oral administration of naphthalene at 300 mg/kg/day to pregnant mice on days 7–14 of gestation (Plasterer et al. 1985), or at doses up to 400 mg/kg/day to

pregnant rabbits on days 6–18 of gestation (PRI 1986). Similarly, naphthalene was not teratogenic in rats at doses up to 450 mg/kg/day during gestation days 6–15 (NTP 1991a). However, there was a slight, but dose-related, increase in fused sternebrae in female pups of rabbits administered doses of 20–120 mg/kg/day on days 6–19 of gestation (NTP 1992b). These effects were seen in 2 of 21 litters at 80 mg/kg/day and 3 of 20 litters at 120 mg/kg/day. No other developmental effects were noted in this study.

No studies were located that evaluated developmental end points in animals after oral exposure to 1-methylnaphthalene or 2-methylnaphthalene.

The highest NOAEL values and all LOAEL values from each reliable study for developmental effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.7 Cancer

No studies were located that documented carcinogenic effects in humans after oral exposure to naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene.

In a 2-year feeding study of rats receiving naphthalene at about 41 mg/kg/day, no tumors were reported (Schmahl 1955). Specific details pertaining to the tissues examined were not provided.

The chronic dietary studies with 1-methylnaphthalene or 2-methylnaphthalene provide limited evidence for the carcinogenicity of these chemicals. Long-term exposure (81 weeks) of mice to 71.6 or 140.2 mg/kg/day 1-methylnaphthalene in the diet was associated with statistically significant increases in bronchiolar/alveolar adenomas in males, but not in females (Murata et al. 1993). Incidences for mice with lung adenomas were 2/49, 13/50, and 12/50 for control through high-dose male mice, and 4/50, 2/50, and 4/49 for female mice. Combined incidence for mice with lung adenomas or adenocarcinomas were 2/49, 13/50, and 15/50 for male mice, and 5/50, 2/50, and 5/50 for female mice. In mice exposed to 2-methylnaphthalene in the diet for 81 weeks, incidences for mice with lung adenomas were 2/49, 9/49, and 5/49 in males groups that received 0, 54.3, or 113.8 mg/kg/day, and 4/50, 4/49, and 5/48 in female groups that received comparable doses (Murata et al. 1997). Only the incidence in the 54.3-mg/kg/day group was elevated to a statistically significant degree.

3.2.3 Dermal Exposure

3.2.3.1 Death

Two cases of hemolytic anemia were observed in infants exposed to naphthalene-treated diapers (Schafer 1951; Valaes et al. 1963). One case was fatal. Jaundice, methemoglobinemia, hemolysis, and cyanosis were noted. In the fatal case the symptoms persisted, even after the naphthalene-containing diapers were no longer used (Schafer 1951). The author suggested that use of baby oil on the infant's skin might have facilitated the naphthalene absorption.

No treatment-related deaths occurred within the 14-day observation period when naphthalene was applied at 2,500 mg/kg to the skin of male and female rats or when doses of up to 1,000 mg/kg/day were applied to the skin for 6 hours/day, 5 days/week for 13 weeks (Frantz et al. 1986; Gaines 1969). There were also no deaths in New Zealand White rabbits after application of 2,000 mg/kg naphthalene to intact and abraded shaved areas of skin in an LD_{50} study (Papciak and Mallory 1990).

No studies were located that documented lethal effects in humans or animals after dermal exposure to 1-methylnaphthalene or 2-methylnaphthalene.

3.2.3.2 Systemic Effects

No studies were located that documented musculoskeletal effects in humans or animals after dermal exposure to naphthalene. The highest NOAEL and all LOAEL values for dermal exposure to naphthalene are recorded in Table 3-3. Data for systemic effects in humans or animals from dermal exposure to 1-methylnaphthalene or 2-methylnaphthlene are restricted to two studies that only examined the lung for lesions following repeated dermal exposure to methylnaphthalene, a mixture of 1-methylnaphthalene and 2-methylnaphthalene (Emi and Konishi 1985; Murata et al. 1992).

Respiratory Effects. No studies were located that documented respiratory effects in humans after dermal exposure to naphthalene.

No histological changes of the lungs were noted in rats dermally treated with doses of up to 1,000 mg/kg/day naphthalene (6 hours/day, 5 days/week) for 13 weeks (Frantz et al. 1986).

Table 3-3 Levels of Significant Exposure to Naphthalene (Nap) Or Methylnaphthalene (1-Mn Or 2-Mn) - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL	LOAEL			Reference Chemical Form
				Less Serious		Serious	
ACUTE EXPOSURE							
Systemic							
Rabbit New Zealand White	once 24hr contact	Dermal		2000 mg/kg	(skin irritation, edema, fissuring)		Papciak and Mallory 1990 NAP
Rabbit	once	Dermal		125 mg/kg	(reversible erythema)		PRI 1985a NAP
Immuno/ Lymphoret							
Gn Pig	3 wk 1x/wk		1000 mg/kg				PRI 1985c NAP

Table 3-3 Levels of Significant Exposure to Naphthalene (Nap) Or Methylnaphthalene (1-Mn Or 2-Mn) - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL	LOAEL			Reference Chemical Form
				Less Serious		Serious	
INTERMEDIATE EXPOSURE							
Systemic							
Rat	90 d 5d/wk 6 hr/d	Resp	1000 mg/kg/day				Frantz et al. 1986 NAP
		Cardio	1000 mg/kg/day				
		Gastro	1000 mg/kg/day				
		Hemato	1000 mg/kg/day				
		Hepatic	1000 mg/kg/day				
		Renal	1000 mg/kg/day				
		Dermal	300 mg/kg/day	1000 mg/kg/day	(increased incidence of excoriated skin and papules)		
Mouse (B6C3F1)	30 wk 2x/wk	Resp		119 mg/kg	(100% incidence of mice with pulmonary alveolar proteinosis)		Murata et al. 1992 1-MN+2-MN

Table 3-3 Levels of Significant Exposure to Naphthalene (Nap) Or Methylnaphthalene (1-Mn Or 2-Mn) - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL	LOAEL			Reference Chemical Form
				Less Serious	Serious		
CHRONIC EXPOSURE							
Systemic							
Mouse (B6C3F1)	61 wk 2x/wk	Resp	30 mg/kg		119 mg/kg	(31/32 mice had pulmonary alveolar proteinosis; an unspecified number died)	Emi and Konishi 1985 1-MN+2-MN

Cardio = cardiovascular; d = day(s); Gastro = gastrointestinal; Gn Pig = Guinea pig; Hemato = hematological; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s); x = time(s). 1-Mn = 1-methylnaphthalene; 2-Mn = 2-methylnaphthalene.

Pulmonary alveolar proteinosis was noted in nearly all female B6C3F1 mice given dermal doses of methylnaphthalene (a mixture of 1-methylnaphthalene and 2-methylnaphthalene) twice a week at a dose level of 119 mg/kg for 30 weeks (Murata et al. 1992) or 61 weeks (Emi and Konishi 1985). Endogenous lipid pneumonia was the term used to describe this lesion in the earlier study. With the longer-duration exposure to 119 mg/kg methylnaphthalene, an unspecified number of mice died early. Pulmonary alveolar proteinosis developed in 3/11 female mice treated twice weekly with dermal doses of 30 mg/kg for 61 weeks, compared with 0/4 controls (Emi and Konishi 1985).

Cardiovascular Effects. No studies were located that documented cardiovascular effects in humans after dermal exposure to naphthalene.

No differences in organ weight or histological changes of the heart were noted in rats dermally treated with 1,000 mg/kg/day naphthalene (6 hours/day, 5 days/week) for 13 weeks (Frantz et al. 1986).

Gastrointestinal Effects. No studies were located that documented gastrointestinal effects in humans after dermal exposure to naphthalene.

No histological changes of the esophagus, stomach, or intestines were noted in rats dermally treated with 1,000 mg/kg/day naphthalene (6 hours/day, 5 days/week) for 13 weeks (Frantz et al. 1986).

Hematological Effects. Hemolytic anemia was reported in infants dermally exposed to diapers or other clothing treated with naphthalene mothballs (Dawson et al. 1958; Schafer 1951; Valaes et al. 1963). Jaundice, fragmentation of erythrocytes, Heinz bodies, methemoglobinemia, and reticulocytosis were observed. Several of the infants had G6PD deficiencies. Individuals with this genetic disorder are particularly susceptible to hemolysis from chemical agents. The application of oil to the skin may have aided absorption of naphthalene, as shown by the increasing severity of symptoms (jaundice and cyanosis) even after the use of the naphthalene-containing diapers ceased (Schafer 1951).

There were no changes in hemoglobin, hematocrit, red blood cell count, leukocyte count, or platelet count at 4 and 13 weeks in rats treated with doses of up to 1,000 mg/kg/day applied to the skin (6 hours/day, 5 days/week) for 13 weeks (Frantz et al. 1986).

Hepatic Effects. The liver was enlarged in two infants who experienced acute hemolysis after dermal exposure to naphthalene (Dawson et al. 1958; Schafer 1951). The relationship between liver enlargement and potential naphthalene-induced hemolysis is unknown.

There were no differences in liver weights or histological damage to the liver in rats dermally treated with doses of up to 1,000 mg/kg/day naphthalene (6 hours/day, 5 days/week) for 13 weeks (Frantz et al. 1986). In addition, the levels of aspartate amino transferase, alanine amino transferase, urea nitrogen, and bilirubin were not elevated in the exposed rats as compared to the controls.

Renal Effects. No studies were located that documented renal effects in humans after dermal exposure to naphthalene.

There were no differences in kidney weights or histological damage to the liver in rats dermally treated with doses of up to 1,000 mg/kg/day naphthalene (6 hours/day, 5 days/week) for 13 weeks (Frantz et al. 1986). In addition, the results of urinalysis conducted at 4 and 13 weeks on the treated rats were not different from the control results, indicating that there was no impairment of kidney function.

Dermal Effects. No studies were located that documented dermal effects in humans after dermal exposure to naphthalene.

A study in rabbits has shown that naphthalene is a mild dermal irritant, causing erythema and fissuring, when directly applied to the shaved, abraded, or nonabraded skin under a dressing; healing occurred within 6–7 days (Papciak and Mallory 1990; PRI 1985a). In rats that were dermally treated for 6 hours/day, 5 days/week, for 13 weeks with 1,000 mg/kg/day naphthalene, there was an increased incidence of excoriated skin lesions and papules (Frantz et al. 1986). However, similar lesions were seen in the controls and lower dose group animals. At the high dose, naphthalene appeared to exacerbate the severity of the lesions. Acute and chronic exposures of animal skin to naphthalene appear to cause dermal irritation.

Ocular Effects. Two case studies were reported in which humans experienced eye irritation and conjunctivitis as a result of naphthalene exposure (van der Hoeve 1906). In one case, a worker accidentally got naphthalene powder in his left eye. The exact amount was unknown, but was described by the worker as large. Despite immediate cleansing of the eye, the subject experienced conjunctivitis and pain shortly after exposure. Symptoms of irritation subsided, but then reappeared 6 weeks later. At

that time, the subject noticed decreased vision in his left eye. When examined by a doctor, the eye had retinal lesions (one fresh and others seemingly older); the entire retina appeared clouded. The subject's vision in the left eye was poorer than in the right. Five years earlier, vision was the same in both eyes.

In the second case study, an adult male who worked in a storage area where naphthalene was used as a pesticide complained of ocular pain, conjunctivitis, and impaired vision (van der Hoeve 1906). Neither the duration nor the mode of exposure was described. The subject most likely was exposed to naphthalene vapors. When examined by a doctor, the subject was found to have retinal bleeding and the beginning of a cataract.

Dermal and ocular contact with naphthalene vapors accompanied by inhalation may have contributed to the development of multiple lens opacities in 8 of 21 workers involved with a dye manufacturing process that used naphthalene as a raw material (Ghetti and Mariani 1956). Workers, who were employed at the plant for up to 5 years, melted naphthalene in open vats, resulting in high atmospheric vapor concentrations.

Mild ocular irritation was observed in the nonrinsed eyes of rabbits after instillation of naphthalene at 0.1 mg/eye (Papciak and Mallory 1990; PRI 1985b). Observed effects were reversible within 7 days after exposure. When the eyes were rinsed with water immediately after exposure, there were no signs of irritation (Papciak and Mallory 1990). Oral administration of naphthalene in rats resulted in cataract formation beginning at the posterior outer cortex, suggesting that this region is the most sensitive part of the lens (Kojima 1992). The lenses of pigmented Brown-Norway rats had changes, such as water cleft formation, during the first week that 10 mg/kg/day naphthalene was orally administered every other day (Murano et al. 1993). These rats were more sensitive to cataract formation than albino Sprague-Dawley rats, presumably because they more effectively metabolized naphthalene to the toxic compound naphthoquinone (Murano et al. 1993).

3.2.3.3 Immunological and Lymphoreticular Effects

No studies were located that documented immunological or lymphoreticular effects in humans after dermal exposure to naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene. An enlarged spleen was noted in two human subjects dermally exposed to unspecified doses of naphthalene (Dawson et al. 1958;

Schafer 1951). However, spleen enlargement is a result of hemolysis rather than a direct effect of naphthalene on the spleen.

In animals, dermal application of pure naphthalene (1,000 mg/kg) 1 time/week for 3 weeks did not result in delayed hypersensitivity reactions in guinea pigs (Papciak and Mallory 1990; PRI 1985c).

No studies were located that documented immunological or lymphoreticular effects in animals after dermal exposure to 1-methylnaphthalene or 2-methylnaphthalene.

A NOAEL for immunological/lymphoreticular effects following dermal exposure to naphthalene is recorded in Table 3-3.

No studies were located that documented the following health effects in humans or animals after dermal exposure to naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene:

3.2.3.4 Neurological Effects

3.2.3.5 Reproductive Effects

3.2.3.6 Developmental Effects

3.2.3.7 Cancer

3.3 GENOTOXICITY

No studies of genotoxic effects in humans exposed to naphthalene were located.

Table 3-4 summarizes results for naphthalene and its metabolites in bacterial mutation assays; *in vitro* eukaryotic gene mutation, cytogenetic, or DNA damage assays; and *in vivo* eukaryotic gene mutation, cytogenetic, or DNA damage assays.

Bacterial Gene Mutation Assays for Naphthalene. Naphthalene was not mutagenic in *Salmonella typhimurium* assays in the presence or absence of rat liver metabolic preparations (Bos et al. 1988; Connor et al. 1985; Florin et al. 1980; Gatehouse 1980; Godek et al. 1985; Kaden et al. 1979; McCann et al. 1975; Mortelmans et al. 1986; Nakamura et al. 1987; Narbonne et al. 1987; NTP 1992a; Sakai et al. 1985). The metabolites, 1-naphthol and 1,4-naphthoquine, were not mutagenic in several *S. typhimurium*

Table 3-4. Results of Genotoxicity Testing of Naphthalene or Metabolites[a]

Assay	Test system	Dose/ concentration	HID or LED	Result	Reference
Bacterial gene mutation assays					
Reverse mutation	*Salmonella typhimurium* TA1535, TA1537, TA98, TA100	100 µg/plate ±S9 activation	100	Negative	McCann et al. 1975
	S. typhimurium TA1535, TA1537, TA98, TA100	0.3–100 µg/plate ±S9 activation	100	Negative	Mortelmans et al. 1986
	S. typhimurium TA1535, TA1537, TA98, TA100	0.3–100 µg/plate ±S9 activation	100	Negative	NTP 1992a
	S. typhimurium TA1537, TA1538	10–200 µg/plate ±S9 activation	100	Negative, toxic above 100 µg/plate	Gatehouse 1980
	S. typhimurium TA98, TA100	10–50 µg/plate ±S9 activation	50	Negative	Bos et al. 1988
	S. typhimurium TA1535, TA1537, TA98, TA100	0.03–30 µmol/plate ±S9 activation	3	Negative, toxic above 3 µmol/plate	Florin et al. 1980
	S. typhimurium TA1535, TA1537, TA98, TA100	250 µg/plate ±S9 activation	250	Negative	Sakai et al. 1995
	S. typhimurium TA1535, TA1537, TA1538, TA98, TA100	3–300 µg/plate ±S9 activation	300	Negative, toxic above 300 µg/plate	Godek 1985
	S. typhimurium TM677	1–2 mM ±S9 activation	2	Negative	Kaden et al. 1979
	S. typhimurium TA98, TA1535	5–1,000 µg/plate ±S9 activation	1,000	Negative	Narbonne et al. 1987
	S. typhimurium UTH8413, UTH8414, TA98, TA100	100–2,000 µg/plate ±S9 activation	2,000	Negative	Conner et al. 1985
	S. typhimurium TA1535, TA1537, TA98, TA100	1,000 µg/plate ±S9 activation	1,000	Negative (1-naphthol)	McCann et al. 1975
	S. typhimurium TA98, TA1535	5–1,000 µg/plate ±S9 activation	1,000	Negative (1-naphthol)	Narbonne et al. 1987
	S. typhimurium TA1535, TA1537, TA98, TA100	250 µg/plate ±S9 activation	250	Negative (1,4-naphthoquinone)	Sakai et al. 1995

Table 3-4. Results of Genotoxicity Testing of Naphthalene or Metabolites[a]

Assay	Test system	Dose/ concentration	HID or LED	Result	Reference
Bacterial gene mutation assays (continued)					
	S. typhimurium TA97a, TA98, TA100, TA104	0–100 nmol/plate ±S9 activation	17.5	Positive (1,2-naphtho-quinone), 1.8- to 3.4-fold increase without S9; +S9 results similar to -S9 results	Flowers-Geary et al. 1996
SOS response	*S. typhimurium* TA1535/p5K1002 (uMuC-lacZ)	83 µg/mL ±S9 activation	83	Negative	Nakamura et al. 1987
	Escherichia coli K12 inductest (λ lysogen GY5027; uvrB-, envA-)	2,000 µg/plate ±S9 activation	2,000	Negative	Mamber et al. 1984
SOS chromotest	*E. coli* PQ37 (sfiA::lacZ fusion)	0.156–10.0 µg/assay ±S9 activation	10	Negative	Mersch-Sundermann et al. 1993
Pol A- or Rec assay	*E. coli* WP2/WP10 (uvrA-, recA-)	2,000 µg/mL ±S9 activation	2,000	Negative	Mamber et al. 1983
	E. coli WP2/WP67 (uvrA-, pol A-)	Dose not specified ±S9 activation	NS	Negative	Mamber et al. 1983
Pol A- or Rec assay	*E. coli* WP2/WP3478 (pol A-)	Dose not specified ±S9 activation	NS	Negative	Mamber et al. 1983
Mutatox (reversion to luminescence)	*Vibrio fischeri* M169	Up to 5,000 µg/tube ±S9 activation	0.203 0.625	Negative without S9 activation Positive with S9 activation	Arfsten et al. 1994
***In vitro* eukaryotic gene mutation, cytogenetic, or DNA damage assays**					
Mutation at hprt and tk loci	Human B-lymphoblastoid cell line MCL-5	40 µg/mL	40	Negative	Sasaki et al. 1997
	Human B-lymphoblastoid cell line MCL-5	40 µg/mL	40	Negative (1,4-naphthoquinone)	Sasaki et al. 1997
Chromosomal aberrations	Chinese hamster ovary cells	15–75 µg/mL ±S9 activation	30 75	Positive with S9 activation Negative without S9 activation	NTP 1992a
Chromosomal aberrations	Preimplantation whole mouse embryos	0.16 mM ±S9 activation	0.16	Positive, more pronounced with S9 activation	Gollahon et al. 1990 [abstract only]

Table 3-4. Results of Genotoxicity Testing of Naphthalene or Metabolites[a]

Assay	Test system	Dose/ concentration	HID or LED	Result	Reference
***In vitro* eukaryotic gene mutation, cytogenetic, or DNA damage assays (continued)**					
Sister chromatid exchange	Human mononuclear leukocytes	100 µM ± human liver microsomes	100	Negative	Tingle et al. 1993; Wilson et al. 1995
Sister chromatid exchange	Human mononuclear leukocytes	0–100 µM ± human liver microsomes	10	Positive (1,2- and 1,4-naphthoquinone) Negative (naphthalene 1,2-epoxide)	Wilson et al. 1996
Sister chromatid exchange	Chinese hamster ovary cells	9–90 µg/mL ±S9 activation	27	Positive with S9 in the second of two trials and without S9 in both trials	NTP 1992a
Alkaline elution (*in vitro*)	Rat hepatocytes	3 mM, 3-hour exposure	3 mM	Negative for increased incidence of DNA single-strand breaks	Sina et al. 1983
Unscheduled DNA synthesis (*in vitro*)	Rat primary hepatocytes	0.16–5,000 µg/mL	16	Negative, toxic above 16 µg/mL	Barfknecht et al. 1985
	Rat primary hepatocytes	0.5–1,000 nM/mL	1,000	Negative (1-naphthol, 2-naphthol)	Probst et al. 1981
Cell transformation	Fischer rat embryo cells (F1706P96)	0.1, 0.5 µg/mL	0.5	Negative	Freeman et al. 1973
	Syrian baby hamster kidney cells (BHK-21C13)	0.08–250 µg/mL +S9	250	Negative	Purchase et al. 1978
	Mouse (BALB/c) whole mammary gland cultures	0.001–1.0 µg/gland	0.1	Negative, cytotoxic above 0.1 µg/gland	Tonelli et al. 1979
	Mouse BALB/c 3T3 cell culture	15–150 µg/mL	150	Negative, toxic at highest dose	Rundell et al. 1983
	Human diploid fibroblasts (WI-38)	0.08–250 µg/mL +S9	250	Negative	Purchase et al. 1978
***In vivo* eukaryotic gene mutation, cytogenetic, or DNA damage assays**					
Somatic mutation, recombination	*Drosophila melanogaster*	1, 5, 10 mM (feeding larvae)	5	Positive, loss of heterozygosity of two recessive wing genes (about 2-fold increase in number of wing spots)	Delgado-Rodriguez et al. 1995
Micronuclei induction	Male ICR Swiss mice: bone marrow cells	50, 250, and 500 mg/kg gavage	500	Negative	Harper et al. 1984

Table 3-4. Results of Genotoxicity Testing of Naphthalene or Metabolites[a]

Assay	Test system	Dose/ concentration	HID or LED	Result	Reference
***In vivo* eukaryotic gene mutation, cytogenetic, or DNA damage assays (continued)**					
	Male and female CD-1 mice: bone marrow cells	250 mg/kg intraperitoneal	250	Negative	Sorg 1985
Micronuclei induction	Salamander larvae (*Pleurodeles waltl*): erythrocytes	0.125–0.5 ppm in the tank water	0.25	Positive at 0.5 ppm, weakly positive at 0.25 ppm	Djomo et al. 1995
Alkaline elution (*in vivo*)	DNA from hepatocytes of female rats given single oral doses	359 mg/kg oral	359	Negative for DNA single-strand breaks	Kitchin et al. 1992, 1994
Unscheduled DNA synthesis (*in vivo*)	Hepatocytes from rats given single oral doses	600, 1,000, and 1,600 mg/kg gavage	1,600	Negative	RTC 1999
DNA fragmentation	DNA fragmentation in liver or brain tissue from mice given single doses	0, 3, 32, and 158 mg/kg (0.01, 0.1, 0.5 of LD_{50}=316 mg/kg)	32	Positive (1.0- to 1.5-fold and 1.8- to 2.2-fold increase in DNA fragmentation at 32 and 158 mg/kg, respectively)	Bagchi et al. 2002
DNA fragmentation	DNA fragmentation in liver or brain tissue from rats given daily doses for up to 120 days	0, and 110 mg/kg in corn oil	110	Positive (1.9- to 2.5-fold maximal increases in DNA fragmentation in brain and liver tissue)	Bagchi et al. 1998a
DNA fragmentation	DNA fragmentation in liver or brain tissue from p53-deficient and standard mice given single oral doses	0, 3, 32, and 158 mg/kg (0.01, 0.1, and 0.5 of LD_{50}=316 mg/kg)	158 (std) 3 (-p53)	Positive (1.8- to 3.9-fold increases in DNA fragmentation in brain and liver tissue; p53-deficient (tumor suppressor gene) strain was more sensitive	Bagchi et al. 2000
Neoplastic transformation (*in vivo*)	F344 partially hepatectomized rats (sex not specified)	100 mg/kg gavage (in corn oil)	100	Negative for gamma-glutamyl trans-peptidase foci	Tsuda et al. 1980

[a]Metabolites are noted in result column.

DNA = dioxyribonucleic acid; HID = highest ineffective dose for negative tests; LED = lowest effective dose for positive tests; NS = not specified; SOS = an emergency system to repair single strand DNA breaks; std = standard deviation

strains in the presence or absence of metabolic activation (McCann et al. 1975; Narbonne et al. 1987; Sakai et al. 1985). Naphthalene was not mutagenic, with or without metabolic activation, in the Pol A- or Rec assays in several *Escherichia coli* strains (Mamber et al. 1983). Naphthalene did not damage DNA (as assayed by the induction of the SOS-repair system) in *E. coli* PQ37 (Mersch-Sundermann et al. 1993), in *E. coli* K12 (Mamber et al. 1984), or in *S. typhimurium* TA1535/p5K1002 (Nakamura et al. 1987).

1,2-Naphthoquinone induced reverse mutations in several *S. typhimurium* strains without a metabolic activation system (Flowers-Geary et al. 1996), and naphthalene, in the presence of rat liver metabolic activation, induced reverse mutations in the marine bacterium *Vibrio fischeri* (Arfsten et al. 1994).

In Vitro Eukaryotic Gene Mutation, Cytogenetic, or DNA Damage Assays for Naphthalene. *In vitro* eukaryotic gene mutation assays are restricted to a single report that naphthalene and 1,4-naphthoquinone (1,2-naphthoquinone was not tested) did not induce mutations at the hprt and tk loci in human lymphoblastoid cells (Sasaki et al. 1997). However, naphthalene (in the presence of rat liver metabolic activation) induced chromosomal aberrations in Chinese hamster ovary cells (NTP 1992a) and preimplantation whole mouse embryos (Gollahon et al. 1990). Naphthalene also induced sister chromatid exchanges (in the presence or absence of rat liver metabolic activation) in Chinese hamster ovary cells (NTP 1992a), but did not do so in human mononuclear leukocytes in the presence or absence of human liver microsomes (Tingle et al. 1993; Wilson et al. 1995). In contrast, 1,2-naphthoquinone and 1,4-naphthoquinone (but not 1,2-naphthalene oxide), in the absence of metabolic activation, induced sister chromatid exchanges in human leukocytes at concentrations (10 and about 50 µM) that depleted cellular glutathione levels and induced about 35-45% cell death (Wilson et al. 1996). Naphthalene did not induce cell transformations in several mammalian cell types (see Table 3-4) or DNA single-strand breaks (Sina et al. 1983) or unscheduled DNA synthesis (Barfknecht et al. 1985; Probst et al. 1981) in rat hepatocytes.

In cell-free test systems (not included in Table 3-4), 1,2-naphthoquinone formed N7 adducts with deoxyguanosine (McCoull et al. 1999) and caused DNA strand scission in the presence of NADPH and copper via reactive oxygen species from a Cu(II)/Cu(I) oxidation/reduction cycle (Flowers et al. 1997).

In Vivo Eukaryotic Gene Mutation, Cytogenetic, or DNA Damage Assays for Naphthalene. Naphthalene was mutagenic in *Drosophila melanogaster* (Delgado-Rodriquez et al. 1995), but no *in vivo* mutagenicity tests of naphthalene or its metabolites are available in mammalian systems (Table 3-4). Naphthalene induced micronuclei in erythrocytes of salamander (*Pleurodeles waltl*) larvae exposed to concentrations of 0.5 mM, but did not induce micronuclei in bone marrow of mice given single oral doses

(50, 250, or 500 mg/kg) or intraperitoneal doses (250 mg/kg) (Harper et al. 1984; Sorg 1985). Naphthalene did not cause increased single-stranded DNA breaks in hepatocytes of rats given single oral doses of 359 mg/kg (Kitchin et al. 1992, 1994), unscheduled DNA synthesis in hepatocytes from rats given single doses as high as 1,600 mg/kg (RTC 1999), or transformation foci (γ-glutamyl transpeptidase-positive) in livers of F344 partially hepatectomized rats given single 100 mg/kg doses, but did cause DNA fragmentation in brain and liver tissue from mice given single doses of 32 or 158 mg/kg (Bagchi et al. 2000, 2002) and rats exposed to 110 mg/kg/day for up to 120 days (Bagchi et al. 1998a). In the DNA fragmentation assays, the effect was accompanied by increased lipid peroxidation in the same tissues. It is unclear whether the apparent DNA damage in these assays was due to direct effects of naphthalene metabolites or reactive oxygen species or was secondary to cell death induced at an extranuclear site.

No studies were located that examined possible genotoxic effects of naphthalene or its metabolites in sensitive target tissues of naphthalene in rodents (lung and nasal epithelial tissue).

Genotoxicity Assays for 1-Methylnaphthalene and 2-Methylnaphthalene. No studies were located that documented genotoxic effects of 1-methylnaphthalene or 2-methylnaphthalene in humans or animals by any route of exposure. Data are limited to one *in vitro* study where 1-methylnaphthalene and 2-methyl-naphthalene failed to induce chromosomal aberrations or sister chromatid exchanges in human peripheral lymphocytes (Kulka et al. 1988). In an *in vitro* microbial assay employing *S. typhimurium*, mutagenic activity was not detected with either compound, with either the presence or absence of microsomal activation (Florin et al. 1980). These studies are presented in Table 3-5.

3.4 TOXICOKINETICS

Little information is available that documented the toxicokinetics of naphthalene in humans by any route of exposure. No information on the toxicokinetics of 1-methylnaphthalene or 2-methylnaphthalene in humans was located. The available animal data pertaining to naphthalene are described in the following sections. The relevance of this information to the toxicokinetics of naphthalene in exposed humans, however, is not known.

No toxicokinetic data on 1-methylnaphthalene-exposed animals were located. Animal data pertaining to 2-methylnaphthalene were limited.

Table 3-5. Genotoxicity of 1-Methylnaphthalene and 2-Methylnaphthalene *In Vitro*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
1-Methylnaphthalene				
Prokaryotic organisms:				
Salmonella typhimurium (TA98, TA100, TA1535, TA1537)	Gene mutation	–	–	Florin et al. 1980
Mammalian cells:				
Human lymphocytes	Chromosomal aberration, sister chromatid exchange	–	–	Kulka et al. 1988
2-Methylnaphthalene				
Prokaryotic organisms:				
S. typhimurium (TA98, TA100, TA1535, TA1537)	Gene mutation	–	–	Florin et al. 1980
Mammalian cells:				
Human lymphocytes	Chromosomal aberration, sister chromatid exchange	–	–	Kulka et al. 1988

– = negative result

3.4.1 Absorption

Based on the presence of adverse effects following exposure, humans and animals can absorb naphthalene by pulmonary, gastrointestinal, and cutaneous routes. However, the rate and extent of naphthalene absorption are unknown in many instances.

3.4.1.1 Inhalation Exposure

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). Unfortunately, the rate and extent of naphthalene absorption were not determined in these studies. Presumably naphthalene moves across the alveolar membrane by passive diffusion through the lipophilic matrix.

No animal data that documented the absorption of naphthalene after inhalation were located. The only data observed in animal studies involved localized effects in the lungs and nasal passages. Thus, it is not possible to conclude that they were the consequence of absorbed naphthalene. However, absorption can be presumed to occur based on the human data.

No information has been located that documented the absorption of 1-methylnaphthalene or 2-methyl-naphthalene in humans or animals after inhalation exposure.

3.4.1.2 Oral Exposure

Several case reports indicate that naphthalene ingested by humans can be absorbed in quantities sufficient to elicit toxicity (Bregman 1954; Chusid and Fried 1955; Gidron and Leurer 1956; Gupta et al. 1979; Haggerty 1956; Kurz 1987; MacGregor 1954; Mackell et al. 1951; Ojwang et al. 1985; Santhanakrishnan et al. 1973; Shannon and Buchanan 1982; Zuelzer and Apt 1949). However, no studies have been located that report the rate or extent of absorption. Absorption of naphthalene presumably occurs by passive diffusion through the lipophilic matrix of the intestinal membrane.

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.

No information that documented the absorption of naphthalene after oral administration to animals has been located. The occurrence of ocular effects in rats and rabbits indicates that gastrointestinal absorption does occur (Kojima 1992; Murano et al. 1993; Srivastava and Nath 1969).

No information was located that documented the absorption of 1-methylnaphthalene in humans or animals after oral administration. Systemic effects observed after the ingestion of 1-methylnaphthalene demonstrate that intestinal absorption does occur in rats (Murano et al. 1993).

No information has been located that documented absorption in humans after oral exposure to 2-methylnaphthalene. Small doses of 2-methylnaphthalene appear to be rapidly absorbed from the gastrointestinal tract in guinea pigs. 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).

3.4.1.3 Dermal Exposure

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 which had been stored in contact with naphthalene (mothballs or naphthalene flakes). The 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 which 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-time 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 authors proposed that naphthalene was absorbed more slowly from the sandy soil than 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 1.6% organic matter.

No studies were located that examined the absorption of 1-methylnaphthalene or 2-methylnaphthalene in humans or animals after dermal administration.

3.4.2 Distribution

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 1986g). 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.

Information is available for the distribution of naphthalene in swine after oral exposure, the distribution of naphthalene in rats after dermal exposure, and the distribution of 2-methylnaphthalene in guinea pigs after oral exposure. No data were located for the inhalation exposure routes and no data were identified on the distribution of 1-methylnaphthalene by any route of exposure.

3.4.2.1 Inhalation Exposure

No studies were located that examined the distribution of naphthalene, 1-methylnaphthalene, or 2-methyl-naphthalene in humans or animals after inhalation exposure.

3.4.2.2 Oral Exposure

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; 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±0.04% 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, that in the liver 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.

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.) At 24 hours, the value fell to 0.43 µg and at 48 hours, to 0.04 µg. 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.

Radiolabelled 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 amount in blood at 3 hours was 0.75 µg and that for the lungs was 0.69 µg; at 6 hours, the blood had a concentration of 0.71 µg and the lung had 0.76 µg. The half-life of 2-methylnaphthalene in the blood was 10.4 hours. The decay of naphthalene in the other tissues examined was described as biphasic.

3.4.2.3 Dermal Exposure

No information was located that documented the distribution of naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene in humans after dermal 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.

No information that documented the distribution of 1-methylnaphthalene or 2-methylnaphthalene in dermally exposed animals was located.

3.4.2.4 Other Routes of Exposure

After intraperitoneal 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.

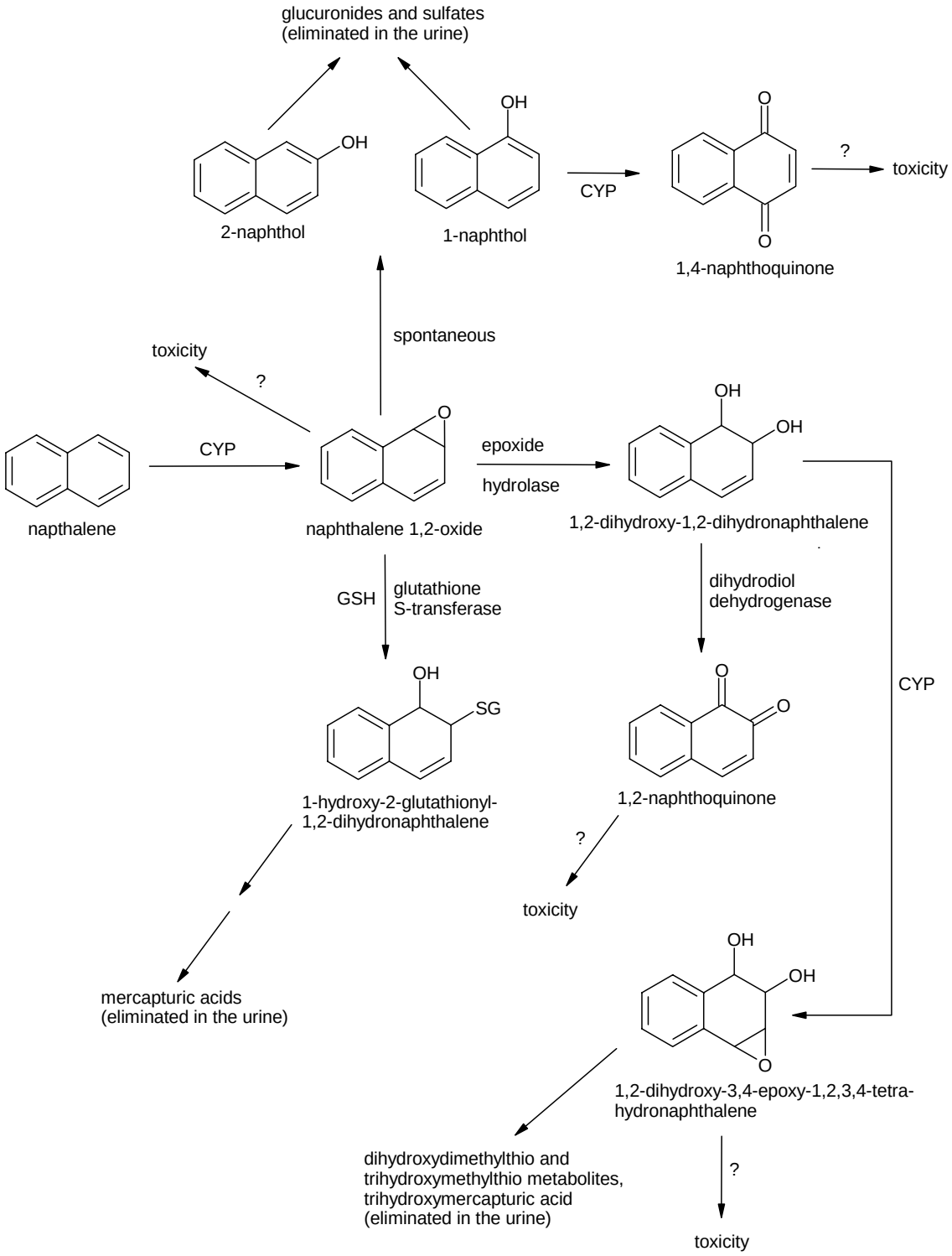
3.4.3 Metabolism

The metabolism of naphthalene in mammalian systems has been studied extensively and is depicted in Figure 3-3. The metabolic scheme in Figure 3-3 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 recent 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 cytochrome P-450 (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.

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 Clara cells (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 Clara 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

Figure 3-3. 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

*Adapted from Buckpitt et al. (2002) and Waidyanatha et al. (2002)

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. 1992a). 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 intraperitoneal 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 might be 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. (1992a).

In addition to being converted to the naphthols, the 1,2-epoxide can be conjugated with glutathione via glutathione-S-transferase catalysis. Figure 3-3 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 Clara cell injury in mice (Warren et al. 1982; West et al. 2000a). 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 Clara cell cytotoxicity (West et al. 2000a).

The 1,2-epoxide can also be enzymatically hydrated by epoxide hydrolase to form 1,2-dihydroxy-1,2-dihydronaphthalene (Figure 3-3). 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 lung 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 Clara 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 (D'Arcy 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 is not specified in Figure 3-3). 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).

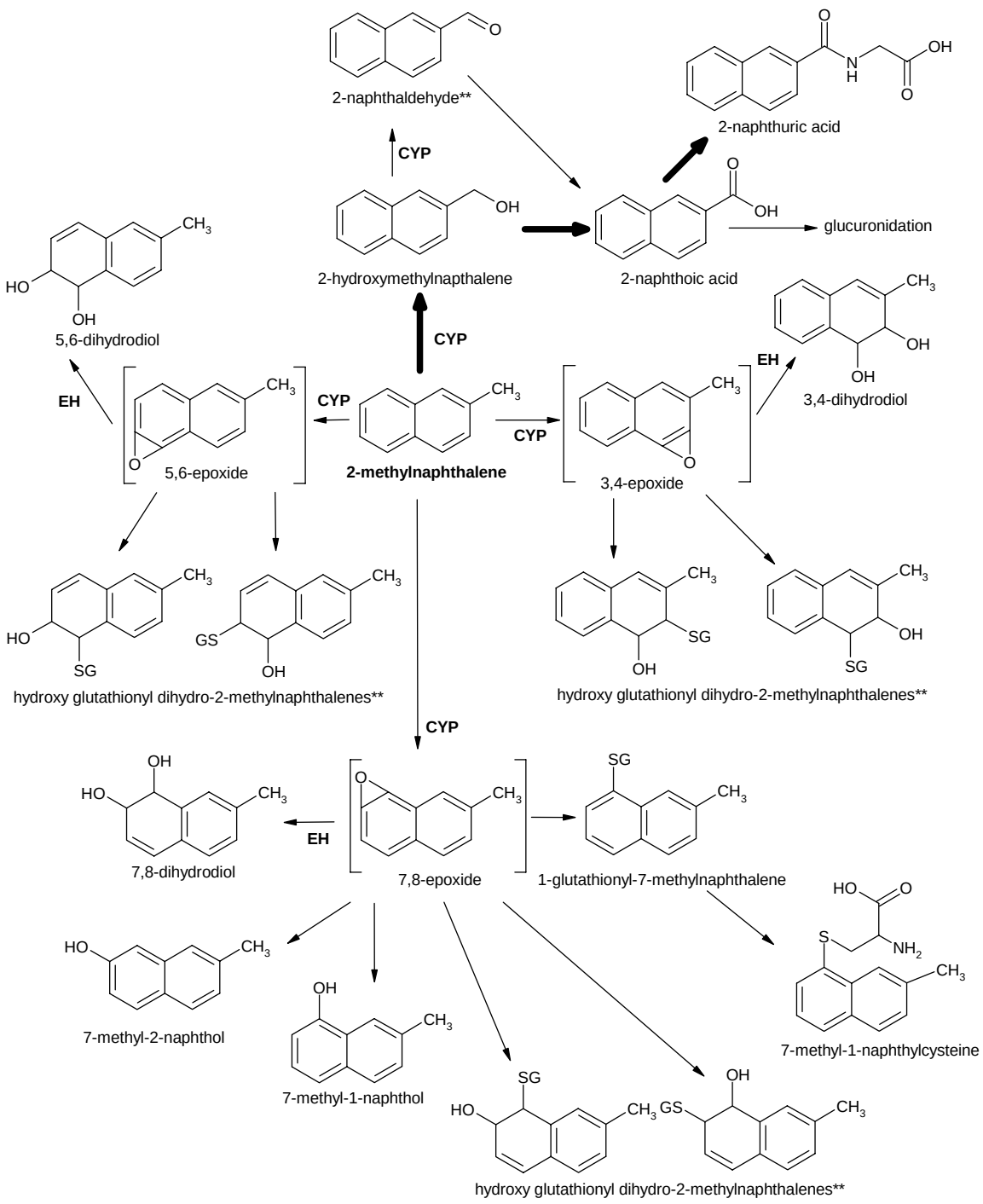
Support for the *in vivo* formation of another potentially reactive metabolite, 1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydronaphthalene, comes from the identification of several urinary metabolites, including a number of trihydroxytetrahydromethylthio derivatives (Horning et al. 1980) and a trihydroxytetrahydro-mercapturic 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 3-3 proposes an oxidative transformation of dihydrodiol derivative to the tetrahydrodiol epoxide derivative via CYP catalysis.

The methyl substituent of 1-methylnaphthalene and 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 3-4. Oxidation at the methyl group (the predominant path), or at several competitive positions on the rings, is catalyzed by CYP monooxygenases (Figure 3-4). No information was located that documented the metabolism of 1-methylnaphthalene. It may be similar to that for 2-methylnaphthalene with oxidation of the side chain and the ring.

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 3-4). 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 3-4). 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 3-4). 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

Figure 3-4. Metabolism of 2-Methylnaphthalene*



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

*Adapted from Buckpitt and Franklin (1989), EPA (2003r); Shultz et al. (2001), and Teshima et al. (1983)
**Metabolites identified *in vitro* only

spontaneously. The hydroxy glutathionyl dihydro-2-methylnaphthalenes (Figure 3-4) 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 3-4 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 3-4 (Shultz et al. 2001).

Three other minor metabolites formed via the 7,8-epoxide pathway are shown in Figure 3-4. 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). The three diols were identified as 3,4-dihydrodiol, 5,6-dihydrodiol, and 7,8-dihydrodiol (Breger et al. 1983).

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 intraperitoneal administration of 400 mg/kg of 2-methylnaphthalene resulted in mortality in 4/5 mice, whereas treatment without glutathione depletion was not fatal (Griffin et al. 1982). Bronchiolar

necrosis was not observed in male ddY mice given single intraperitoneal 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 animals (5/5) (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).

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

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. 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). No studies were located that documented excretion in humans after inhalation exposure to 1-methylnaphthalene or 2-methylnaphthalene.

No studies were located that documented excretion in animals after inhalation exposure to naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene.

3.4.4.2 Oral Exposure

Little information is available pertaining to the excretion of orally 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 radiolabelled 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.

No information was located that documented excretion in humans after oral exposure to 2-methylnaphthalene. In guinea pigs, 80% of a 10 mg/kg tritium-labeled 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.

No studies were located that examined excretion in humans or animals after oral exposure to 1-methylnaphthalene.

3.4.4.3 Dermal Exposure

No reports have been located which discuss the excretion of naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene in humans following dermal exposure.

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 less than 0.4% of the administered label.

No studies were located that documented excretion in animals after dermal exposure to 1-methyl-naphthalene or 2-methylnaphthalene.

3.4.4.4 Other Routes of Exposure

In mouse studies using the intraperitoneal or subcutaneous exposure routes, several naphthalene metabolites were excreted in the urine. After intraperitoneal 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-di-hydro-1,2-naphthalenediol.

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.

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

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models 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 (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen and Krishnan 1994; Andersen et al. 1987). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parameterization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations

provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

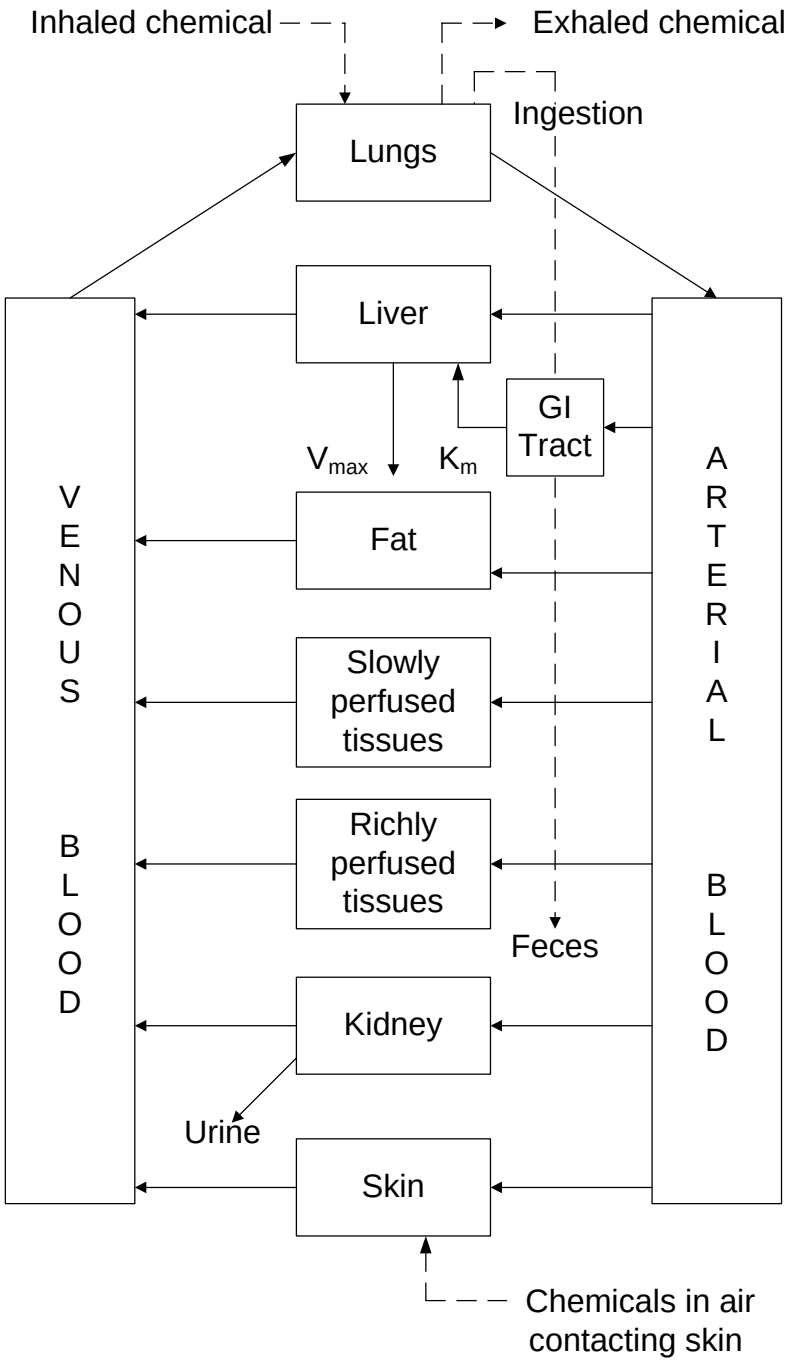
The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) are adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-5 shows a conceptualized representation of a PBPK model.

If PBPK models for naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

This section will discuss the structure and application of the most recent PBPK models for naphthalene that were developed with *in vivo* data for the time-course of naphthalene in blood in rats and mice following inhalation exposure or intravenous administration (Willems et al. 2001). The inhalation data were used to select best-fitting models with the fewest assumptions possible and to optimize model parameters. The intravenous data were used to examine the validity of the final models. These models are refinements of earlier PBPK models for naphthalene in rats and mice, which were developed using parameters estimated from *in vitro* data (Ghanem and Shuler 2000; Quick and Shuler 1999; Sweeney et al. 1996). The most recent models have been used to attempt to explain why naphthalene-induced lung tumors in female B6C3F1 mice, but did not induce lung tumors in F344/N rats in chronic inhalation studies (Abdo et al. 2001; NTP 1992a, 2000). The use of these models to extrapolate dosimetry from rodents to humans is not possible until appropriate validated human physiologically based toxicokinetic (PBTK) models for naphthalene are developed.

Figure 3-5. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Source: adapted from Krishnan et al. 1994

Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

The models do not include nasal compartments that metabolize naphthalene, because no data were available on nasal deposition and epithelial absorption of naphthalene (Willems et al. 2001). Without such data, reliable models for nasal deposition, tissue dosimetry, and nasal-tissue metabolism cannot be developed for naphthalene (models similar to those developed for other nasal toxicants such as acrylic acid [Frederick et al. 2001]). The existence of validated PBTK models with metabolizing nasal compartments would be useful to help to explain why male and female rats develop nasal tumors with chronic inhalation exposure to naphthalene, but mice do not, even though both species develop nonneoplastic lesions in the nasal tissues in which tumors developed in rats (Abdo et al. 2001; NTP 1992a, 2000). In addition, development of human models incorporating anatomical and physiological characteristics of nasal tissue will be useful to decrease uncertainty in extrapolating dose-response relationships for nasal effects in rodents to humans.

The final best-fitting models for rats and mice are comprised of two parts: (1) a diffusion-limited naphthalene submodel with compartments for arterial and venous blood, alveolar space, and tissue and capillary spaces for the lung, liver, kidney, fat, and other organs (with naphthalene metabolism occurring in the liver and lung by the same CYP isozyme with one set of Michaelis-Menten metabolic rate constants); and (2) a flow-limited 1,2- naphthalene oxide submodel describing metabolism and distribution of naphthalene oxide in the same compartments as in the naphthalene submodel (but without tissue capillary spaces) (Willems et al. 2001). Physiological parameters in both submodels (e.g., cardiac output, ventilation rates, tissue volumes, tissue capillary volumes, tissue blood flows) were taken from the literature and scaled to body weights of rats in the NTP (2000) bioassay and reference values for mice. Partition coefficients between the various compartments were calculated from octanol-water partition coefficients. Metabolic rate constants (Vmax and Km) and permeability constants (blood:fat and blood:other tissues) for naphthalene were estimated by fitting the models to naphthalene blood time-course data from the inhalation studies. The naphthalene oxide submodel was essentially the same as that developed by Quick and Shuler (1999) with *in vitro* data, with the exception that it contained a subroutine for reduced glutathione synthesis involving γ-glutamylcysteine synthetase modeled with Michaelis-Menten rate constants and noncompetitive inhibition by reduced glutathione. The metabolic fate of naphthalene oxide in the lung and liver was restricted to dihydrodiol formation via epoxide hydrolase and conjugation to glutathione via glutathione-*S*-transferase. The model did not include spontaneous conversion of naphthalene oxide to 1-naphthol or metabolic transformations to the naphthoquinones. Because no *in vivo* data were available on naphthalene oxide distribution or metabolism, the model predictions for naphthalene oxide tissue dosimetry could not be verified.

Under exposure conditions used in the rat (0, 10, 30, or 60 ppm) and mouse (0, 10, or 30 ppm) NTP (1992a, 2000) chronic inhalation bioassays with naphthalene (6 hours/day), the models predicted that: (1) steady-state lung concentrations of the parent compound, naphthalene, were not very different in rats and mice at equivalent exposure concentrations; (2) cumulative daily naphthalene metabolism in the lung was greater in the mouse than in the rat (by about 1.5- to 2.5-fold) at equivalent exposure concentrations; (3) cumulative daily naphthalene metabolism in the lung (64.9 mg/kg) and estimated maximal lung concentrations of naphthalene oxide (about 12 nmol/mL) for 30-ppm female mice, some of which developed lung tumors, were greater than respective values of 45.9 mg/kg and about 8 nmol/mL in 60-ppm female rats, which did not develop lung tumors; and (4) cumulative daily naphthalene metabolism in the lung was only slightly greater in 30-ppm female mice (64.9 mg/kg) than in the 30-ppm male mice (60.7 mg/kg), which did show statistically significant increased incidence of lung tumors (comparisons of lung concentrations of naphthalene oxide in female and male mice were not reported).

The model simulations are consistent with the hypothesis that the difference in lung tumor response between mice and rats may be due to a combination of greater maximal levels of naphthalene oxide or other metabolites in the mouse lung and, perhaps, a greater susceptibility of the mouse lung to epoxide-induced carcinogenesis. Results with other chemicals, such as ethylene oxide, suggest that the mouse lung may be more susceptible to epoxides than the rat lung (Willems et al. 2001). Differences in predicted cumulative lung metabolism of naphthalene in 30-ppm female mice and 30-ppm male mice were smaller than the difference noted between 30-ppm female mice and 60-ppm female rats; thus the model simulations do not explain the apparent gender difference in tumor response of the mouse lung. The formation of naphthoquinone metabolites was not included in the model. Thus, the model simulations do not provide a basis for identifying which metabolite is responsible for the nonneoplastic and neoplastic responses to naphthalene in the female mouse lung.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Absorption. No studies were located regarding the mechanisms by which naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene are absorbed from the guts, lungs, or skin. Although absorption of these compounds at these sites has been demonstrated, it is unknown if the transport is passive, active, or carried out by a facilitated diffusion mechanism. The relatively small molecular weights and lipophilicity of these compounds indicate that passive diffusion across cell membranes is a possible mechanistic path.

There is some evidence that different vehicles may influence the rate and extent of gastrointestinal or dermal absorption. Naphthalene adsorbed to organic-rich soils was absorbed across the skin more slowly than naphthalene from organic-poor soils (Turkall et al. 1994).

Distribution. As discussed in more detail in Section 3.4.2, there are limited data on the distribution of naphthalene and 2-methylnaphthalene in animals following oral or parenteral administration, but there are no data for these compounds following inhalation exposure or for 1-methylnaphthalene by any exposure route. The available data are inadequate to characterize the mechanisms by which 2-methylnaphthalene may be transported following oral exposure to the lung, the site of toxic action with acute or chronic exposure. No data are available on differences in deposition and absorption of inhaled naphthalene in nasal epithelial tissue, two of which (olfactory epithelium and respiratory epithelium) are key toxicity targets in rats and mice following chronic inhalation exposure to naphthalene.

Metabolism. As discussed in more detail in Section 3.4.3, results from *in vitro* and *in vivo* metabolic studies in mammalian systems indicate that naphthalene metabolism is complex, with multiple competing pathways leading to the formation of several reactive metabolites (e.g., 1,2-naphthalene oxide, 1,2-naphthoquinone, and 1,4-naphthoquinone) and an array of conjugated and nonconjugated metabolites that are excreted predominantly in the urine. Conjugation of the reactive metabolites is viewed as a detoxifying mechanism for the reactive metabolites. With oral exposure, the liver is expected to be the principal site of metabolism, but metabolism of naphthalene at other tissue sites, including the nasal olfactory epithelium, Clara cells in pulmonary epithelial tissue, and eye tissue, has been demonstrated. A first-pass metabolic effect due to liver metabolism is expected with oral exposure, but the degree to which a first-pass effect due to respiratory tissue metabolism occurs with inhalation exposure to naphthalene has not been studied quantitatively.

Section 3.4.3 also discusses in more detail the complexity of 2-methylnaphthalene metabolism, which, in contrast to naphthalene, involves several competing initial steps: oxidation of the methyl side group and oxidation at several positions on the rings. Oxidation of the methyl side group is the principal metabolic pathway, representing about 50–80% of administered doses in animal studies. An array of conjugated and nonconjugated metabolites that are principally excreted in the urine have been identified in animal studies. Although conjugation of metabolites (principally with glutathione) appears to be a detoxication mechanism with acute exposure in animal studies, the involvement of reactive metabolites in the development of pulmonary alveolar proteinosis from chronic exposure to 2-methylnaphthalene is

uncertain (see Section 3.5.2). No studies were located on the metabolism of 1-methylnaphthalene in humans or animals, but it is expected to be similar to 2-methylnaphthalene metabolism based on its similar chemical, physical, and toxicological properties.

Excretion. As discussed in more detail in Section 3.4.4, results from animal studies involving oral or parenteral exposure indicate that naphthalene and 2-methylnaphthalene are principally excreted as metabolites in urine. Excretion in the feces represents a minor excretion pathway for these chemicals, and the possibility of excretion via exhalation of unmetabolized parent compounds has not been examined in available studies. Data for 1-methylnaphthalene were not located, but excretion is likely to be similar to 2-methylnaphthalene given the similarity in chemical and physical properties of these chemicals.

3.5.2 Mechanisms of Toxicity

Some information on the mechanism of toxicity is available for three of the health effects associated with naphthalene exposure: hemolysis, the development of lens opacities (cataracts), and nonneoplastic and neoplastic respiratory tract lesions. Mechanistic hypotheses for these naphthalene-induced effects are discussed below, followed by a discussion of the limited mechanistic information on 1-methyl-naphthalene- and 2-methylnaphthalene-induced pulmonary alveolar proteinosis.

Naphthalene-induced Hemolysis. Humans experience red-cell hemolysis after naphthalene exposure by the inhalation, oral, and dermal routes. In general, animal species are less susceptible than humans. There are no reports of naphthalene-induced hemolysis in either rats or mice; however, hemolysis has been observed in dogs.

Chemically induced red blood cell hemolysis is caused by a breakdown of the system that protects the erythrocyte biomolecules from oxidation. In the erythrocyte, glutathione peroxidase rather than catalase is the major antioxidant enzyme. Glutathione peroxidase (Gpx) is a selenium containing metalloprotein that utilizes reduced glutathione as a cofactor. Oxidized glutathione is reduced by glutathione reductase, a nicotinamide adenine dinucleotide phosphate (NADPH)-requiring enzyme.

The primary source of erythrocyte NADPH is glucose-6-phosphate oxidation by the enzyme G6PD. Individuals who suffer from a genetic defect resulting in a modified enzyme structure (a recessive trait) have a reduced capacity to produce NADPH. Accordingly, they are more susceptible to red cell

hemolysis than individuals without this defect (Gosselin et al. 1984). There is some evidence that heterozygotes may also have an increased susceptibility to red cell hemolysis (Dawson et al. 1958).

When the red blood cell is exposed to oxidizing agents, heme iron is oxidized to the ferric state, producing methemoglobin. This in turn leads to Heinz body formation. It is believed that free radical oxygen modifies membrane lipids leading to increased membrane fragility and lysis. Destruction of the red blood cells decreases erythrocyte counts and stimulates hematopoiesis (leading to increased numbers of reticulocytes). The oxygen carrying capacity of the blood is reduced. Cell lysis releases heme and protein into the blood. Heme breakdown produces bilirubin and biliverdin, causing jaundice. Both erythrocytes and heme breakdown products (urobilinogen) spill into the urine.

Several suggestions can be made regarding the impact of naphthalene on this sequence of events. Since naphthalene is conjugated with glutathione for excretion, it can reduce the supplies of glutathione available for glutathione peroxidase and increase the vulnerability of the cell to oxidation. It is also possible that a naphthalene metabolite may act as an inhibitor for either glutathione peroxidase or glutathione reductase. Glutathione reductase activity was reduced in children who experienced hemolysis following dermal exposure to naphthalene and in related family members (Dawson et al. 1958). Both glutathione peroxidase and glutathione reductase activity were decreased in the lens of rats orally exposed to naphthalene (Rathbun et al. 1990; Tao et al. 1991).

Each of the hypotheses discussed above would serve to increase the sensitivity of any naphthalene-exposed subject to an external oxidizing agent. However, given the severity of the hemolysis that follows naphthalene exposure, it is probable that naphthalene or a naphthalene metabolite also acts as an oxidizing agent in the erythrocyte. Unfortunately, data could not be identified which would correlate the production of any particular metabolite with initiation of red cell peroxidation.

Naphthalene-induced Cataracts. Although there are reports that inhalation, oral, and dermal naphthalene exposure in humans can lead to lens opacities (Grant 1986), the case studies or industrial exposure reports that link naphthalene to cataracts in humans have not been verified by well-conducted epidemiological studies of individuals exposed to naphthalene vapors on a chronic basis. In addition, impurities present in the naphthalene may have contributed to the cataract development in all recorded human cases. Conversely, there are data from a number of well-conducted studies which demonstrate that naphthalene can induce cataracts in animals.

Much of the animal data regarding ocular effects suggest that the toxicity of naphthalene is mediated by the *in situ* formation of 1,2-naphthalenediol in the lens. It has been proposed that metabolism of naphthalene starts in the liver, yielding epoxide metabolites that are subsequently converted to stable hydroxy compounds that circulate to the lens (Van Heyningen and Pirie 1967). The 1,2-naphthalenediol metabolite is subsequently oxidized to 1,2-naphthaquinone and hydrogen peroxide. The quinone metabolite binds to constituents of the lens (protein, amino acids, and glutathione), disrupting its integrity and transparency (Rees and Pirie 1967; Uyama et al. 1955; Van Heyningen and Pirie 1967; Van Heyningen 1976, 1979; Wells et al. 1989).

Intraperitoneal administration of naphthalene (125–1,000 mg/kg), 1-naphthol (56–562 mg/kg), 1,2-naphthoquinone (5–250 mg/kg), and 1,4-naphthoquinone (5–250 mg/kg) caused a dose-related increase in cataracts in C57BL/6 mice, but administration of 2-naphthol (56–456 mg/kg) did not (Wells et al. 1989). The cataractogenic potency of the naphthoquinones was about 10 times that of naphthalene. The cataractogenic potency of 1-naphthol was intermediate to that of naphthalene and the naphthoquinones. The potency of naphthalene was increased by pretreatment with cytochrome P-450 inducers and a glutathione-depleting agent. It was inhibited by pretreatment with a cytochrome P-450 inhibitor. This suggests that the unconjugated oxidized naphthoquinone metabolites are a necessary prerequisite for cataract formation. There are differences in species and strain susceptibility to cataract formation that theoretically relate to the animals' ability to form these metabolites. Naphthalene, 1-naphthol, 1,2-naphthoquinone, and 1,4-naphthoquinone did not form cataracts in DBA/2 mice suggesting the difference between strains is not simply due to metabolite exposure (Wells et al. 1989).

Because hydrogen peroxide is also formed following the oxidation of 1,2-dihyroxynaphthalene, peroxides may play a role in naphthalene-induced ocular damage. Increased levels of ocular lipid peroxides were noted in rats given incremental doses of naphthalene which increased from 100 to 750 mg/kg/day during a 9 week period (Germansky and Jamall 1988). The antioxidants caffeic acid (527 mg/kg) and vitamin E (250 mg/kg), which have free radical protection properties, and the free radical spin trapping agent α-phenyl-N-t-butylnitrone (PBN) (518 mg/kg) diminished the incidence of cataracts in animals given 750 mg/kg naphthalene (Wells et al. 1989). There were no cataracts in the rats given only PBN.

Support for this mechanism of cataract formation was provided by a gavage study in which five rat strains (pigmented and albino) were given 500 mg/kg/day naphthalene for 3 days and 1,000 mg/kg/day for the remainder of the 28-day treatment period (Xu et al. 1992b). After 3 weeks, there was a decrease in reduced glutathione (GSH) in the lens, an increase in protein-glutathione mixed disulfides, and an

increase in high molecular weight insoluble proteins (Xu et al. 1992a, 1992b). The only metabolite detected in the aqueous humor of the lens was 1,2-dihydro-1,2-naphthalenediol. The authors hypothesized that 1,2-dihydro-1,2-naphthalenediol was oxidized to 1,2-naphthalenediol and then to 1,2-naphthoquinone. The 1,2-naphthoquinone is believed to be responsible for the chemical changes in the eyes either through crosslinking reactions or by generating free radicals (Xu et al. 1992a). All of the rats developed cataracts.

The complete mechanism for this sequence of reactions is not clear. In *in vitro* studies of cataract formation, 1,2-dihydro-1,2,naphthalenediol was the only metabolite that resulted in cataracts that were morphologically the same as those generated *in vivo* (Xu et al. 1992a). Although 1,2-naphthalenediol and naphthoquinone also formed cataracts in lens culture studies, the opacities were located in the outer layer of the cortex rather than inside the lens. Also, the permeability of the cultured lens to the metabolites in the media may have contributed to the differences in lesion location.

When the aldose reductase inhibitor, AL01576, was given to rats along with the same naphthalene doses, no cataracts developed (Xu et al. 1992a, 1992b). Aldose reductase is an enzyme found in the lens, liver, and peripheral neurons that reduces aldehyde sugars such as glucose to their corresponding alcohols (McGilvery 1983). It is believed to oxidize 1,2-naphthalenediol to 1,2-naphthoquinone; therefore, when this reaction is inhibited, the quinone hypothetically does not form and there is no eye damage (Xu et al. 1992a). Support for this hypothesis includes observations that aldose reductase inhibitors inhibit cataract formation in naphthalene-exposed 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).

Naphthalene-induced Nonneoplastic and Neoplastic Respiratory Tract Lesions. The mechanisms by which naphthalene affects mouse lung epithelial tissue and mouse and rat nasal epithelial tissue are thought to involve metabolic intermediates that can react with tissue macromolecules: 1,2-naphthalene oxide, 1,2-naphthoquinone, and 1,4-naphthoquinone (Buckpitt et al. 2002). The innate reactivity of 1,2-naphthalene oxide is demonstrated by a half-life of approximately 2–3 minutes in buffer at pH 7.4; the half-life is extended by the presence of albumins to about 11 minutes (Buckpitt et al. 2002; Kanekal et al. 1991). The reactivity of 1,2-naphthoquinone has been demonstrated by its ability to form N7-adducts with deoxyguanosine under acidic conditions (McCoull et al. 1999). A second mode by which 1,2-naphthoquinone may damage tissue macromolecules involves redox cycling of the ortho-quinone

moiety and the subsequent generation of reactive oxygen species, which can lead to lipid peroxidation, consumption of reducing equivalents, oxidation of DNA, or DNA strand breaks (Bolton et al. 2000). 1,2-Naphthoquinone caused hydroxyl radical formation and DNA strand scission in buffered solutions in the presence of NADPH and $CuCl_2$ (Flowers et al. 1997), was directly mutagenic in *S. typhimurium* (Flowers-Geary et al. 1996), and directly induced sister chromatid exchanges in human mononuclear leukocytes (Wilson et al. 1996). The comparative importance of these reactive metabolic intermediates of naphthalene in producing nonneoplastic and neoplastic lesions in lung or nasal epithelial tissue is unknown, although the difference between mice and rats in susceptibility to naphthalene-induced lung damage has been associated with greater rates of naphthalene transformation to epoxides and the formation of a different enantiomeric form of 1,2-naphthalene oxide in mice compared with rats.

A fourth reactive metabolic intermediate, 1,2-dihyroxy-3,4-epoxy-1,2,3,4-tetrahydronaphthalene, has been proposed based on molecular structure characterizations of some urinary metabolites (Horning et al. 1980; Pakenham et al. 2002), but these metabolites represent minor metabolic fates of naphthalene and the potential importance of their proposed precursor in naphthalene toxicity is unstudied to date.

Mice are markedly more susceptible than rats to acute naphthalene-induced Clara cell injury (Buckpitt et al. 1992; West et al. 2001), as well as to lung inflammation and tumor development from chronic inhalation exposure (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).

Although these observations on epoxide formation suggest that naphthalene may be metabolized to epoxide intermediates at faster rates and with different stereoselectivity in the mouse lung than in the human lung, the toxicologic significance of this species difference is uncertain. The uncertainty arises due to the possibility (and potential toxicological importance) of species differences in several steps in downstream metabolism including glutathione conjugation of the epoxide, transformation to the dihydrodiol via epoxide hydrolase, and transformations to 1,2- or 1,4-naphthoquinone. For example,

human liver microsomes have been reported to be more proficient at converting naphthalene to the dihydrodiol metabolite than rat and mouse liver microsomes (Kitteringham et al. 1996). These results suggest that epoxide hydrolase activities may be higher in humans than mice (although they do not necessarily reflect activities in the pertinent naphthalene target tissues) and that this may decrease the potential for epoxide-induced tissue damage in humans relative to mice (see Figure 3-3). However, this difference may cause relatively greater formation of 1,2-naphthoquinone (from the dihydrodiol via dihydrodiol dehydrogenase) in human tissue than in mouse tissue. While the toxicologic significance of such a difference is uncertain, it is possible that humans may be more susceptible than mice, due to the possible involvement of 1,2-naphthoquinone in naphthalene-induced lung injury as suggested by a report that 1,2-naphthoquinone was the predominant naphthalene metabolite covalently bound to proteins obtained from freshly isolated mouse Clara cells incubated for 1 hour with 0.5 mM naphthalene (Zheng et al. 1997). To date, mechanistic understanding of species differences in naphthalene bioactivation in the lung is too incomplete to definitively identify which naphthalene metabolite is responsible for the development of nonneoplastic or neoplastic lung lesions, or to rule out the possible human relevance of naphthalene-induced lung lesions in mice.

Species differences in susceptibility to naphthalene-induced nonneoplastic and neoplastic lesions in the olfactory and respiratory epithelia of the nose have not been correlated with differences in rates of transformation to 1,2-epoxide derivatives in extracts of olfactory tissue (Buckpitt et al. 1992; Plopper et al. 1992a). Rates of epoxide formation showed the order, mouse > rat > hamster, but rats were the most susceptible to acute nasal injury from naphthalene, showing olfactory epithelial necrosis and exfoliation following single intraperitoneal doses as low as 200 mg/kg naphthalene, compared with 400 mg/kg in mice and hamsters (Plopper et al. 1992a). These observations suggest that the reasons for species differences in susceptibility to naphthalene nasal toxicity are complex and do not solely involve differences in the formation of the 1,2-epoxide metabolic intermediates.

Involvement of the naphthoquinone metabolites is possible, but studies comparing species in their ability to form or accumulate reacted derivatives of naphthoquinones (or 1,2-naphthalene oxide) in nasal tissues (i.e., protein adducts) are not available. In blood of rats following gavage administration of single oral doses of naphthalene (100–800 mg/kg), levels of hemoglobin and albumin adducts with 1,2-naphthalene oxide were greater than levels of adducts of 1,2- and 1,4-naphthoquinone (Troester et al. 2002; Waidyanatha et al. 2002). These findings suggest that levels of the epoxide in the rats' blood were greater than levels of the naphthoquinones, but do not provide information on the relative amounts of these reactive metabolites in the target tissue, the nose.

Current information is inadequate to (1) identify which metabolite(s) are responsible for nonneoplastic or neoplastic nasal lesions that develop in rodents following chronic inhalation exposure, (2) explain why nasal tumors develop in rats but not in mice, or (3) rule out the possible human relevance of naphthalene-induced nasal lesions in rats or mice.

Evidence to support a nongenotoxic mode of action in naphthalene carcinogenicity involving sustained cell proliferation following repeated naphthalene-induced tissue damage includes the negative results in the genotoxicity database (see Section 3.3) suggesting that naphthalene and its metabolites (with the likely exception of 1,2-naphthoquinone) are not mutagens, and the findings that naphthalene-induced tumors in mice and rats occur in the same general tissues as those displaying nonneoplastic lesions. Evidence to support a genotoxic mode of action includes the consistently positive results for genotoxic action by 1,2-naphthoquinone and the limited and scattered positive results for genotoxic action by naphthalene in the presence of metabolic activation. Current evidence is not adequate to rule out the possibility of naphthalene genotoxic action or to determine pertinent threshold levels for genotoxic action, due to the absence of studies examining genotoxic end points in naphthalene target tissues, the nose and lung. As suggested by Moore and Harrington-Brock (2000), answering critical questions in human cancer risk assessment involves an understanding of the mode(s) of action of tumor induction in the target tissue(s) at environmentally-relevant concentrations. Such understanding can come from experiments examining genotoxic endpoints in target tissues. These data are not available for naphthalene.

In summary, the available evidence regarding the mechanism(s) by which naphthalene produces neoplastic and nonneoplastic lesions in the respiratory tract of rodents suggests the involvement of reactive metabolites. The identity of this metabolite(s), and evidence of its presence in known target tissues, remains unknown. The finding that mice are more susceptible than rats to naphthalene-induced lung toxicity may correlate with the *in vivo* generation of this reactive intermediate in target tissues. Whether the mechanism by which naphthalene produces neoplastic and nonneoplastic changes in the respiratory tract of rodents involves genotoxicity remains unknown.

1-Methylnaphthalene or 2-Methylnaphthalene-induced Pulmonary Alveolar Proteinosis. Exposure of mice to 1-methylnaphthalene or 2-methylnaphthalene in the diet for 81 weeks induced increased incidences of pulmonary alveolar proteinosis (Murata et al. 1993, 1997). The absence of nonneoplastic lesions in other lung regions or in other tissues indicates that the alveolar region of the lung is a critical and specific toxicity target of chronic oral exposure to 1-methylnaphthalene or 2-methylnaphthalene.

Increased incidences of pulmonary alveolar proteinosis have also been observed in mice exposed to dermal doses of methylnaphthalene (a 2:1 mixture of 2-methylnaphthalene and 1-methylnaphthalene) applied twice weekly for 20–61 weeks (Emi and Konishi 1985; Murata et al. 1992).

There is evidence to suggest that type II pneumocytes are specific cellular targets of the methylnaphthalenes. Pulmonary hyperplasia and hypertrophy of type II pneumocytes in alveolar regions with proteinosis was observed by light microscopy in mice that were repeatedly exposed to dermal doses of methylnaphthalene (119 mg/kg methylnaphthalene twice a week for 30 weeks [Murata et al. 1992]). In this same study, electron microscopic examination showed that alveolar spaces were filled with numerous myelinoid structures that resembled lamellar bodies of type II pneumocytes. This extracellular material was associated with mononucleated giant cells (called balloon cells) containing numerous myelinoid structures, lipid droplets, and electron dense ascicular crystals. The authors hypothesized that, in response to 1-methylnaphthalene or 2-methylnaphthalene, type II pneumocytes produce increased amounts of lamellar bodies due to hyperplasia and hypertrophy, and eventually transform into balloon cells. Balloon cell rupture has been hypothesized to lead to the accumulation of the myelinoid structures in the alveolar lumen. Ultrastructural studies of the pathogenesis of pulmonary alveolar proteinosis from chronic exposure to 2-methylnaphthalene or 1-methylnaphthalene alone were not available. However, the lesions detected by light microscopy following chronic oral exposure to 2-methylnaphthalene or 1-methylnaphthalene alone were very similar to the lesions detected following chronic dermal exposure to the mixture. These similarities suggest that the mechanistic hypotheses prompted by observations for the mixture are relevant to the individual methylnaphthalenes.

The mechanism of targeting type II pneumocytes is consistent with what is generally known regarding the etiology of pulmonary alveolar proteinosis in humans. The disease in humans, characterized by the accumulation of surfactant material in the alveolar lumen, has been hypothesized to be caused by either excessive secretion of surfactant by type II pneumocytes, or disruption of surfactant clearance by macrophages (Lee et al. 1997; Mazzone et al. 2001; Wang et al. 1997). The condition in humans has been associated with pulmonary dysfunction, characterized by decreased functional lung volume, reduced diffusing capacity, and symptoms such as dyspnea and cough. Pulmonary alveolar proteinosis has not been associated with airflow obstruction (EPA 2003; Lee et al. 1997; Mazzone et al. 2001; Wang et al. 1997).

The development of pulmonary alveolar proteinosis in mice appears to require prolonged oral exposure to 2-methylnaphthalene (or 1-methylnaphthalene). Exposure to a dietary concentration of 0.075% 2-methyl-

naphthalene for 81 weeks induced increased incidences of the lesion, but 13-week exposure to concentrations as high as 1.33% 2-methylnaphthalene did not (Murata et al. 1997). No further studies of the temporal development of methylnaphthalene-induced pulmonary alveolar proteinosis are available.

It is unknown whether the parent compounds or metabolites are responsible for the development of methylnaphthalene-induced pulmonary alveolar proteinosis. Type II pneumocytes are enriched in CYP monooxygenases (Castranova et al. 1988), which are involved in metabolizing 2-methylnaphthalene, and it is possible that metabolites may play a role in the pathogenesis of pulmonary alveolar proteinosis. Studies designed to test this hypothesis, however, have not been conducted.

In contrast to chronic oral exposure, which targets alveolar type II pneumocytes, acute intraperitoneal injection of 2-methylnaphthalene into mice targets bronchiolar Clara cells, inducing Clara cell abnormalities, focal or complete sloughing of Clara cells, or complete sloughing of the entire bronchiolar lining (Buckpitt et al. 1986; Griffin et al. 1981, 1982, 1983; Honda et al. 1990; Rasmussen et al. 1986). Mechanistic studies have not provided clear evidence that metabolites are involved in this response to acute exposure to 2-methylnaphthalene. For example, pretreatment of male C57BL/6J mice with phenobarbital (an inducer of CYP2B; 75 mg/kg, 4 days prior) or 3-methylcholanthrene (an inducer of CYP1A; 80 mg/kg, 2 days prior) prior to injection with 400 mg/kg 2-methylnaphthalene reduced the severity of bronchiolar necrosis in all mice compared to those injected without pretreatment (Griffin et al. 1982). However, CYP inhibitors, such as piperonyl butoxide (a mixed monooxygenase inhibitor; 1,000 mg/kg, 30 minutes prior) and SKF 525-A (an inhibitor of CYP1B; 25 mg/kg, 30 minutes prior), had no effect on the severity of the lung lesions. The mechanism of acute Clara cell toxicity of 2-methyl-naphthalene may be similar to that of naphthalene, which involves CYP-mediated metabolism via ring epoxidation to reactive species such as the 1,2-naphthalene oxide and 1,2-naphthoquinone (Cho et al. 1995; Greene et al. 2000; Lakritz et al. 1996; Van Winkle et al. 1999). This hypothesis is supported by the finding that 2-methylnaphthalene is less acutely toxic than naphthalene (Buckpitt and Franklin 1989; Cho et al. 1995) and that only a small fraction of 2-methylnaphthalene (15-20%) undergoes metabolic ring epoxidation (Breger et al. 1983; Melancon et al. 1985). Information on the mechanism of the acute response of Clara cells is not expected to be directly related to the pathogenesis of pulmonary alveolar proteinosis from chronic oral or dermal exposure to 2-methylnaphthalene, because in mice chronically exposed to 2-methylnaphthalene or 1-methylnaphthalene for 81 weeks, no evidence for exposure-related bronchiolar Clara cell lesions was found (Murata et al. 1993, 1997). This finding is not surprising, as Clara cells have been shown to develop resistance to the acute toxicity of naphthalene (Lakritz et al.

1996). The possible development of Clara cell resistance to the acute toxicity of 2-methylnaphthalene, however, has not been studied.

Data are limited to support the hypothesis that rats are less sensitive than mice to the lung damage caused by acute exposure to 2-methylnaphthalene. Wistar rats given intraperitoneal doses of 142 mg/kg 2-methylnaphthalene did not develop lung lesions (Dinsdale and Verschoyle 1987). However, bronchiolar necrosis was induced in Swiss-Webster mice injected with the same dose (Rasmussen et al. 1986) and in C57BL/6J and DBA/2J mice injected with 100 mg/kg 2-methylnaphthalene (Griffin et al. 1981, 1982, 1983). No data are available for interspecies comparisons of the chronic toxicity of 1-methynaphthalene or 2-methylnaphthalene.

3.5.3 Animal-to-Human Extrapolations

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. As discussed in Section 3.5.2, studies with microsomes from human and animal cells indicate that there are species differences in specific steps of naphthalene metabolism (Buckpitt et al. 1992; Kitteringham et al. 1996; Lanza et al. 1999), but mechanistic understanding of these differences is too incomplete to effectively argue that they rule out the possible human relevance of naphthalene-induced lung lesions in mice or nasal lesions in rats or mice. Rodents and humans also display distinct differences in nasal anatomy and respiratory physiology that may cause different deposited doses, and subsequently different responses, in human nasal tissue relative to rats or mice. However, the anatomical and physiological differences alone are insufficient to rule out the possible human relevance of naphthalene-induced nasal lesions in rats or mice. For example, rat and human hybrid computational fluid dynamics and PBPK models, developed for acrylic acid, another rodent nasal toxicant, predicted that tissue concentrations of acrylic acid in human and rat nasal tissues would be similar when exposure conditions were the same (Frederick et al. 2001). Current PBPK models for naphthalene do not include nasal compartments that metabolize naphthalene, because no data were available on nasal deposition and epithelial absorption of naphthalene (Willems et al. 2001). In the absence of this type of data or a pertinent validated human PBPK model, it is reasonable to assume that naphthalene-induced nonneoplastic and neoplastic lesions observed in nasal tissues of rats and mice are relevant to humans. Development of rat, mouse, and human hybrid computational fluid dynamics and PBPK models that include metabolizing nasal compartments and the application of the models to extrapolating rat or mouse

nasal doses to humans will likely decrease uncertainty in extrapolating naphthalene health hazards from rodents to humans.

In animals orally exposed to naphthalene, the critical effects appear to be decreased weight gain and clinical signs of toxicity in pregnant rats with acute exposure and decreased body weight in rats with intermediate-duration exposure. Mechanisms associated with these effects are unstudied. Reliable data to preclude the relevance of these effects to humans were not located.

Pulmonary alveolar proteinosis induced in mice following chronic oral exposure to 1-methylnaphthalene 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-methylnaphthalene or 2-methylnaphthalene were not located.

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Colborn and Clement (1992), was also used in 1996 when Congress mandated the EPA to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), and in 1998, the EDSTAC completed its deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and

descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

No studies were located regarding endocrine disruption in human or animals after exposure to naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene.

No *in vitro* studies were located regarding endocrine disruption of naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene.

3.7 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. 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. Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6, Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage

may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

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 1987a). 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 1991a; Plasterer et al. 1985; PRI 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.

Children with genetically determined glucose-6-phosphate dehydrogenase (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 1987a).

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.

An association between elevated maternal exposure to naphthalene and increased maternal cord-blood levels of one of four T cell types, IL-4, has recently been reported (Lehmann et al. 2002). The study looked for possible associations between maternal indoor exposure to 28 volatile organic chemicals (including naphthalene) and putative immune status at birth assessed by cord-blood levels of cytokine-producing T cells [interleukin-4 (IL-4), interleukin-2 (IL-2), interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α)]. Levels of 28 volatile organic chemicals in air samples, collected during a 4-week postnatal period in bedrooms of 85 newborn children, were measured as surrogate indices of maternal

indoor exposure. A logistic regression analysis found an elevated odds ratio (OR=2.9; 95% CI 1.0–8.2) for elevated naphthalene air concentrations (>75th percentile) and elevated percentage of IL-4-producing T cells in cord blood. The analysis adjusted for possible confounding factors of family allergic (i.e., atopic) history and maternal smoking during pregnancy. Several other statistically significant associations were found for changes in levels of different types of T cells and air levels of other chemicals, including methylcyclopentane, trichloroethylene, and tetrachloroethylene. The significance of the observed variations in cord blood T cell levels to the immune status of the newborn children is unknown. The findings from this study are inadequate to determine if maternal exposure to naphthalene may influence the immune status of newborn children.

Studies that have examined age-related effects of toxicokinetic variables specifically related to naphthalene are restricted to a study with results indicating that neonatal mice may be more susceptible than adult mice to lung injury from single intraperitoneal doses of 25, 50, or 100 mg/kg naphthalene (Fanucchi et al. 1997). Epithelial damage in terminal bronchioles (principally in the Clara 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 Clara 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 Clara cell proliferation and tumor formation in adult animals, but 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).

No direct information was located on the relative susceptibility of children or young animals to 1-methylnaphthalene 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 poor prognosis for survival than do adults (EPA 2003r; Mazzone et al. 2001).

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. 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 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene are discussed in Section 3.8.1.

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 1989). 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 effects caused by naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene are discussed in Section 3.8.2.

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 3.10, Populations That Are Unusually Susceptible.

Additional information concerning biomarkers for effects on the immune, renal, and hepatic systems can be found in the CDC/ATSDR Subcommittee Report on Biological Indicators of Organ Damage (CDC/ATSDR 1990), and on the neurological system in the Office of Technology Assessment Report on Identifying and Controlling Poisons of the Nervous System (OTA 1990). Additional details concerning the health effects caused by naphthalene can be found in Section 3.2.

3.8.1 Biomarkers Used to Identify or Quantify Exposure to Naphthalene, 1-Methylnaphthalene, and 2-Methylnaphthalene

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 less than 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 1986g; 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.

An analytical method is available to determine levels of 2-methylnaphthalene and its derivatives in rat urine (Melancon et al. 1982). This method would probably also be useful in measuring 2-methyl-naphthalene 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.

3.8.2 Biomarkers Used to Characterize Effects Caused by Naphthalene, 1-Methylnaphthalene, and 2-Methylnaphthalene

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.

Clara 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.

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

3.9 INTERACTIONS WITH OTHER CHEMICALS

When either 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 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, 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 mixed function oxidase activity and glutathione conjugation in naphthalene-induced pulmonary and ocular lesions.

Mixed function oxidase inducers also affect the metabolism of 2-methylnaphthalene. Inducers that influence cytochrome P-450 increase the oxidation of the side chain and the concentration of one dihydrodiol. Induction of cytochrome P-450 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 Clara cells than acute exposure to naphthalene.

In general, interactions with environmental contaminants, such as polycyclic aromatic hydrocarbons, should be expected at hazardous waste sites. Most hazardous waste sites (with the notable exception of certain pharmaceutical sites) would not be expected to contain substantial volumes of certain types of contaminants, such as antioxidants or cytochrome P-450 inhibitors.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene than will most persons exposed to the same level of naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene, or compromised function of organs affected by naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene. Populations who are at greater risk due to their unusually high exposure to naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene are discussed in Section 6.7, Populations with Potentially High Exposures.

The hemolytic response to naphthalene is enhanced by the presence of inherited erythrocyte 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.

Results from a recent study indicate that female mice are more susceptible than male mice to lung injury from acute parenteral exposure to naphthalene (Van Winkle et al. 2002). Male and female Swiss-Webster mice were given intraperitoneal injections of 0 or 200 mg/kg naphthalene in corn oil, and lungs were removed at 1, 2, 3, 6, and 24 hours after treatment. Acute lung injury was determined by (1) high-resolution microscopic assessment of differential permeability to fluorescent nuclear dyes in cells along the long axis of conducting airway trees of microdissected right middle lung lobes and (2) high-resolution histopathology of sections of Karnovsky-fixed left lung lobes. Clara cell injury occurred in the terminal bronchioles of both male and female mice. Clara cell injury in terminal bronchioles, however, occurred earlier, affected cells farther up the airway tree, and showed a different temporal pattern of changes in female mice compared with male mice. Twenty-four hours after injection, Clara cell injury in the lobar bronchus of female mice was evidenced by numerous vacuolated cells, whereas normal bronchiolar epithelium containing Clara 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 microdissected regions of airways from male and female mice by high performance liquid chromatography (HPLC) analysis 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. This metabolic difference may be related to the apparent gender difference in susceptibility to acute lung injury from naphthalene. It is unknown whether or not the gender difference in susceptibility to acute lung injury is relevant to nasal or lung lesions formed with chronic-duration exposure to naphthalene.

There are no data that indicate whether there are populations that are unusually susceptible to the toxic effects of 1-methylnaphthalene or 2-methylnaphthalene.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following texts provide specific information about treatment following exposures to naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene:

Kurz JM. 1987. Naphthalene poisoning: Critical care nursing techniques. Dimens Crit Care Nurs 6:264-270.

Melzer-Lange M, Walsh-Kelly C. 1989. Naphthalene-induced hemolysis in a black female toddler deficient in glucose-6-phosphate dehydrogenase. Pediatr Emerg Care 5:24-26.

Siegel E, Wason S. 1986. Mothball toxicity. Pediatr Clin North Am 33:369-374.

Stutz DR, Janusz SJ. 1988. Hazardous materials injuries: A handbook for pre-hospital care. Second edition. Beltsville, MD: Bradford Communications Corporation.

3.11.1 Reducing Peak Absorption Following Exposure

If inhalation of naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene has occurred, movement to fresh air is recommended. In cases where a small amount (e.g., one mothball, 0.5–3.6 g) of naphthalene has been ingested, measures are implemented to empty the stomach contents. Syrup of Ipecac, which may be used for this purpose, is administered after ingestion to induce vomiting and is most effective if initiated within a 2-hour period after exposure (Siegel and Wason 1986). If large quantities of naphthalene have been ingested, syrup-of-ipecac-induced vomiting is usually followed by gastric aspiration using a large gauge lavaculator (to remove mothballs) (Kurz 1987). This will only be of value if the naphthalene particles are small enough to be aspirated. Measures are usually taken to protect the respiratory tract from aspiration of gastric contents. Activated charcoal can be given to bind dissolved naphthalene in the gastrointestinal tract. Further treatment with a cathartic (e.g., magnesium sulfate) to speed fecal excretion is recommended (Melzer-Lange and Walsh-Kelly 1989). Milk or fatty meals ingested within 2–3 hours after exposure may increase absorption (Siegel and Wason 1986).

In order to reduce absorption of naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene through the skin, areas of skin that have come in contact with the compound should be washed with soap and water. Application of oil based lotions should be avoided. If these compounds are splashed into the eyes, irrigation with large amounts of water for 15–30 minutes may be useful to wash away unabsorbed material (Stutz and Janusz 1988).

3.11.2 Reducing Body Burden

Some evidence exists that naphthalene metabolites may be retained in the body in adipose tissue (EPA 1986g). Naphthalene was identified in 40% of the samples evaluated for the Human Adipose Tissue Survey (EPA 1986g). Naphthalene metabolites were detected in urine up to 13 days following exposure (Mackell et al. 1951).

The most frequently documented acute toxic effect of naphthalene in humans is red cell hemolysis. In cases of clinically significant hemolysis, accelerated urinary excretion of naphthol metabolites is recommended to protect the kidney from products of hemolysis (EPA 1989d). In cases of renal failure, hemodialysis may be effective in controlling extracellular fluid (plasma) composition (EPA 1989d). It should be noted that this method is not very effective in removing lipophilic compounds from blood. Ocular effects have also been reported in humans; however, there are no specific treatments for reducing the toxic effects on the eyes. Respiratory effects have been observed in animals but these effects have not been reported in humans. Due to lack of data, it is difficult to speculate regarding the benefits of treatments that enhance elimination of naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene and their metabolites as a basis for reducing toxic effects.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

Existing data indicate that lung, nose, and eye toxicity may be mediated by reactive metabolites for naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene, although the evidence for the involvement of reactive metabolites is greater than the evidence for methylnaphthalenes. More information is needed on the bioactivation of naphthalene and transport mechanisms before methods for blocking those mechanisms can be developed.

Many of the symptoms of acute naphthalene poisoning in humans are a direct consequence of red blood cell hemolysis. Blood transfusions, packed red blood cell transfusions, and exchange transfusions (particularly in infants) can be used to replenish the concentration of red blood cells and diminish the risks of cellular anoxia (Bregman 1954; Chusid and Fried 1955; MacGregor 1954; Mackell et al. 1951). Bicarbonate is also administered to hemolysis patients to increase the alkalinity of the urine and thereby minimize deposition of hemoglobin in the kidney tubules (Chusid and Fried 1955; Gidron and Leurer 1956).

3.12 ADEQUACY OF THE DATABASE

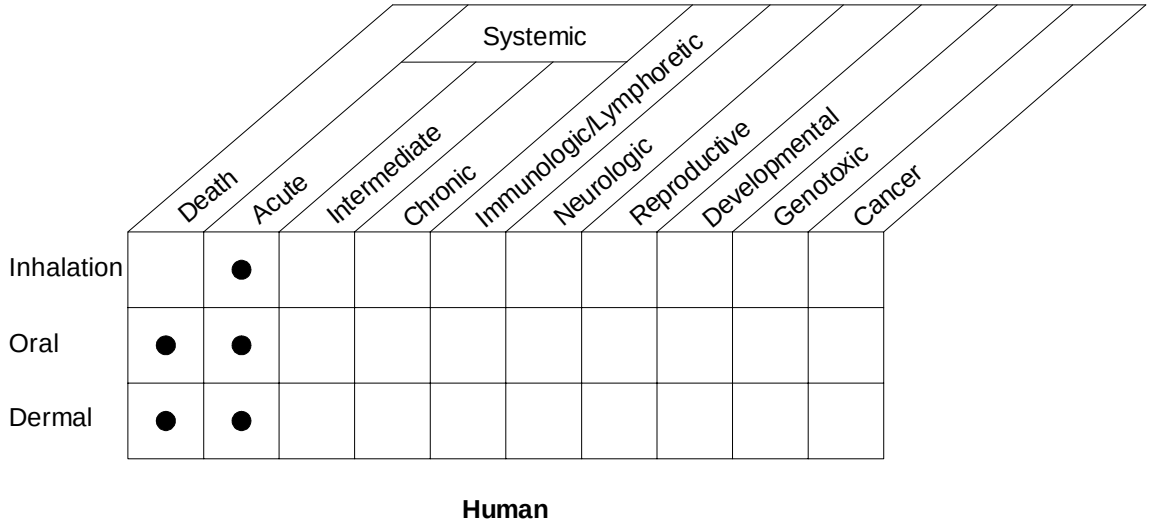
Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of naphthalene, 1-methylnaphthalene, and 2-methyl-naphthalene is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

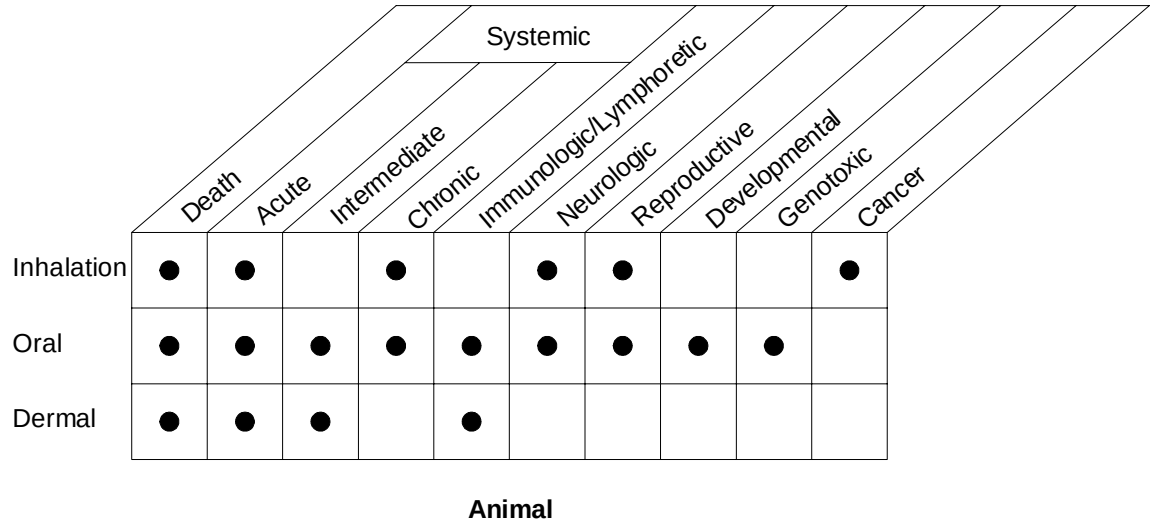
3.12.1 Existing Information on Health Effects of Naphthalene, 1-Methylnaphthalene, and 2-Methylnaphthalene

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene are summarized in Figures 3-6, 3-7, and 3-8, respectively. The purpose of this figure is to illustrate the existing information concerning the health effects of naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in

Figure 3-6. Existing Information on Health Effects of Naphthalene



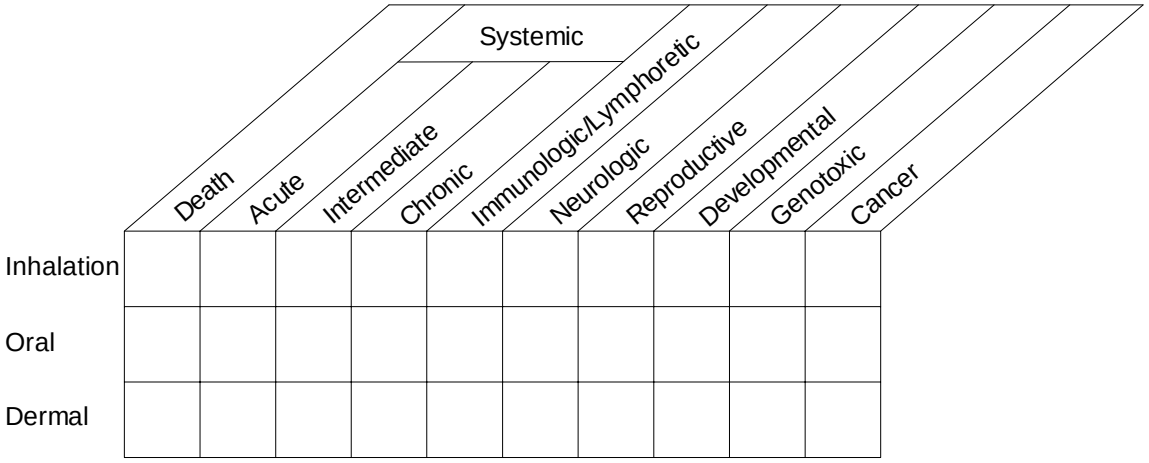
Human



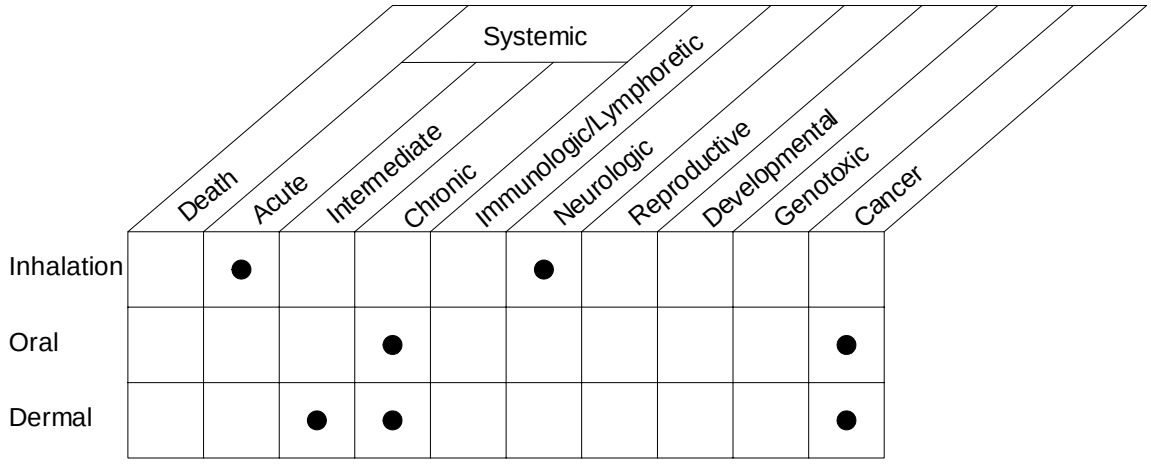
Animal

● Existing Studies

Figure 3-7. Existing Information on Health Effects of 1-Methylnaphthalene



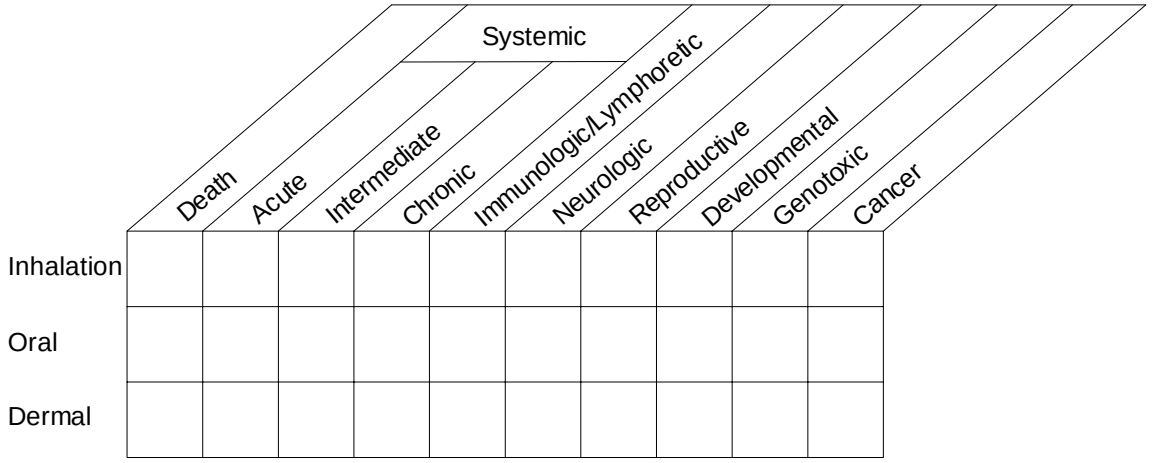
Human



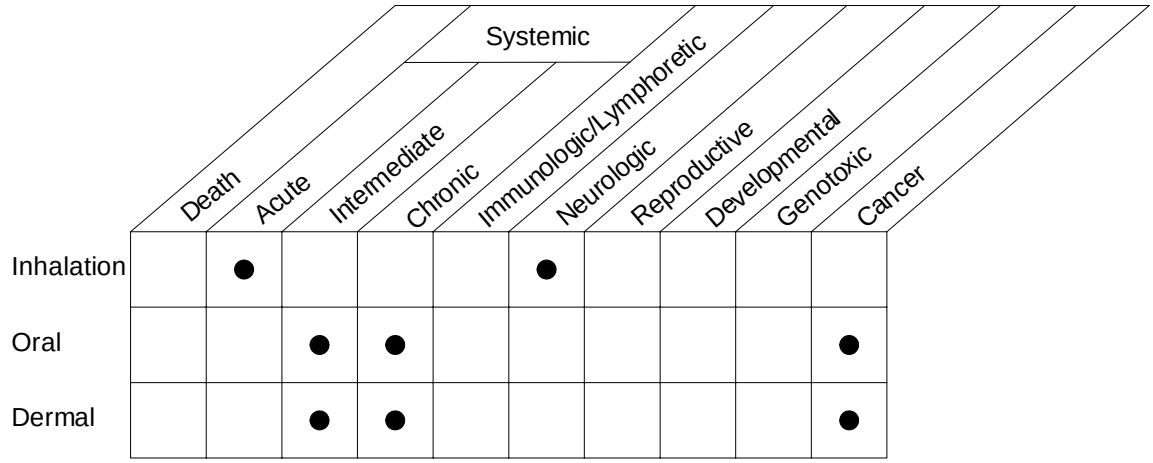
Animal

● Existing Studies

Figure 3-8. Existing Information on Health Effects of 2-Methylnaphthalene



Human



Animal

● Existing Studies

this figure be interpreted as a "data need". A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Figure 3-6 shows that the database on naphthalene toxicity in humans is not extensive. There are case reports and case series of deaths, acute hemolytic anemia, and ocular effects in humans, but these reports lack quantitative information on exposure levels. Epidemiologic studies designed to examine possible associations between intermediate- or chronic-duration human exposure to naphthalene by any route of exposure and neoplastic or nonneoplastic health effects are not available. Animal data on naphthalene exist in several areas. Oral toxicity data are adequate for deriving acute- and intermediate-duration oral MRLs, but adequate chronic-duration oral toxicity studies in animals are not available. Available toxicology and carcinogenesis studies of chronic inhalation exposure to naphthalene in rats and mice are adequate for deriving a chronic-duration inhalation MRL for naphthalene and assessing the potential carcinogenicity of naphthalene, but available acute- and intermediate-duration inhalation toxicity studies are not adequate for deriving MRLs.

Figures 3-7 and 3-8 show that no information was located on the health effects of 1-methylnaphthalene or 2-methylnaphthalene in humans via inhalation, oral, or dermal exposure. These figures also reflect that data in animals are limited to cancer and toxicity studies of intermediate- and chronic-duration oral exposure of mice to 1-methylnaphthalene or 2-methylnaphthalene, a single poorly reported acute inhalation exposure study of hematologic end points in dogs exposed by inhalation to 1-methylnaphthalene or 2-methylnaphthalene, a study that reported decreased pain sensitivity, but no effects on the ability to balance on a rotating rod, in rats exposed for 4 hours by inhalation to 1-methylnaphthalene or 2-methylnaphthalene, and cancer and toxicity studies of intermediate- and chronic-duration dermal exposure of mice to a mixture of 1-methylnaphthalene and 2-methylnaphthalene.

3.12.2 Identification of Data Needs

Acute-Duration Exposure. A number of reports of human exposure to acute inhalation, oral, or dermal doses of naphthalene have established the erythrocyte as a toxicity target (Dawson et al. 1958; Haggerty 1956; Kurz 1987; Linick 1983; MacGregor 1954; Mackell et al. 1951; Melzer-Lange and

Walsh-Kelly 1989; Ojwang et al. 1985; Schafer 1951; Shannon and Buchanan 1982; Valaes et al. 1963). However, the data from these reports were not useful in predicting toxic or lethal dose levels by any of these routes because the exposure levels were not defined.

The acute oral toxicity of naphthalene has been studied in animals but there are limited data for acute inhalation and dermal exposures.

The most frequently reported adverse effects associated with acute oral exposure are ocular lesions (primarily cataracts). These have been observed in rabbits (Srivastava and Nath 1969; Van Heyningen and Pirie 1967) and rats (Kojima 1992; Murano et al. 1993; Rathburn et al. 1990; Tao et al. 1991; Yamauchi et al. 1986) and occur following exposure to high (>500 mg/kg) doses. Acute oral exposure of pregnant rats to naphthalene doses of 150 or 450 mg/kg/day (but not 50 mg/kg/day) during gestation produced maternal toxicity including clinical signs (lethargy and prone position) and marked decreases in body weight gain (NTP 1991a), but clear effects on the developing fetus have not been found at maternal oral doses as high as 450 mg/kg/day in rats (NTP 1991a), 300 mg/kg/day in mice (Plasterer et al. 1985), or 120 (NTP 1992b) or 400 mg/kg/day (PRI 1985i,1986) in rabbits. Slightly reduced numbers of mouse pups per litter were observed when naphthalene in corn oil was orally administered to pregnant mice (Plasterer et al. 1985); however, no effects were seen when pregnant rabbits were orally administered naphthalene at even higher doses but delivered in methylcellulose rather than in an oil vehicle (PRI 1986). It is unclear if these differences are due to species differences in sensitivity or to possible differences in the effects of the two vehicles on naphthalene absorption. Effects on liver (Rao and Pandya 1981) and lung (Shopp et al. 1984) weights have been reported, but no treatment-related histopathological lesions were observed in these acute oral exposure studies. Lethal doses have been identified in mice (Plasterer et al. 1985; Shopp et al. 1984) and rats (Gaines 1969).

The finding of transient clinical signs of toxicity in orally-exposed pregnant rats (NTP 1991a) serves as the basis of the acute-duration oral MRL for naphthalene. The MRL was calculated from a minimal LOAEL of 50 mg/kg/day using an uncertainty factor of 90 (3 for the use of a minimal LOAEL, 10 for extrapolation from animals to humans, and 3 for human variability). An uncertainty factor of 3 was used for human variability because the critical effect is based on effects in a sensitive animal subpopulation. Dermal or inhalation developmental toxicity studies in animals are not available. Pregnant rats appear to be more sensitive for the effects observed (clinical signs in response to gavage exposure and decreased body weight gain) than nonpregnant rats. In 13-week gavage studies with nonpregnant rats (NTP 1980b), similar persistent clinical signs were not observed following administration of doses as high as

200 mg/kg/day, but were observed at 400 mg/kg/day. In nonpregnant rats exposed for 13 weeks, significant body weight decreases occurred at 200 mg/kg/day throughout exposure, but not at 100 mg/kg/day (NTP 1980b) or in nonpregnant mice exposed for 13 weeks to 133 mg/kg/day (Shopp et al. 1984) or 200 mg/kg/day (NTP 1980a). Mice in the NTP (1980a) study showed transient signs of toxicity (lethargy, rough hair coats, and decreased food consumption), but these only occurred between weeks 3 and 5 in the 200-mg/kg/day group.

Data are inadequate for deriving an acute-duration inhalation MRL for naphthalene. Data are restricted to a 14-day (6 hours/day, 5 days/week) range-finding study in B6C3F1 mice (NTP 1992a), which only examined hematologic end points and did not histologically examine expected critical toxicity targets (lung and nasal cavity epithelial tissue) (NTP 1992a), and a study (West et al. 2001) with Swiss Webster mice and Sprague-Dawley rats, which involved single 4-hour exposure periods. The more recent study, however, only histologically examined the lung and did not examine nasal tissue. A comprehensive inhalation study involving an acute repeated exposure scenario and examining the other critical target (the nose, based on the findings from chronic mouse and rat bioassays) is not currently available. Results from such a study may be useful for deriving an acute-duration inhalation MRL for naphthalene.

Hemolysis is the best documented effect of acute naphthalene exposures in humans, but it has not been observed in studied strains of rats (F344) or mice (CD-1, B6C3F1). Dose-response data for hemolysis from a susceptible animal species (such as dogs or the Jackson Laboratory hemolytic anemia mouse) may be useful to obtain data that could be used for considering changes to the acute-duration oral MRL. Data from both inhalation and oral exposure protocols would be useful.

No acute-duration studies are available on 1-methylnaphthalene or 2-methylnaphthalene exposure in humans using the inhalation, oral, or dermal routes. Two acute inhalation studies in animals were identified. The first study reported that 1-methylnaphthalene (pure) administered in a kerosene aerosol was associated with increased reticulocyte and lymphocyte counts in splenectomized dogs and practical grade 1-methylnaphthalene was associated with increased leucocyte and neutrophil counts (Lorber 1972). Neither grade of 1-methylnaphthalene had any effect on hematocrit values. None of these parameters were affected when 2-methylnaphthalene aerosols were used. The physiological significance of these findings is not apparent and the exposure levels in the study were not clearly specified. As such, the data are not suitable for use in deriving an MRL for 1-methylnaphthalene or 2-methylnaphthalene. The second study measured decreased sensitivity to pain in rats exposed by inhalation for 4 hours to 1-methylnaphthalene (44 ppm) or 2-methylnaphthalene (61 ppm), but found no effects on the ability to balance on

a rotating rod at exposure levels as high as 70 ppm 1-methylnaphthalene or 90 ppm 2-methylnaphthalene (Korsak et al. 1998). The biological significance of these findings is uncertain, and, in the absence of corroborative evidence of acute neurotoxicity, the findings are not suitable for deriving acute inhalation MRLs for 1-methylnaphthalene or 2-methylnaphthalene.

Parenteral studies in animals revealed that a single intraperitoneal injection of 2-methylnaphthalene (1,000 mg/kg) was lethal in mice (Griffin et al. 1981). When a glutathione-depleting agent (diethyl maleate) was administered prior to administration of 2-methylnaphthalene, a lower dose of 2-methylnaphthalene (400 mg/kg) was also lethal. A single intraperitoneal injection of 1-methylnaphthalene (426 mg/kg) was not lethal in mice (Griffin et al. 1982). Systemic effects have been reported and were limited to effects on the respiratory system (Rasmussen et al. 1986). Exfoliation of the bronchiolar epithelium in mice was reported following a single intraperitoneal injection of 2-methylnaphthalene (Buckpitt et al. 1986; Griffin et al. 1981, 1983). A single intraperitoneal injection of 2-methylnaphthalene (1,000 mg/kg) did not cause liver or kidney lesions (Griffin et al. 1981, 1983).

Because populations living near hazardous waste sites might be exposed to 1-methylnaphthalene or 2-methylnaphthalene for short periods, comprehensive toxicity studies of acute exposure in animals by the inhalation and oral routes to determine potential target tissues and dose-related effects would be useful in assessing possible health hazards to humans. The studies would be most useful if they included a battery of neurological end points and comprehensive histological examination of nasal and lung tissue.

Intermediate-Duration Exposure. Quantitative data were not provided in any intermediate-duration inhalation case studies of human naphthalene exposure and, in one case, there was simultaneous exposure to paradichlorobenzene (Harden and Baetjer 1978; Linick 1983).

The results from three intermediate-duration oral toxicity studies in animals (two in mice and one in rats) identified body weight changes as the most sensitive biologically significant effect on which to base the intermediate-duration oral MRL for naphthalene. Comprehensive intermediate-duration oral toxicity studies found no evidence for naphthalene-induced lesions in any tissue or organs in male or female Fischer 344 rats exposed to doses up to 400 mg/kg/day (NTP 1980b) or in male or female B6C3F1 mice exposed to doses up to 200 mg/kg/day (NTP 1980a). The only biologically significant effect found in these studies was decreased body weight (>10% decreased compared with control values) in rats at doses of 200 and 400 mg/kg/day. The other intermediate-duration oral study (with CD-1 mice) focused on a battery of immunologic tests, but did not include comprehensive histopathologic examination of tissues

(Shopp et al. 1984). No biologically significant effects were found except for decreases in weights of several organs (brain, liver, and spleen) in mice exposed to 133 mg/kg/day, but not to 53 or 5.3 mg/kg/day. The lack of naphthalene-induced lesions in these organs in the NTP (1980a, 1980b) studies suggests that the brain, liver, and spleen are not sensitive targets of naphthalene following intermediate-duration oral exposure. Statistically significant changes were reported in several hematological parameters, hepatic enzyme activities, and serum chemical parameters (Shopp et al. 1984), but these changes are not considered to be biologically significant or adverse. The acute-duration oral MRL was adopted as the intermediate-duration oral MRL for naphthalene, because a potential intermediate-duration oral MRL (see Section 2.3 and Appendix A) based on the NOAEL for decreased body weight changes in rats exposed by gavage 5 days/week for 13 weeks (NTP 1980b) was slightly larger than the acute MRL value.

No data were suitable for the development on an intermediate-duration inhalation MRL for naphthalene.

Intermediate-duration dermal toxicity data are restricted to an report that dermal exposure of male and female Sprague-Dawley rats (occluded exposure 6 hours/day, 5 days/week) to technical-grade naphthalene at doses up to 1,000 mg/kg/day for 13 weeks did not affect comprehensive ophthalmologic, hematologic, serum chemistry, or urinalysis parameters (Frantz et al. 1986). In addition, exposure did not produce increased incidences of histological lesions in 34 tissues that were examined (however, the nasal cavity was not included). The only exposure-related effect found was an increased incidence of excoriated skin and papules at the site of exposure at the highest dose level (1,000 mg/kg/day).

Intermediate-duration studies on 1-methylnaphthalene or 2-methylnaphthalene exposure in humans or animals using the inhalation, oral, or dermal routes are restricted to a study that found no pulmonary alveolar proteinosis in male or female mice exposed to diets containing up to 1.33% 2-methylnaphthalene for 13 weeks (Murata et al. 1997). The reporting of the experimental protocol and results from this study, however, is too limited to reliably use the results as a basis for an intermediate-duration oral MRL for 2-methylnaphthalene. New intermediate-duration toxicity studies using the inhalation route of exposure may be the most useful to better assess the health hazard of intermediate-duration exposure to naphthalene, based on the findings that the alveolar region of the lung is the most sensitive tissue in mice chronically exposed to 1-methylnaphthalene or 2-methylnaphthalene in the diet (Murata et al. 1993, 1997).

Chronic-Duration Exposure and Cancer. There is one report of cataracts occurring in humans following chronic-duration inhalation exposure to naphthalene (Ghetti and Mariani 1956) but no information on effects from exposures by the oral or dermal routes. The only studies of cancer in humans exposed to naphthalene are two case series reports of cancer; one report of four laryngeal cancer cases (all of whom were smokers) among workers in a naphthalene purification plant in East Germany (Wolf 1976, 1978), and another report of 23 cases of colorectal carcinoma admitted to a hospital in Nigeria (Ajao et al. 1988). NTP (2002b), EPA (2002b), and IARC (2002) concurred that these studies provide inadequate evidence of naphthalene carcinogenicity in humans. No cohort mortality or morbidity studies or case-control studies examining possible associations between naphthalene exposure and increased risk of cancer (or other health effects) are available.

There are two comprehensive chronic-duration inhalation toxicology and carcinogenicity studies of naphthalene in animals, one in rats (Abdo et al. 2001; NTP 2000) and one in mice (NTP 1992a). These studies identify respiratory tissues as the most sensitive toxicity targets of chronic-duration exposure to inhaled naphthalene in animals: nonneoplastic and neoplastic lesions in the nose of rats, nonneoplastic lesions in the nose of mice, and nonneoplastic and neoplastic lesions in the lungs of mice. Exposure-related lesions in other tissues were not found in these studies. NTP (2002b) and IARC (2002) concurred that these studies provide sufficient evidence of naphthalene carcinogenicity in animals. The chronic-duration inhalation MRL for naphthalene is based on the LOAEL of 10 ppm for nonneoplastic lesions in the olfactory epithelium and respiratory epithelium of the nose of rats.

No appropriate studies were located for deriving an MRL for chronic-duration oral exposure to naphthalene. One chronic study was located that examined the toxicity of naphthalene in rats (Schmahl 1955). No treatment-related effects were reported at a dose level of 41 mg/kg/day for 700 days. The study was not suitable as the basis for deriving a chronic MRL or for assessing carcinogenicity because only one dose level was evaluated (apparently below the maximum tolerated dose), histopathological examination was limited, and dosing was not precisely controlled.

New chronic oral or dermal toxicity studies would be useful to better determine the possible carcinogenicity and noncancer toxicity of naphthalene via these routes of exposure.

Epidemiology studies, case reports, or controlled-exposure studies examining the potential health effects of human chronic exposure to 1-methylnaphthalene or 2-methylnaphthalene by any route of exposure are not available.

No chronic-duration studies are available on 1-methylnaphthalene or 2-methylnaphthalene exposure in animals using the inhalation routes.

A chronic-duration study of 1-methylnaphthalene in the diet that identified a LOAEL of 71.6 mg/kg/day for the occurrence of pulmonary alveolar proteinosis in mice (Murata et al. 1993) was used as the basis of the oral MRL for 1-methylnaphthalene. A chronic-duration oral study of 2-methylnaphthalene in the diet (Murata et al. 1997) that identified a LOAEL of 50.3 mg/kg/day for pulmonary alveolar proteinosis in mice was the basis of the chronic oral MRL for 2-methylnaphthalene. Support for pulmonary alveolar proteinosis as the critical effect for the chronic oral MRLs for 1-methylnaphthalene and 2-methyl-naphthalene comes from dermal chronic-duration studies with methylnaphthalene (a mixture of 1- and 2-methylnaphthalene), which reported increased incidences of this lesion in mice dermally exposed to 30 or 119 mg/kg of methylnaphthalene for 30–61 weeks (Emi and Konishi 1985; Murata et al. 1992). Increased incidences of lung adenomas were found in several exposed groups in the oral chronic-duration studies, but the evidence for carcinogenicity is considered to be limited. The tumorigenic response was predominantly benign and was only consistently seen in male mice exposed to 1-methylnaphthalene. The available data on the methylnaphthalenes appear inadequate to determine the potential carcinogenicity in humans.

A new chronic-duration oral study in rats or another animal species may help to better assess the potential carcinogenicity and noncancer toxicity of the methylnaphthalenes. Because the lung is the most sensitive toxicity target of the methylnaphthalenes in mice exposed orally or dermally, it is plausible that chronic inhalation exposure may also target the lung. The availability of repeated-exposure inhalation carcinogenicity and toxicity studies would help to better determine this possibility.

Genotoxicity. As discussed in Section 3.3, results in bacterial mutation assays were predominantly negative (see Table 3-4 for citations) with the exceptions that the metabolite, 1,2-naphthoquinone, was mutagenic in *S. typhimurium* without metabolic activation (Flowers-Geary 1996), and naphthalene was mutagenic in *V. fischeri* with metabolic activation (Arfsten et al. 1994).

Results from a limited number of *in vitro* eukaryotic genotoxicity assays are mixed. Negative results were obtained for mutations and sister chromatid exchanges in cultured human cells exposed to naphthalene, for DNA single strand breaks and unscheduled DNA synthesis in rat hepatocytes, and for cell transformation in several types of mammalian cells (see Table 3-3 for citations). Positive results

included increased chromosomal aberrations in Chinese hamster ovary cells and preimplantation whole mouse embryos exposed to naphthalene, and increased sister chromatid exchanges in human mononuclear leukocytes exposed to 1,2- or 1,4-naphthoquinone and in Chinese hamster ovary cells exposed to naphthalene (see Table 3-3 for citations). Other studies in cell-free systems reported that 1,2-naphthoquinone formed N7 adducts with deoxyguanosine (McCoull et al. 1999) and caused DNA strand scission in the presence of NADPH and copper via reactive oxygen species from an oxidation/reduction cycle (Flowers et al. 1997).

In vivo genotoxicity assays with naphthalene are also limited and do not provide consistently negative or positive results for naphthalene genotoxicity. Positive results were obtained for somatic mutations in *D. melanogaster*, micronuclei in salamander larvae erythrocytes, and DNA fragmentation in liver and brain tissue from mice and rats orally exposed to naphthalene (see Table 3-3 for citations). Negative results were obtained for micronuclei formation in bone marrow of mice given oral or intraperitoneal injections of naphthalene, DNA single strand breaks and unscheduled DNA synthesis in hepatocytes of rats given oral doses of naphthalene, and neoplastic transformations in liver cells of partially hepatectomized rats given oral doses of naphthalene (see Table 3-3 for citations).

The available data suggest that genotoxic action by the naphthalene metabolite, 1,2-naphthoquinone, is plausible and that the mutagenic/genotoxic potential of naphthalene and its metabolites may be weak. Assays of possible genotoxic action in sensitive target tissues of naphthalene in rodents (lung and nasal epithelial tissue), however, are not available. New studies examining genotoxic end points in lung and nasal epithelial tissue following inhalation exposure to naphthalene would help to better determine the potential genotoxicity of naphthalene and it metabolites.

For the methylnaphthalenes, data in humans are limited to one study that reported no effects on human chromosomes in tests evaluating the effects of 1-methylnaphthalene or 2-methylnaphthalene on human peripheral lymphocytes *in vitro* (Kulka et al. 1988). 1-Methylnaphthalene and 2-methylnaphthalene were also determined to be nonmutagenic in four strains of *S. typhimurium* (Florin et al. 1980). Additional mutagenicity studies using an *in vivo* approach would be useful to better assess the genotoxicity potentials of 1-methylnaphthalene and 2-methylnaphthalene.

Reproductive Toxicity. No information is available on the reproductive effects of naphthalene in humans, although the occurrence of hemolytic anemia in the 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). Animal studies involving naphthalene exposure during gestation reported no reproductive effects in rabbits administered doses of up to 120 mg/kg/day by gavage or in rats given doses of up to 450 mg/kg/day, although doses of 150 mg/kg/day and greater were maternally toxic to rats. There was a decrease in the number of live mouse pups per litter with a dose of 300 mg/kg/day given during gestation (Plasterer et al. 1985) and *in vitro* studies of naphthalene embryotoxicity in the presence of liver microsomes support the concept that naphthalene metabolites may be harmful to the developing embryo (Iyer et al. 1991). No exposure-related lesions in reproductive tissues were found in intermediate-duration oral exposure studies in rats (NTP 1980b) and mice (NTP 1980a) or in chronic inhalation studies in rats (Abdo et al. 2001; NTP 2000) or mice (NTP 1992a). One- or two-generation reproductive toxicity studies evaluating reproductive performance variables in male and female animals exposed to naphthalene are not available. Results from such studies may help to better determine the potential reproductive toxicity of naphthalene.

No studies are available on the reproductive toxicity of 1-methylnaphthalene or 2-methylnaphthalene in humans or animals following inhalation, oral, or dermal exposure, with the exceptions of the reports that 81-week oral exposure to 1-methylnaphthalene or 2-methylnaphthalene did not induce lesions in reproductive tissues of male or female mice (Murata et al. 1993; 1997). One- or two-generation reproductive toxicity studies evaluating reproductive performance variables in male and female animals exposed to 1-methylnaphthalene or 2-methylnaphthalene are not available. Results from such studies may help to better determine the potential reproductive toxicity of the methylnaphthalenes.

Developmental Toxicity. There is no information on the potential developmental effects of naphthalene in humans, although, as mentioned previously, naphthalene and/or its metabolites can cross the placental barrier and cause hemolytic anemia in newborns (Anziulewicz et al. 1959; Zinkham and Childs 1957, 1958). Studies of the developmental effects of orally administered naphthalene in rats (NTP 1991a), mice (Plasterer et al. 1985), and rabbits (NTP 1992b; PRI 1985i, 1986) have been negative, except for a slight nonsignificant increase in fused sternebrae in female rabbit pups from a small number of litters at doses of 80 and 120 mg/kg/day (NTP 1992b). No developmental toxicity studies involving inhalation or dermal exposure to naphthalene are available. The availability of such studies would help to better determine the developmental toxicity potential of naphthalene.

No studies are available on the developmental toxicity of 1-methylnaphthalene or 2-methylnaphthalene in humans or animals following inhalation, oral, or dermal exposure.

Immunotoxicity. There have been no comprehensive studies of the immunotoxicity of naphthalene in humans exposed by the inhalation, oral, or dermal routes. The animal oral exposure data indicate that naphthalene did not affect humoral or cell-mediated immunity in mice (Shopp et al. 1984). Minor effects on the thymus and spleen were noted in mice and rats (NTP 1980b; Shopp et al. 1984), but in no case were animals of both sexes affected. Because there are few data pertaining to the immunotoxicity of naphthalene, a battery of *in vitro*/*in vivo* screening assays of immune function may be useful to determine whether more detailed and longer-term studies are needed.

No studies are available on the immunotoxicity of 1-methylnaphthalene or 2-methylnaphthalene in humans or animals following inhalation, oral, or dermal exposure. However, the reported increase in the level of monocytes in mice following long-term oral exposure to 1-methylnaphthalene (Murata et al. 1993) may deserve additional study. As with naphthalene, a battery of *in vitro*/*in vivo* screening assays of immune function may be useful to determine whether more detailed and longer-term studies are needed.

Neurotoxicity. The direct effects of naphthalene on the central nervous system have not been investigated in either humans or animals. Neurotoxic effects seen in humans exposed to naphthalene via inhalation or oral exposure may be a consequence of the diminished oxygen-carrying capacity of the blood which results from red cell hemolysis (Bregman 1954; Gupta et al. 1979; Kurz 1987; Linick 1983; MacGregor 1954; Ojwang et al. 1985; Zuelzer and Apt 1949). Persistent clinical signs of toxicity (lethargy and prone position) were seen in pregnant rats following gavage administration of naphthalene at dose levels of 150 or 450 mg/kg/day; at 50 mg/kg/day, the signs were only observed during the first 2 days of dose administration (NTP 1991a). Comparable effects were not observed in F344/N rats exposed to doses of up to 400 mg/kg/day for 13 weeks or in B6C3F1 mice at doses of up to 200 mg/kg/day (NTP 1980a, 1980b). With inhalation exposure, no treatment-related gross or histopathological lesions of the brain were observed in mice (NTP 1992a) or rats (Abdo et al. 2001; NTP 2000) exposed for 2 years to naphthalene concentrations as high as 30 or 60 ppm, respectively. Clinical observations revealed no gross behavioral changes indicative of neurological impairment. Additional studies involving batteries of neurological end points following oral and/or inhalation exposure may help to better determine the potential neurotoxicity of naphthalene and explain why pregnant rats appear to be more susceptible to the behavioral effects of acute-duration exposures to naphthalene.

No studies on the neurotoxicity of 1-methylnaphthalene or 2-methylnaphthalene in humans following inhalation, oral, or dermal exposure were located with the exception of a single study that found decreased sensitivity to pain in rats exposed by inhalation for 4 hours to 1-methylnaphthalene (44 ppm) or

2-methylnaphthalene (61 ppm), but no effects on rotarod performance at exposure levels as high as 70 ppm 1-methylnaphthalene or 90 ppm 2-methylnaphthalene (Korsak et al. 1998). The biological significance of these findings is uncertain. Additional studies involving batteries of neurological end points may help to better determine the potential neurotoxicity of the methylnaphthalenes.

Epidemiological and Human Dosimetry Studies. A small number of reports have equivocally suggested that workers exposed to naphthalene for long periods of time may have an elevated risk of cataract development (Ghetti and Mariani 1956; Lezenius 1902). This information, coupled with the cataractogenic effects of naphthalene in orally exposed rats (Kojima 1992; Xu et al. 1992b; Yamauchi et al. 1986) and rabbits (Rossa and Pau 1988; Srivastava and Nath 1969; Van Heyningen and Pirie 1967) in acute- and intermediate-duration studies, suggests that studies of occupationally-exposed workers would help to determine its potential to produce ocular toxicity in humans. The incidence of tumors, anemia, and reproductive problems in this population could be determined at the same time. Available case reports of cancer in naphthalene-exposed humans provide inadequate evidence of naphthalene carcinogenicity. Currently, no cohort mortality or morbidity studies or case-control studies examining possible associations between naphthalene exposure and increased risk of cancer (or other health effects) are available. If human populations that are specifically and repeatedly exposed to naphthalene can be identified, epidemiological studies of these populations may help to better assess the potential chronic-duration toxicity and carcinogenicity of naphthalene.

No epidemiological or human dosimetry studies on the effects of 1-methylnaphthalene or 2-methylnaphthalene were located. Exposure to these compounds, particularly through dermal contact or inhalation, can occur in workplaces where the compounds are produced or used. Populations living near hazardous waste sites can potentially be exposed by the oral, inhalation, and dermal routes. If an appropriate population can be identified, it may be helpful to conduct epidemiological studies to determine if there are toxic effects (particularly on the lungs) resulting from exposure to these substances.

Biomarkers of Exposure and Effect.

Exposure. There are methods to determine the presence of naphthalene in adipose tissue and these methods have been used in a national monitoring program for the analysis of naphthalene in the adipose tissue of the general population (EPA 1986g). Metabolites of naphthalene, such as naphthols and naphthoquinones, have been detected in the urine of a patient 4 days after ingestion of naphthalene (Zuelzer and Apt 1949), but not in another patient at 17 days after ingestion (Mackell et al. 1951).

1-Naphthol is present in the urine of workers occupationally exposed to naphthalene. Maximum 1-naphthol levels occurred immediately after the end of the work period and in some cases had returned to baseline levels 8 hours later (Bieniek 1994). New techniques have been developed to measure cysteinyl adducts formed from reactions of hemoglobin and albumin with reactive metabolites of naphthalene (Troester et al. 2002; Waidyanatha et al. 2002). The adducts are expected to be useful in estimating internal doses of these metabolites, and with further development, they may become useful biomarkers of exposure.

Effect. There are no known specific biomarkers of effects for naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene. Hemolytic anemia has been frequently associated with human exposure to naphthalene, but may also be the result of exposure to other chemicals. Pulmonary alveolar proteinosis in mice has been associated with chronic oral exposure to 1-methylnaphthalene and 2-methylnaphthalene. The condition has been described in humans, but has not been associated with human exposure to 1-methylnaphthalene or 2-methylnaphthalene. Currently, these effects (hemolytic anemia or pulmonary alveolar proteinosis) do not hold promise as specific biomarkers of effect for naphthalene or methyl-naphthalenes. Identification of specific biomarkers of effect such as particular protein adducts in naphthalene-affected target tissues in animals (e.g., nasal epithelium tissue) may be useful to test whether similar biomarkers of effect may exist in naphthalene-exposed human populations.

Absorption, Distribution, Metabolism, and Excretion. Although human absorption of naphthalene has not been quantitatively characterized, case reports indicate that humans can absorb toxicologically significant amounts of this compound by the oral, inhalation, or dermal routes (Bregman 1954; Chusid and Fried 1955; Dawson et al. 1958; Gidron and Leurer 1956; Gupta et al. 1979; Haggerty 1956; Kurz 1987; Linick 1983; MacGregor 1954; Mackell et al. 1951; Ojwang et al. 1985; Santhanakrishnan et al. 1973; Schafer 1951; Shannon and Buchanan 1982; Valaes et al. 1963; Zuelzer and Apt 1949). Laboratory animals such as rats, mice, and rabbits also absorb the chemical via their skin and gastrointestinal and respiratory tracts (NTP 1992a; Rao and Pandya 1981; Shopp et al. 1984; Srivastava and Nath 1969; Turkall et al. 1994; van Heyningen and Pirie 1967). Naphthalene adsorbed to organic-rich soils is absorbed across the skin more slowly than naphthalene from organic-poor soils (Turkall et al. 1994). The compound apparently partitions between the soil organic carbon and the hydrophobic components of the epidermis and dermis. More information concerning the mechanism of absorption (facilitated versus passive transport) across nasal and pulmonary epithelial membranes, the gastrointestinal tract, and the skin may be helpful in estimating the effect of dose on absorption coefficients and in better determining the effect of the medium of exposure (water, oil, food, etc.) on oral

or dermal absorption. Empirical measurements of permeability coefficients for naphthalene in blood or air with various tissues from various species may be useful to further develop PBPK models for naphthalene.

As discussed in Sections 3.4.3 and 3.5.2, extensive research on the bioactivation and metabolic transformations of naphthalene in mammalian systems has identified several reactive metabolites that are potentially responsible for the nasal, pulmonary, and ocular toxicity of naphthalene (1,2-naphthalene oxide, 1,2-naphthoquinone, and 1,4-naphthoquinone), but the relative importance of these metabolites in affecting these toxicity targets remains uncertain. Because nasal respiratory and olfactory epithelia are the most sensitive targets in rodents following acute or chronic inhalation exposure, better understanding of the deposition, absorption, and metabolism of inhaled naphthalene in different regions of nasal epithelia, and the degree to which species (particularly rodents and primates) differ in these processes, may be useful for decreasing uncertainty in extrapolating human health hazards from data for rodents exposed to naphthalene. *In vivo*, *in vitro*, and modeling research approaches are likely to create better understanding of these processes, which may also provide explanations for observed species differences in response to naphthalene. For example, both rats and mice developed nonneoplastic nasal lesions following chronic inhalation exposure to naphthalene concentrations as low as 10 ppm, but only rats developed nasal tumors (Abdo et al. 2001; NTP 1992a, 2000). Other examples are the findings that *in vitro* rates of epoxide formation from naphthalene in extracts of nasal olfactory tissue showed the order, mouse>rat>hamster, but rats were more susceptible to acute nasal injury from naphthalene than mice or hamsters (Buckpitt et al. 1992; Plopper et al. 1992a). Mechanistic explanations for these differences are not currently available.

The most recently developed PBPK models for naphthalene in mice and rats (Willems et al. 2001) do not include nasal compartments that metabolize naphthalene and do not include the spontaneous conversion of 1,2-naphthalene oxide to 1-naphthol or metabolic transformations to the naphthoquinones. Additional toxicokinetic data are needed to further refine these models to include these potentially important processes. Application of such further refined models, and the development of comparable models for humans, may be useful to decrease uncertainty in extrapolating dose-response relationships for nasal effect in rodents to humans.

No studies were located on the absorption, metabolism, and excretion of 1-methylnaphthalene in humans or animals following inhalation, oral, or dermal exposure. There was one study of 2-methylnaphthalene in guinea pigs (Teshima et al. 1983). Parenteral studies in animals show that 2-methylnaphthalene is

converted to both monohydrated compounds and dihydrodiols (Breger et al. 1981, 1983; Melancon et al. 1982). In addition, 2-naphthoic acid and the glycine or the cysteine conjugates were identified in rats (Melancon et al. 1982) and guinea pigs (Teshima et al. 1983). Studies by relevant exposure routes would further characterize the toxicokinetics of these compounds and may enhance the understanding of the potential risk associated with exposure to these compounds.

Comparative Toxicokinetics. Data suggest that there are strain- and species-specific effects associated with naphthalene toxicity. Laboratory animals, such as rats and mice, do not exhibit red cell hemolysis after exposure to naphthalene, while humans and dogs do (NTP 1980a, 1980b, 1992a; Shopp et al. 1984; Zuelzer and Apt 1949). Mice and rats both develop nonneoplastic nasal lesions after chronic inhalation exposure to naphthalene, but only rats develop nasal tumors, and only mice develop nonneoplastic lung lesions or lung tumors (Abdo et al. 2001; NTP 1992a; 2000). There are differences in susceptibility to the acute pulmonary toxicity of naphthalene among mice, rats, hamsters, and guinea pigs (Buckpitt et al. 2002; Plopper et al. 1992a, 1992b). Differences in the susceptibility of rats and mice, and of different mouse strains, to the cataractogenic properties of naphthalene have also been reported (Wells et al. 1989). These differences may relate to differences in tissue distribution of specific CYP isoenzymes, rates of formation of reactive metabolites, rates of transformation of reactive metabolites to nonreactive metabolites, or partitioning of the parent compound or metabolites within and between tissues. For example, the difference in susceptibility to the acute pulmonary toxicity of naphthalene between mice and rats has been correlated with higher rates of metabolic formation and different stereoselectivity of epoxide metabolites in mice compared with rats (Buckpitt et al. 1992; 1995; 2002). In contrast, differences among rat, mice and hamsters in susceptibility to naphthalene-induced nasal lesions were not correlated with species differences in rates of epoxide formation from naphthalene in extracts of olfactory epithelial tissue (Plopper et al. 1992; see Section 3.5.2). Further evaluation of these differences and comparative studies of distribution and metabolic patterns among species may help to decrease uncertainty in extrapolating estimates of human health hazards from data for animals exposed to naphthalene.

There are no data available concerning the toxicokinetics of 1-methylnaphthalene or 2-methylnaphthalene in humans following inhalation, oral, or dermal exposure. There are no data from studies of 1-methylnaphthalene in animals, but there are limited data for 2-methylnaphthalene (Breger et al. 1983; Griffin et al. 1982; Melancon et al. 1982, 1985; Teshima et al. 1983). New studies that evaluate toxicokinetic parameters in several animal species may be useful to decrease uncertainty in the chronic oral MRLs for

1-methylnaphthalene and 2-methylnaphthalene, which are based on the occurrence of pulmonary alveolar proteinosis in mice.

Methods for Reducing Toxic Effects. Available methods are sufficient for reducing peak absorption of naphthalene following ingestion (Melzer-Lange and Walsh-Kelly 1989; Siegel and Wason 1986; Stutz and Janusz 1988). No antidotal methods are available that would be useful for treatment of naphthalene exposure based on any proposed hypothesis pertaining to the mechanism of action. Additional studies to characterize the metabolic activation of naphthalene and the role of circulating reactive metabolites from nontarget tissues may be useful in developing methods for interfering with the mechanism of action. Further studies to identify ways to reduce or prevent accumulation of toxic metabolites in target tissues may be warranted when mechanisms of naphthalene toxic action are better understood.

There are no compound-specific methods for reducing the toxic effects of 1-methylnaphthalene and 2-methylnaphthalene. Additional information on the toxicokinetics and mechanism of action for these compounds may be beneficial in identifying possible approaches for reducing compound toxicity.

Children's Susceptibility. Data needs relating to both prenatal and childhood exposures, and developmental effects expressed either prenatally or during childhood, are discussed in detail in the Developmental Toxicity subsection above.

As discussed in Section 3.7, cases of naphthalene-induced hemolytic anemia in children have been frequently reported (Owa 1989; Owa et al. 1993; Santucci and Shah 2000; Valaes et al. 1963). Newborns and infants are thought to be more susceptible than older people because hepatic enzymes involved in conjugation and excretion of naphthalene metabolites are not well developed after birth, and children with genetically determined G6PD deficiency are thought to be especially susceptible to chemically-induced hemolytic anemia (EPA 1987a). There are no studies that have specifically examined the influence of age on naphthalene toxicokinetic capabilities in humans. Although the availability of such studies may increase the understanding of the specific physiological basis for the apparent susceptibility of newborns, they are unlikely to be conducted. Experiments examining the most sensitive targets in animals (see below) are likely surrogates.

Although 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 1991a; Plasterer et al. 1985; PRI 1986). Additional developmental toxicity studies in animals with inhalation or dermal exposure would determine if naphthalene exposure by these routes represents a greater developmental hazard than oral exposure.

Neonatal mice (7 days old) appear to be more susceptible than adult mice to lung injury induced by acute intraperitoneal injection of naphthalene (Fanucchi et al. 1997). The mechanistic basis of this difference is currently unknown, but does not appear to be explained by differences in CYP catalytic capabilities to produce epoxide metabolites, since CYP activities were 2.5 time lower in neonates than in adults. Downstream metabolic capabilities, however, were not examined in this study. Comparison of neonatal and adult tissues in these metabolic steps may help to explain this apparent susceptibility of neonatal mice. Based on findings that *in utero* exposure to other CYP-bioactivated chemicals caused Clara cell tumors in adult offspring, Fanucchi et al. (1997) postulated that naphthalene exposure during the neonatal period may lead to loss of regulatory mechanisms resulting in Clara cell proliferation and tumor formation in adult animals. Direct evidence for naphthalene in support of this hypothesis, however, is not available. Additional research may help to determine whether or not *in utero* or neonatal naphthalene exposure will cause increased incidence of lung tumors in adult mice.

Child health data needs relating to exposure are discussed in Section 6.8.1, Identification of Data Needs: Exposures of Children.

3.12.3 Ongoing Studies

Dr. Alan Buckpitt and colleagues at the University of California, Davis have been conducting studies in several areas related to naphthalene toxicology including (1) identifying specific naphthalene-protein adducts in lungs of mice, rats, and Rhesus macaques and characterizing the time course of their generation and disappearance; (2) identifying cellular and molecular events involved in the development of naphthalene-induced acute lung injury by comparing lung tissue from rodents, Rhesus macaques, and humans; and (3) comparing the cellular distribution and catalytic activities of CYP monooxygenases in lung tissues from various species.

Dr. Charles Plopper and colleagues at the University of California, Davis have been conducting studies comparing acute naphthalene-induced lung injury in neonatal mice and adult mice and the biochemical

effects of *in utero* or neonatal exposure to lung toxicants on the development of bronchiolar repair capabilities. This work is part of an effort to increase understanding of molecular mechanisms involved in lung diseases that may originate in childhood exposures.

Dr. Leena Nylander French and colleagues at the University of North Carolina, Chapel Hill have been conducting studies to test the hypothesis that low levels of exposure to benzene or naphthalene can be detected using samples of keratinized epidermis removed by tape stripping.

Dr. Y. Awasthi and colleagues at the University of Texas, Galveston are studying the roles of glutathione S-transferases in protecting against ocular cytotoxicity and apoptosis caused by several oxidants, including naphthalene. Studies include the use of genetically altered knock-out mice strains, which are deficient in specific types of glutathione-*S*-transferases.

Dr. Barry Stripp and colleagues at the University of Pittsburgh are studying the role of proliferative cells originating from the neuroepithelial body in repair of airway epithelial cell damage in mice exposed to ozone or naphthalene.

Dr. John Markley and colleagues at the University of Wisconsin, Madison are studying the 1-, 2-, and 3-dimensional molecular structures of toluene 4-monooxygenase, an enzyme that catalyzes NADH- and O_2-dependent conversion of toluene to p-cresol, as well as the oxidation of numerous hydrocarbons, including naphthalene.