FINAL

Report on Carcinogens Background Document for

Naphthalene

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FOREWORD

The Report on Carcinogens (RoC) is prepared in response to Section 301 of the Public Health Service Act as amended. The RoC contains a list of all substances (i) that either are known to be human carcinogens or may reasonably be anticipated to be human carcinogens; and (ii) to which a significant number of persons residing in the United States are exposed. The Secretary, Department of Health and Human Services (DHHS) has delegated responsibility for preparation of the RoC to the National Toxicology Program (NTP) who prepares the Report with assistance from other Federal health and regulatory agencies and non-government institutions.

Nominations for listing in or delisting from the RoC are reviewed by a formal process that includes a multi-phased, scientific peer review and multiple opportunities for public comment. The review groups evaluate each nomination according to specific RoC listing criteria. This Background Document was prepared to assist in the review of the nomination of Naphthalene. The scientific information in this document comes from publicly available, peer reviewed sources. Any interpretive conclusions, comments or statistical calculations, etc made by the authors of this document that are not contained in the original citation are identified in brackets [ ]. If any member(s) of the scientific peer review groups feel this Background Document does not adequately capture and present the relevant information they will be asked to write a commentary for this Background Document that will be included as an addendum to the document. In addition, a meeting summary that contains a brief discussion of the respective review group's review and recommendation for the nomination will be added to the Background Document, also as an addendum.

A detailed description of the RoC nomination review process and a list of all nominations under consideration for listing in or delisting from the RoC can be obtained by accessing the NTP Home Page at http://ntp-server.niehs.nih.gov. The most recent RoC, the 9th Edition, was published in May, 2000 and may be obtained by contacting the NIEHS Environmental Health Information Service (EHIS) at http://ehis.niehs.nih.gov (800-315-3010).

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Criteria for Listing Agents, Substances or Mixtures in the Report on Carcinogens

U.S. Department of Health and Human Services
National Toxicology Program

Known to be Human Carcinogens:

There is sufficient evidence of carcinogenicity from studies in humans, which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated to be Human Carcinogens:

There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible but that alternative explanations such as chance, bias or confounding factors could not adequately be excluded; or

There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species, or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals; however, the agent, substance or mixture belongs to a well defined, structurally-related class of substances whose members are listed in a previous Report on Carcinogens as either a *known to be human carcinogen, or reasonably anticipated to be human carcinogen* or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

Executive Summary

Introduction
Naphthalene is a polyaromatic hydrocarbon found in coal tar. It was nominated by the National Institute of Environmental Health Sciences based on the results of the National Toxicology Program (NTP) two-year inhalation studies, which concluded that naphthalene was carcinogenic in rats and female mice. Naphthalene was evaluated by the International Agency for Research on Cancer (2000) and classified as possibly carcinogenic to humans (Group 2B) based on sufficient evidence of carcinogenicity in animals.

Human Exposure
Use. The principal use of naphthalene in the United States is as an intermediate in the production of phthalic anhydride, which is an intermediate in the production of phthalate plasticizers, pharmaceuticals, insect repellents, and other materials. Naphthalene also is used as an intermediate in the production of 1-naphthyl-*N*-methylcarbamate insecticides, beta-naphthol and synthetic leather tanning chemicals, and surfactants (e.g. naphthalene sulfonates), and crystalline naphthalene is used as a moth repellent and toilet bowl deodorant.

Production. Naphthalene is produced from either coal tar (of which it is the most *abundant* constituent) or petroleum. Production of naphthalene in 2000 was 235 million pounds, most of which (219 million pounds) was produced from petroleum.

Environmental Exposure. The main source of exposure to naphthalene is through inhalation in ambient and indoor air. The average daily intake of naphthalene from ambient air has been estimated to be 19 μg, based on an average naphthalene concentration of 0.95 $\mu g/m^3$ in urban and suburban air and an inhalation rate of 20 m^3/day. Accidental ingestion of naphthalene-containing household products has been reported, especially in children. Dermal exposure to naphthalene may occur through handling or wearing of clothing stored with naphthalene-containing moth repellents.

Occupational exposure. The National Occupational Exposure Survey, conducted from 1981 to 1983, estimated that 112,702 workers potentially were exposed to naphthalene. Workers identified by the U.S. Environmental Protection Agency (EPA) as potentially exposed to naphthalene include beta-naphthol makers, celluloid makers, coal tar workers, dye chemical makers, fungicide makers, hydronaphthalene makers, moth repellent workers, phthalic anhydride makers, smokeless powder makers, tannery workers, textile chemical workers, and aluminum reduction plant workers. Air concentrations of naphthalene have been measured in many studies and vary with the type of industry. A survey by the National Institute for Occupational Safety and Health (NIOSH) in 1980 reported air concentrations of naphthalene as high as 10.2 $\mu g/m^3$ in an area sample and 19.3 $\mu g/m^3$ in a personal sample.

Regulations. Naphthalene is regulated by the EPA (under the Clean Air Act, Clean Water Act, Safe Drinking Water Act, Resource Conservation and Recovery Act, Superfund Amendments and Reauthorization Act, and Toxic Substances Control Act), the Occupational Safety and Health Administration (OSHA) and the U.S. Food and Drug Administration. OSHA has established an eight-hour time-weighted-average permissible exposure level for naphthalene of 10 ppm (50 mg/m^3), which is consistent with recommendations by NIOSH and the American Conference of Governmental Industrial Hygienists.

Human Cancer Studies

Two case-series studies of cancer occurring in individuals exposed to naphthalene have been reported: laryngeal and other cancers occurring in naphthalene-exposed workers in Germany and colorectal carcinoma occurring among individuals in Africa who had used a naphthalene compound for medicinal reasons. The available data are insufficient for evaluation of the carcinogenicity of naphthalene in humans.

Studies in Experimental Animals

The NTP published two-year carcinogenicity studies of naphthalene administered by inhalation to $B6C3F_1$ mice (NTP 1992) and F344/N rats (NTP 2000). These studies showed no evidence of carcinogenic activity of naphthalene in male $B6C3F_1$ mice; some evidence of carcinogenic activity in female $B6C3F_1$ mice, based on increased incidence of pulmonary alveolar/bronchiolar adenoma; and clear evidence of carcinogenic activity in male and female F344/N rats, based on increased incidences of respiratory epithelial adenoma and olfactory epithelial neuroblastoma of the nose. The strain A mouse lung tumor bioassay showed a slight, but not statistically significant, increase in alveolar adenoma in female mice exposed to naphthalene, and the number of tumors per tumor-bearing lung was significantly increased. There was no evidence that naphthalene was carcinogenic in rats by routes of administration other than inhalation; however, these studies are considered inadequate, as the numbers of animals in these experiments were small, and there were no controls.

Genotoxicity

Naphthalene has been tested for genotoxicity in bacterial, non-mammalian, and mammalian systems. In general, naphthalene is not mutagenic in bacteria or in mammalian cell systems. Naphthalene did not induce mutations in bacteria (*Salmonella typhimurium* or *Escherichia coli*) or in human lymphoblastoid cells. In non-mammalian *in vivo* systems, naphthalene induced mutations in fruitflies (*Drosophila melanogaster)* and micronuclei in salamander larvae *(Pleurodeles waltl)*. In mammalian *in vitro* cells systems, naphthalene did not induce cell transformation (in mouse mammary gland cells or rat or mouse embryo cells), DNA strand breaks (in rat hepatocytes), or kinetochore-positive micronuclei (in human lymphoblastoid cells), which are a marker for chromosomal loss. However, positive results were observed for chromosomal aberrations (in Chinese hamster ovary [CHO] cells), sister-chromatid exchange (in CHO cells), and kinetochore-negative micronuclei (in human lymphoblastoid cells), which are a marker for chromosomal breakage. *In vivo* exposure to naphthalene induced oxidative stress and DNA damage in Sprague-Dawley rats.

Other Relevant Data

Absorption, excretion, and metabolism in animals and humans. Naphthalene is rapidly absorbed and metabolized when inhaled or administered dermally or orally to animals. Naphthalene is excreted in the urine as the unchanged parent compound, as metabolites (including 1-naphthol, 2-naphthol, naphthoquinones, and dihydroxynaphthalenes), or as glutathione (GSH), cysteine, glucuronic acid, and sulfate conjugates. Urinary naphthalene metabolites found in workers at a coke plant correlated significantly with naphthalene concentrations in personal air samples, indicating that naphthalene is absorbed in humans. The first step in the metabolism of naphthalene is the formation of naphthalene-1,2-oxide by cytochrome P450 in the presence of NADPH, which is converted to the trans-1,2-diol and other products. The electrophilic naphthalene intermediates are metabolized to naphthoquinones and possibly to free-radical intermediates; *in vivo*, these metabolites contribute to depletion of GSH, and excess metabolites may bind covalently to tissue macromolecules. Higher rates of metabolism in microdissected airways have been reported to occur in mice than in rats or hamsters.

Toxicity. The toxicity of naphthalene is manifested primarily in the hematologic system in humans and dogs (hemolytic anemia), the pulmonary system in rodents (lung injury) and the eye in humans and rodents (lens opacity and cataracts).

Potential mechanisms of carcinogenicity. Naphthalene induced lung neoplasia in female B6C3F$_1$ mice and nasal tumors in male and female F344/N rats. The mechanism of action has not been elucidated. Toxicity of naphthalene to lung and other tissues has been attributed to formation of the 1*R*,2*S*-naphthalene oxide; a strong correlation has been reported between the rates of formation of 1*R*,2*S*-naphthalene oxide in various tissues and tissue-selective toxicity. Naphthalene-induced oxidative damage and DNA breakage, which have been observed in rat liver and brain tissue, may contribute to the toxicity and carcinogenicity of naphthalene. Mice appear to be more susceptible to induction of lung neoplasia by epoxides and epoxide-forming chemicals than are rats. Differences between rats and mice in the metabolism of naphthalene by nasal epithelia and in nasal anatomy may contribute to the species differences in susceptibility to these tumors.

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1 Introduction

Naphthalene is the most abundant constituent of coal tar. Naphthalene is produced by condensation and separation of coal tar from coke-oven gases or from petroleum by dealkylation of methylnaphthalenes. In the United States, most naphthalene is produced from petroleum. The principal use of naphthalene is as an intermediate in the production of phthalic anhydride, which is used as an intermediate in the production of phthalate plasticizers, resins, dyes, insect repellents, and other materials. It also is used in some moth repellents and toilet bowl deodorizers.

Naphthalene was nominated by the National Institute of Environmental Health Sciences for possible listing in the Report on Carcinogens based on the results of National Toxicology Program (NTP) two-year inhalation studies of naphthalene, which concluded that there was clear evidence of carcinogenicity in male and female F344/N rats (respiratory epithelial adenoma and olfactory epithelial neuroblastoma of the nose) and some evidence of carcinogenicity in female $B6C3F_1$ mice (pulmonary alveolar/bronchiolar adenomas). Naphthalene was also recently evaluated by an IARC Working Group (IARC 2000).

1.1 Chemical identification

Naphthalene ($C_{10}H_8$, mol wt 128.17, CASRN 91-20-3) also is known as naphthalin, naphthene, naphthaline, mothballs, moth flakes, tar camphor, white tar, and camphor tar (NTP 2001). Its RTECS number is QJ0525000, and its DOT number is UN1334, UN2304 (Chemfinder 2002). The structure of naphthalene is illustrated in Figure 1-1.

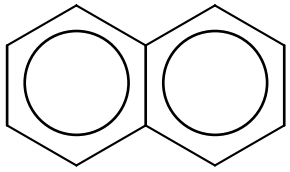


Figure 1-1. Structure of naphthalene

1.2 Physical-chemical properties

Naphthalene occurs as white monoclinic plates, scales, powder, balls, or cakes, with the distinctive odor usually associated with mothballs. It is insoluble in water and soluble in alcohol, benzene, ether, chloroform, carbon tetrachloride, and fixed and volatile oils. It reacts with strong oxidizers and may attack some forms of plastics, rubber, and coatings. Naphthalene is sensitive to heat and volatilizes at room temperature. It sublimes appreciably at temperatures above its melting point and is volatile with steam. It carries two nonequivalent sets of hydrogen atoms; two isomers of every monosubstituted naphthalene are known (HSDB 2002a, NTP 2001). The physical and chemical properties of naphthalene are summarized in Table 1-1.

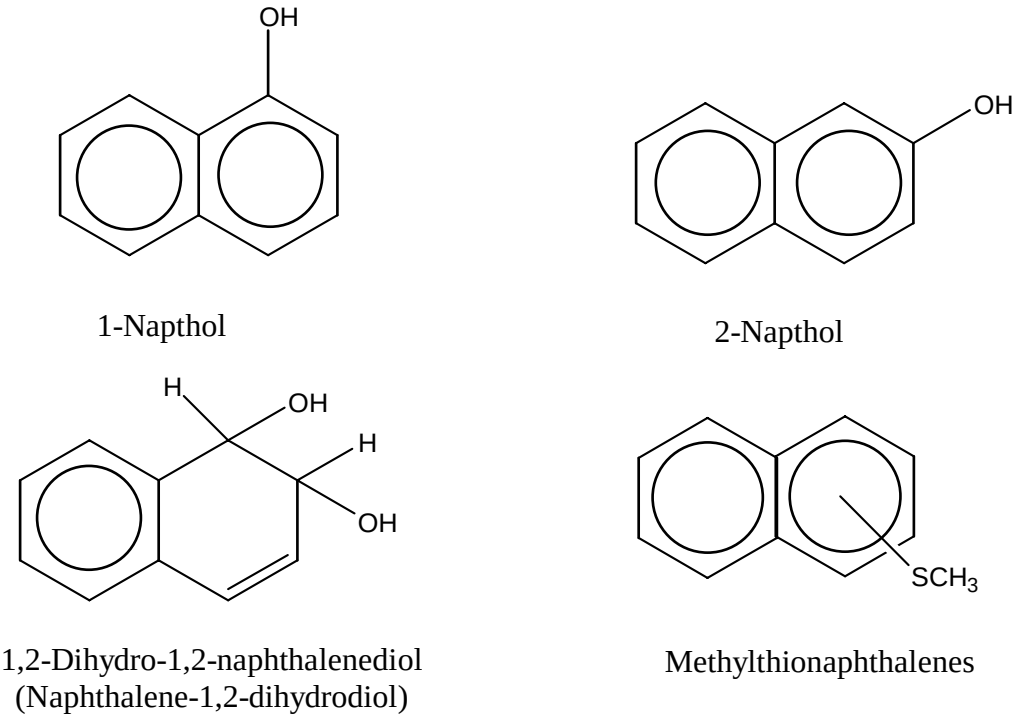
Table 1-1. Physical and chemical properties of naphthalene

Property	Information	Reference
Molecular weight	128.17	Budavari *et al.* 1996
Color	white colorless to brown	Budavari *et al.* 1996 NIOSH 2001
Odor	odor of mothballs	Budavari *et al.* 1996
Physical state	monoclinic prismatic plates, white scales, powder, balls or cakes	Budavari *et al.* 1996
Melting point (°C)	80.2	Budavari *et al.* 1996
Boiling point (°C)	217.9	Budavari *et al.* 1996
Flash point, (°C)	79	Budavari *et al.* 1996
Density at 20°C/4°C (g/cm^3)	1.162	Budavari *et al.* 1996
Vapor pressure (mm Hg)	0.08	NIOSH 2001
Solubility: water benzene chloroform or carbon tetrachloride 95% ethanol ether methanol fixed and volatile oils	 < 1 mg/mL 1 g/3.5 mL 1 g/2 mL 10–50 mg/mL soluble 1 g/13 mL soluble	 NTP 2001 Budavari *et al.* 1996 Budavari *et al.* 1996 NTP 2001 Budavari *et al.* 1996 Budavari *et al.* 1996 Budavari *et al.* 1996
Octanol-water partition coefficient: log K_{ow}	3.30	HSDB 2002a

1.3 Identification of urinary metabolites

The urinary metabolites of naphthalene following oral administration to rats and rabbits and intraperitoneal (i.p.) administration in mice, rats, and guinea pigs were identified. All species excreted 1- and 2-naphthol, 1,2-dihydro-1,2-naphthalenediol, 1-naphthylsulfate, and, except for guinea pigs, 1-naphthylglucuronic acid. Rats and rabbits excreted 1,2-dihydro-2-hydroxy-1-naphthylglucuronic acid, whereas guinea pigs excreted unconjugated 1,2-naphthalenediol (ATSDR 1995).

The structures of four major urinary metabolites of naphthalene are shown in Figure 1-2.



1-Napthol

2-Napthol

1,2-Dihydro-1,2-naphthalenediol
(Naphthalene-1,2-dihydrodiol)

Methylthionaphthalenes

Figure 1-2. Major metabolites of naphthalene

2 Human Exposure

2.1 Uses

The principal use of naphthalene in the United States is as an intermediate in the production of phthalic anhydride. Phthalic anhydride is an intermediate in the production of phthalate plasticizers, resins, phthaleins, dyes, pharmaceuticals, insect repellents, and other materials. Naphthalene also is used as an intermediate in the production of 1-naphthyl-*N*-methylcarbamate insecticides, beta-naphthol and synthetic leather tanning chemicals, and surfactants such as naphthalene sulfonates, which are used as dispersants or wetting agents in paint, dye, and paper-coating formulations. It formerly was used as an intermediate in 1-naphthylamine synthesis. Crystalline naphthalene is used as a moth repellent and toilet bowl deodorant. It was used in the early 1900s as an antiseptic, expectorant, and antihelminthic. It was administered for gastrointestinal tract diseases and applied externally for the treatment of skin disorders (ATSDR 1995, HSDB 2002a).

In 1987, the consumption pattern for naphthalene was 60% for production of phthalic anhydride, 10% for production of 1-naphthyl-*N*-methylcarbamate insecticides and related products (tetralin and 1-naphthol), 10% for production of dispersant chemicals, 6% in moth repellents, 5% for production of synthetic tanning agents, 5% for miscellaneous uses, and 4% for exports (HSDB 2002a). In 1999, the consumption pattern had not changed significantly, with 59% used for production of phthalic anhydride, 21% for production of surfactant and dispersant chemicals, 11% for production of 1-naphthyl-*N*-methylcarbamate insecticides, and 9% in moth repellents and for other purposes (ChemExpo 1999). The Chemical Economics Handbook (CEH 2000) also reported consumption patterns for naphthalene for 1995 and 2000 and projected them for 2004. Table 2-1 summarizes naphthalene consumption patterns for those years.

Table 2-1. Naphthalene consumption patterns

	Consumption in million pounds (thousand metric tons)		
Use	**1995**	**2000**	**2004 (projected)**
Phthalic anhydride	145 (66)	146 (66)	147 (67)
Naphthalene sulfonates[a]	47 (21)	59 (27)	65 (29)
Pesticides[b]	38 (17)	30 (14)	10 (5)
Dyestuff intermediates	0 (0)	0 (0)	0 (0)
Other[c]	5 (2)	6 (3)	6 (3)

Source: CEH 2000.

[a]Includes alkylnaphthalene sulfonates and naphthalene sulfonate–formaldehyde condensates, which are used in concrete additives and synthetic tanning agents.

[b]Includes carbaryl and moth repellents.

[c]Includes diisopropyl naphthalene, naphthalene dicarboxylic acid, tetrahydronaphthalene, decahydronaphthalene, and chloronaphthalenes.

2.2 Production

Naphthalene is produced from either coal tar or petroleum (ATSDR 1995) and is the most abundant constituent of coal tar, which contains about 11% naphthalene by dry weight. Naphthalene crystallizes from the middle, or "carbolic oil," fraction of distilled tar and is purified by hot pressing, which may be followed by washing with sulfuric acid, sodium hydroxide, and water, then by fractional distillation or by sublimation (HSDB 2002a). Naphthalene content in crude oil is as follows: 100 to 2,800 mg/kg in oil from coal; 402 to 900 mg/kg in oil from petroleum and 203 to 1,390 mg/kg in oil from shale (WHO 1998).

Since 1960, the most common commercial production process in the United States has been recovery of naphthalene from petroleum. Petroleum is dealkylated of its methyl naphthalenes in the presence of hydrogen at high temperatures and pressure; naphthalene then is recovered by fractionation, decolorized, and purified by crystallization. Naphthalene produced by this process is about 99% pure (ATSDR 1995).

U.S. naphthalene production and production capacity have been estimated by several sources. The Hazardous Substances Data Bank (HSDB 2002a) identified four producers of naphthalene in the United States in 1989: Allied-Signal Inc., in Ohio; Chemical Exchange Industries, Inc., in Texas; Koppers Industries, Inc., in West Virginia; and Texaco Inc., in Delaware. The Chemical Market Reporter (Greenberg 2000) reported production capacities for three U.S. companies that produced naphthalene in 1999; Advanced Aromatics produced naphthalene from aromatic petroleum fractions, whereas Allied-Signal and Koppers recovered naphthalene from coal tar. The respective production capacities of these three companies in 1999 were 40, 100, and 170 million pounds of chemical-grade naphthalene (ChemExpo 1999). In 2000, Koppers was the only U.S. company still producing naphthalene. In March 2000, Recochem Inc., based in Montreal, Canada, purchased Allied-Signal's naphthalene facility in Ohio, and Advanced Aromatics stopped producing naphthalene in order to concentrate on the development and marketing of naphthalene derivatives (Greenberg 2000).

U.S. production of naphthalene peaked in 1968, with the production of 900 million pounds (409,000 metric tons). By 1982, production had significantly decreased to 354 million pounds (161,000 metric tons). Production capacity has remained level in recent years, with an estimated capacity of 349 million pounds (159,000 metric tons) in 1992 (ATSDR 1995). The Chemical Economics Handbook (CEH 2000) has estimated that production of naphthalene in the United States in 2000 was 235 million pounds (107 metric tons) which is 75% of the estimated U.S. production capacity. The CEH also estimated that 241 million pounds (109 metric tons) were consumed in 2000 (CEH 2000). The U.S. International Trade Commission reported production of naphthalene until 1983, and the CEH estimated production levels from 1975 to 2000. Table 2-2 summarizes naphthalene production from coal tars and petroleum.

Table 2-2. Production of naphthalene in the United States

Year	Production (millions of pounds)		
	From Coal Tar	From Petroleum	Total
1965	464	347	811
1970	428	291	719
1975	351	110	461
1976	354	107	461
1977	350	151	501
1978	346	157	503
1979	326	163	489
1980	314	136	450
1981	351	142	493
1982	230	126	356
1983	223	95	318
1984	190	85	275
1985	184	55	239
1986	185	55	240
1987	181	55	236
1988	181	50	231
1989	180	50	230
1990	180	50	230
1991	180	50	230
1992	172	30	202
1993	180	20	200
1994	221	16	237
1995	231	16	247
1996	223	17	240
1997	222	18	240
1998	228	17	245
1999	225	17	242
2000	219	16	235

Source: USITC reported production from coal tar for 1965, 1970, 1981, and 1982 and from petroleum for 1965, 1970, 1975, and 1977-1982. The remaining estimates are from the CEH 2000.

Historically, from 1989 to 1998, naphthalene demand grew 0.5% per year. Future growth is expected to be 1% per year through 2003. Demand for naphthalene sulfonates, used primarily as concrete super-plasticizer additives to increase flow properties, has grown steadily in recent years, but more slowly in the past few years. Demand was 246 million pounds in 1998 and 248 million pounds in 1999, and is projected at 265 million pounds in 2003 (ChemExpo 1999).

2.3 Analysis

Biological samples are analyzed mostly by gas chromatography/mass spectrometry (GC/MS). Naphthalene undergoes short-term bioaccumulation in tissues, but biochemical processes in the body lead to its eventual elimination. New immunological analysis methods are being developed; however, these are not ready for research and clinical practice. Table 2-3 summarizes analytical methods for determining naphthalene in biological samples.

Naphthalene in environmental samples most commonly is detected by GC and high-performance liquid chromatography (HPLC). Table 2-4 summarizes analytical methods determining naphthalene concentrations in environmental samples.

2.4 Environmental occurrence

2.4.1 Air

Most of the naphthalene that enters the environment is discharged to the air (92.2%). The largest amount released (50%) is in fugitive emissions and exhaust from the combustion of wood and fossil fuels. The second-largest source of naphthalene releases is off-gassing from naphthalene-containing moth repellents. Virtually all the naphthalene contained in moth repellents is emitted into the atmosphere at room temperature. In 1989, 12 million pounds of naphthalene was released into the air from moth repellent use. Naphthalene also enters the environment from coal tar pitch fumes, unvented kerosene space heaters, smoke from forest fires, and tobacco fumes. An unfiltered American cigarette contains 2.8 μg of naphthalene, and smoke from a filtered “little cigar” contains 1.2 μg of naphthalene (ATSDR 1995, HSDB 2002a).

The U.S. Environmental Protection Agency’s (EPA’s) Toxic Release Inventory (TRI) reported that in 1999, 2,707,249 lb of naphthalene was released to air from manufacturing and processing facilities in the United States. Because only certain types of facilities are required to report releases, the TRI data are not exhaustive and should be used with caution (TRI99 2001).

Table 2-3. Analytical methods for determining naphthalene in biological samples

Sample matrix	Preparation method	Analytical method	Detection limit	Recovery (%)	Reference
Adipose tissue	extract, bulk lipid removal, Florisil fractionation	high-resolution gas chromatography (HRGC)/MS	9 ng/g	no data	ATSDR 1995
Adipose tissue	extract with hexane, Florisil cleanup	capillary column GC/MS	10 ng/g	90, human	ATSDR 1995
Human milk	purge with helium, desorb thermally	capillary column	no data	no data	ATSDR 1995
Human urine (1-naphthol analysis)	no data	thin-layer chromatography or GC/unspecified spectroscopy	no data	no data	Bieniek 1994
Human urine (naphthalene metabolites)	untreated	LC-MS with pneumatically assisted electrospray interface (ESI)	0.1 mg/L (α-naphthol); 0.02 mg/L (α-naphthylglucuronide); 0.01 mg/L (β-naphthylsulphate)	no data	Andreoli *et al.* 1999
Burned tobacco	extract with methanol/water and cyclohexane, enrich in dimethyl sulfoxide, fractional distillation and evaporation under dry nitrogen	gas-liquid chromatography/MS	no data	85–96	ATSDR 1995

Source: ATSDR 1995, updated with additional references.

Table 2-4. Analytical methods for determining naphthalene in environmental samples

Sample matrix	Preparation method	Analytical method	Detection limit	Recovery (%)	Reference
Air	adsorb (Charcoal or Chromosorb); desorb (carbon disulfide)	GC/flame ionization detector (FID)	1–10 μg/sample; 4 μg/sample	no data	ATSDR 1995
Air	adsorb (solid sorbent); desorb (carbon disulfide)	HPLC/ultraviolet (UV) spectrometry	0.6–13 μg/sample	no data	ATSDR 1995
Air	adsorb (solid sorbent); desorb (carbon disulfide)	GC/FID	0.3–0.5 μg/sample	no data	ATSDR 1995
Air	collect in charcoal tube, extract with acetonitrile	HPLC/fluorescence detection	0.080 μg/filter or 0.070 μg/tube	no data	Hansen *et al.* 1991
Indoor air	medium flow rate samples: extract with methylene chloride, exchange to cyclohexane, clean up, exchange to acetonitrile	HPLC/ultraviolet (UV) spectrometry	250 pg/μL	no data	ATSDR 1995
Indoor air	medium flow rate samples: extract with methylene chloride	GC/MS	no data	no data	ATSDR 1995
Water	purge and trap	HRGC/photoionization detection (PID)	0.06 μg/L	102 ± 6.3	ATSDR 1995
Drinking-, ground- and surface water	purge (inert gas); trap (Chromosorb W); desorb into capillary GC column	GC/MS	0.04 μg/L	no data	ATSDR 1995
Drinking water and raw source water	purge (inert gas); trap (Chromosorb W); desorb into capillary GC column	GC/PID	0.01–0.06 μg/L	no data	ATSDR 1995
Drinking Water	extract in liquid-solid extractor; elute with methylene chloride; dry; concentrate	HPLC/UV/FD	2.20 μg/L	no data	ATSDR 1995
Drinking water	purge and trap	packed column GC/PID	0.01–0.05 μg/L	92	ATSDR 1995

Sample matrix	Preparation method	Analytical method	Detection limit	Recovery (%)	Reference
Drinking water	purge and trap	capillary column GC/MS	0.02–0.2 μg/L	98–104	ATSDR 1995
Drinking water	purge and trap	capillary column GC/PID	no data	102	ATSDR 1995
Wastewater, municipal and industrial	extract with methylene chloride; dry; concentrate	HPLC/UV or GC/FID	0.01–0.06 μg/L	no data	EPA 1996b, 1996f, 1999a
Wastewater, municipal and industrial	extract with methylene chloride; dry; concentrate	GC/MS	1.6 μg/L	no data	EPA 1999b
Wastewater, municipal and industrial	add isotope labeled analogue; extract with methylene chloride; dry over sodium sulfate; concentrate	GC/MS	10 μg/L	no data	EPA 1999c
Wastewater	extract with methylene chloride, exchange to cyclohexane, clean up, exchange to acetonitrile	HPLC/UV	1.8 μg/L	21.5–100	ATSDR 1995
Solid Waste matrices[b]	purge (inert gas); trap (Tenax or Chromosorb W); desorb into capillary GC column	GC/PID	0.06 μg/L	no data	EPA 1996a
Solid Waste matrices[b]	purge (inert gas); trap (Tenax or Chromosorb W); desorption or headspace sampling or direct injection	GC/MS	0.04–0.1 μg/L	no data	EPA 1996c
Air Sampling media, water samples, solid waste matrices, soil samples	liquid-liquid extraction or Soxhlet extraction or ultrasonic extraction or waste dilution or direct injection	GC/MS	10 μg/L (aqueous); 660 μg/kg (soil/sediment) Estimated quantitation limit	no data	EPA 1996d
Soils, sludges, solid wastes	Thermal extraction; concentrate; thermal desportion	TE/GC/MS	0.01–0.5 mg/kg	no data	EPA 1996e
Wastewater, soil, sediment, solid waste	liquid-liquid extraction (water); Soxhlet or ultrasonic extraction (soil/sediment/waste)	GC/FT-IR	20 μg/L	no data	EPA 1996g

Source: ATSDR 1995, updated with additional references.

[a] Identification limit in water. Detection limits for actual samples are several orders of magnitude higher, depending upon the sample matrix and extraction procedure employed.

[b] Includes: groundwater, aqueous sludges, caustic and acid liquors, waste solvents, oily waters, mousses, tars, fibrous wastes, polymeric emulsions, filter cakes, spent carbons, spent catalysts, soils, and sediments.

2.4.2 Water

About 5% of naphthalene entering the environment is released to water. Most of this is from coal tar production and distillation. Other sources of naphthalene in water are oil spills and effluents from the wood-preserving industry (ATSDR 1995).

The Toxic Release Inventory reported that in 1999, 42,067 lb of naphthalene was released to water from manufacturing and processing facilities in the United States. Because the TRI data are not exhaustive, they should be used with caution (TRI99 2001).

2.4.3 Soil

About 2.7% of naphthalene entering the environment is discharged to land. The major source of land releases is coal tar production, with minor contributions from naphthalene production, sludge disposal from sewage treatment facilities, and use of naphthalene-containing organic chemicals (ATSDR 1995).

The Toxic Release Inventory reported that in 1999, 447,129 lb of naphthalene was released to land and 166,064 lb to underground land injection release from manufacturing and processing facilities in the United States. Because the TRI data are not exhaustive, they should be used with caution (TRI99 2001).

2.5 Environmental fate

2.5.1 Air

The most important process by which naphthalene is removed from the atmosphere is degradation through reaction with photochemically produced hydroxyl radicals. The rate of this reaction is 2.17×10^{-11} cm^3/molecule-sec, with a half-life of 3 to 8 hours. The major by-products are 1- and 2-naphthol and 1- and 2-nitronaphthalene. Although photolysis is expected to occur, no experimental data on photolysis of naphthalene were found. In polluted urban air, reaction of naphthalene with NO_3 radicals may result in additional loss from the atmosphere. Naphthalene also will react with N_2O_5, nitrate radical, and ozone in the atmosphere (ATSDR 1995, HSDB 2002a).

2.5.2 Water

Photolysis, volatilization, biodegradation, and adsorption all may be important loss mechanisms for naphthalene discharged into water. The half-life of naphthalene is estimated to be around 71 hours in surface water and 550 days in deeper water (5 meters). Biodegradation of naphthalene is expected to be a dominant fate process in aquatic ecosystems. In oil-contaminated water not exposed to sunlight (because the water is murky or the water depth is great), biodegradation of naphthalene has a half-life of 7 days. In unpolluted water, biodegradation occurs slowly, with a half-life of 1,700 days (ATSDR 1995, HSDB 2002a).

2.5.3 Soil

The sorption of naphthalene to soil will be low to moderate, depending on the soil's organic carbon content, and is not considered to be the major fate process. Naphthalene is expected to biodegrade to carbon dioxide in aerobic soils, with salicylate as an intermediate product. Abiotic degradation of naphthalene is not expected to occur in soil

(ATSDR 1995). Naphthalene's biodegradation rate in soils varies considerably. The estimated half-life of naphthalene in a solid-waste site was 3.6 months. Its half-life was 11 to 18 days in soils with 0.2% to 0.6% organic carbon and 92% to 94% sand and 2 to 3 days in sandy loam with 0.5% to 1% organic carbon (ATSDR 1995). When naphthalene was incubated in two sandy loam soils at concentrations typical of those in waste-disposal sites, 30% of the naphthalene was lost by volatilization in 48 hours, and its half-life in the two soils was 2.1 and 2.2 days (HSDB 2002a).

2.6 Environmental exposure

The main source of exposure to naphthalene is through inhalation in ambient and indoor air. Exposure to small amounts of naphthalene through drinking water also may occur. Naphthalene was detected in 2 of 13,980 samples of food analyzed in six states. Accidental ingestion of naphthalene-containing household products has been reported, especially in children. In 1990, 72 poison control centers in the United States reported 2,400 cases of accidental naphthalene exposure. Dermal exposure to naphthalene may occur through handling or wearing of clothing stored with naphthalene-containing moth repellents (ATSDR 1995).

2.6.1 Air

Exposure of the general public to naphthalene in indoor air most likely occurs through the use of naphthalene-containing moth repellents and smoking. Kerosene heaters may be another source of indoor exposure. Levels of naphthalene in indoor air were measured in various homes at levels ranging from 0.860 to 1,600 $\mu g/m^3$. However, according to the author, the upper range limit may be an error (ATSDR 1995).

The general public is exposed to naphthalene in ambient air, particularly in areas with heavy traffic, near petroleum refineries or coal tar distillation facilities, or where evaporative losses from the storage, transport, transfer, or disposal of fuel oil occurs. The average reported concentration of naphthalene in 67 air samples at several locations in the United States was 0.991 ppb (5.19 $\mu g/m^3$). The median naphthalene level in air at urban sites in 11 U.S. cities was 0.18 ppb (0.94 $\mu g/m^3$) (ATSDR 1995).

An average naphthalene concentration of 170 $\mu g/m^3$ in ambient air was measured in a residential area in Ohio, while a concentration of 3.3 $\mu g/m^3$ was measured in California. Ambient air at five hazardous waste sites and one landfill in New Jersey ranged from 0.42 to 4.6 $\mu g/m^3$ (0.08 to 0.88 ppb), while the average concentration inside cars in commuter traffic was reported at 4.5 $\mu g/m^3$ (ATSDR 1995).

Based on an average naphthalene concentration of 0.95 $\mu g/m^3$ in urban and suburban air and an inhalation rate of 20 m^3/day, daily intake of naphthalene from ambient air has been estimated at 19 μg (ATSDR 1995).

2.6.2 Water

Naphthalene rarely is detected in drinking water. Only one area in the United States reported naphthalene in the drinking water at levels up to 1.4 μg/L. Naphthalene has been detected in the surface water and groundwater in the United States. Data collected from

EPA's water quality storage and retrieval (STORET) database indicate that for 1980 to 1982, 7% of 630 ambient water samples contained naphthalene. The median concentration in these samples was less than 10 μg/L. Naphthalene was detected in 11% of 86 urban runoff samples at concentrations ranging from 0.8 to 2.3 μg/L. Naphthalene also was detected in 35% of groundwater samples at five wood-treatment facilities at an average concentration of 3,312 μg/L (ATSDR 1995).

Based on a concentration range of 0.001 to 2 μg/L, naphthalene exposure from drinking water is estimated at 0.002 to 4 μg per day (ATSDR 1995).

2.6.3 Soil

Naphthalene has been reported at low concentrations in uncontaminated soils, with levels ranging from 0 to 3 μg/kg in untreated agricultural soils. In contaminated soils, naphthalene levels reached 6.1 μg/g in soil contaminated with coal tar, up to 66 μg/kg in sludge-treated soils, and 16.7 mg/kg in soil from a former tar-oil refinery. However, exposure of the general public to naphthalene in soil is not expected (ATSDR 1995).

2.6.4 Other

Naphthalene has rarely been detected in food products in the United States. In a study in six states, naphthalene was detected in two out of 13,980 food samples. Naphthalene concentrations were reported ranging from 5 to 176 ng/g in oysters, 4 to 10 ng/g in mussels, and from less than 1 to 10 ng/g in clams from waters in the United States (ATSDR 1995).

Naphthalene was detected in ash from municipal refuse and hazardous waste incinerators at levels ranging from 6 to 28,000 μg/kg and 0.17 to 41 mg/kg, respectively. The level of naphthalene in smoke from an unfiltered cigarette was 3 μg, while the level in sidestream smoke was 46 μg/cigarette (ATSDR 1995).

2.7 Occupational exposure

EPA has identified these individuals as having potential exposure to naphthalene in the workplace: beta-naphthol makers, celluloid makers, coal tar workers, dye chemical makers, fungicide makers, hydronaphthalene makers, moth repellent workers, phthalic anhydride makers, smokeless powder makers, tannery workers, textile chemical workers, and aluminum reduction plant workers. The highest reported vapor concentrations occurred in an area described as "naphthalene melt present" and ranged from 1,600 to 1,100,000 μg/m^3 (0.3 to 220 ppm) (EPA 1980). Concentrations in other industrial areas were lower. The air concentrations of naphthalene in an aluminum reduction plant were 0.72 to 311.3 μg/m^3 (0.1 to 59.5 ppb) as vapor and 0.090 to 4.00 μg/m^3 as a particulate (Bjørseth *et al.* 1978a). Levels at a coke oven were 11.35 to 1,120 μg/m^3 (2 to 214 ppb) as vapor and 0 to 4.40 μg/m^3 as a particulate (Bjørseth *et al.* 1978b). The air concentrations of naphthalene in work areas of a silicon carbide plant ranged from 1.3 to 58 μg/m^3 (0.2 to 11 ppb) (Dufresne *et al.* 1987). In another study, average naphthalene concentrations were 0.08 μg/m^3 (0.015 ppb) as vapor and 11.43 μg/m^3 as a particulate in paving/roofing/steel/silicon carbide industries, 16.30 μg/m^3 as a particulate in refractory

brick industries [no vapor concentration was reported], 0.01 μg/m^3 (0.002 ppb) as vapor and 75.40 μg/m^3 as a particulate in silicon carbide industries, and 0.52 μg/m^3 (0.1 ppb) as vapor and 1,111 μg/m^3 as a particulate in aluminum refinery industries (Lesage *et al.* 1987).

Hicks (1995) reported average naphthalene concentrations collected from the breathing zones of workers ranging from 2.3 μg/m^3 in hot mix plants to 7.5 μg/m^3 in roofing manufacturing industries. In the same study, dermal wipe samples collected from the back of the hand or forehead of selected workers showed detectable levels in less than 10% of the samples, with concentrations ranging from 5.5 to 520 ng/cm^3.

A survey by the National Institute for Occupational Safety and Health (NIOSH) in 1980 reported air concentrations of naphthalene as high as 10.2 μg/m^3 in an area sample and 19.3 μg/m^3 in a personal sample. The National Occupational Exposure Survey, conducted from 1981 to 1983, estimated that 112,702 workers potentially were exposed to naphthalene (ATSDR 1995).

2.8 Biological indices of exposure

Little information is available about metabolism of naphthalene in humans; however, urinary metabolites in various animal species have been reported (see Sections 1.3 and 6.1). Naphthol (isomer not specified) was found in the urine of a patient four days after naphthalene ingestion. In another study, urine of an 18-month-old child was found to contain 1-naphthol, 2-naphthol, 1,2-naphthoquinone, and 1,4-naphthoquinone nine days after exposure to naphthalene (ATSDR 1995).

1-Naphthol was found in the urine of workers employed in a plant that distilled naphthalene oil with concentrations ranging from 0.4 to 34.6 mg/L. A good statistical correlation was shown between naphthalene exposure between 0.2 and 6 mg/m^3 and urinary excretion of 1-naphthol (Bieniek 1994). Follow up studies show that most naphthalene metabolizes to 1-naphthol, 2-naphthol, and 1,4-naphthoquinone. Significant differences were found between the urine concentrations of 1- and 2-naphthol in workers exposed in a coke plant compared to non-exposed workers. A strong correlation ($r = 0.76$) was also found between the naphthalene in breathing-zone air and concentrations of 1-naphthol and 2-naphthol in post-shift urine. While occupational exposure to naphthalene can be determined by using 1-naphthol as a biomarker; using 1-naphthol to determine general exposure to naphthalene may be misleading because 1-naphthol is also excreted after exposure to the common insecticide carbaryl. Urinary 1-naphthol and 2-naphthol, however, seem to be useful biomarkers to determine naphthalene exposure (Bieniek 1997). A later study showed that smoking should be considered when using 1-naphthol and 2-naphthol as biomarkers for naphthalene exposure. Urinary levels were three and seven-fold higher for 1-naphthol and 2-naphthol, respectively, among smokers than among non-smokers. Genetic polymorphisms of CYP2E1 and GSTM1 also affected urinary levels of these naphthols. Diet and age were not a factor in the use of these urinary metabolites (Yang *et al.* 1999).

Using radiolabeled (^{14}C) naphthalene, *in vivo* percutaneous absorption studies were performed using spiked JP-8, the major jet fuel used by the U.S. Army and Air Force,

and excised pig ear skin and human skin. Naphthalene permeated significantly through both skins, and permeation rates were found to be proportional to its concentration in JP-8 (Kanikkannan *et al.* 2001a). JP-8 + 100 is a new fuel introduced by the U.S. Air Force that contains JP-8 and additives. Permeation of naphthalene in JP-8 + 100 was significantly higher in pig ear skin than that of JP-8 (Kanikkannan *et al.* 2001b).

In 1982, the National Human Adipose Tissue Survey detected naphthalene in wet adipose tissue with a frequency of 40%, at a concentration range of less than 9 to 63 ppb. Six of eight samples of mothers' milk from four U.S. urban areas had detectable levels of naphthalene (HSDB 2002a).

2.9 Regulations

EPA regulates naphthalene under the Clean Air Act (CAA) as a hazardous air pollutant and under the Clean Water Act (CWA) and the Safe Drinking Water Act (SWDA) as a water pollutant. EPA also regulates naphthalene under the Resource Conservation and Recovery Act (RCRA) and as a toxic chemical under the Superfund Amendments and Reauthorization Act (SARA) and subjects it to general threshold limits. Naphthalene is subject to reporting and recordkeeping rules under the Toxic Substances Control Act (TSCA). NIOSH has set an eight-hour time-weighted-average (TWA) recommended exposure limit (REL) of 10 ppm (50 mg/m^3), with a short-term exposure limit (STEL) ceiling of 15 ppm (75 mg/m^3). The American Conference of Governmental Industrial Hygienists (ACGIH) has set a threshold limit value of 10 ppm (52 mg/m^3), with a STEL ceiling of 15 ppm (79 mg/m^3). The Occupational Safety and Health Administration (OSHA) regulates naphthalene, with an eight-hour TWA permissible exposure level (PEL) of 10 ppm (50 mg/m^3).

EPA regulations are summarized in Table 2-5, and OSHA regulations in Table 2-6. No U.S. Food and Drug Administration regulations were found for naphthalene, although sodium mono- and di-methyl naphthalene sulfonates are allowed as food additives.

Table 2-5. EPA regulations

Regulatory action	Effect of regulation or other comments
40 CFR 60—PART 60—STANDARDS OF PERFORMANCE FOR NEW STATIONARY SOURCES. Promulgated: 36 FR 24877, 12/23/71. Subparts NNN and RRR.	The intent of these standards is to minimize the emissions of volatile organic compounds like naphthalene through the application of best demonstrated technology.
40 CFR 61—PART 61—NATIONAL EMISSION STANDARDS FOR HAZARDOUS AIR POLLUTANTS. Promulgated: 38 FR 8826, 04/06/73. U.S. Code: 7401, 7412, 7414, 7416, and 7601.	This part lists substances that, pursuant to section 112 of the CAA, have been designated as hazardous air pollutants (HAPs). Naphthalene is classified as a hazardous air pollutant.

Regulatory action	Effect of regulation or other comments
40 CFR 63—PART 63—NATIONAL EMISSION STANDARDS FOR HAZARDOUS AIR POLLUTANTS FOR SOURCE CATEGORIES. Promulgated: 57 FR 61992, 12/29/92. U.S. Code: 7401 *et seq.*	Standards that regulate specific categories of stationary sources that emit (or have potential to emit) air pollutants, such as naphthalene, are listed in this part pursuant to section 112(b) of the CAA. Areas where naphthalene is identified as a HAP are in the polymers and resin industries, wood manufacturing operations, off-site waste and recovery operations, and petroleum refineries.
40 CFR 116—PART 116—DESIGNATION OF HAZARDOUS SUBSTANCES. Promulgated: 43 FR 10474, 03/13/78. U.S. Code: 33 U.S.C. 1251 et seq.	This regulation designates hazardous substances under section 311(b)(2)(a) of the FWPCA. The regulation applies to discharge of naphthalene to surface waters.
40 CFR 117—PART 117—DETERMINATION OF REPORTABLE QUANTITIES FOR HAZARDOUS SUBSTANCES. U.S. Code: FWPCA 311(b)(2)(A) and 501(a) as amended by the CWA of 1977.	Discharges to water of amounts equal to or greater than the reportable quantity (RQ) must be reported to EPA. The RQ for environmental releases to water of naphthalene is 10 lb (4.54 kg).
40 CFR 141—PART 141—NATIONAL PRIMARY DRINKING WATER REGULATIONS. Promulgated: 40 FR 59570, 12/24/75. U.S. Code: Public Health Service Act sections 1413–1416, 1445, and 1450 as amended by 1974 SDWA; U.S.C. 300.	To protect a safe drinking water supply, community and non-transient, non-community water systems must monitor for naphthalene.
40 CFR 172—PART 172—Subpart B—Table of Hazardous Materials and Special Provisions. Promulgated: 55 FR 52582, 12/21/90. Naphthalene has a UN number of 1334.	The Hazardous Materials Table in this section designates the materials listed therein as hazardous materials for the purpose of transportation of those materials. Naphthalene is identified in the table.
40 CFR 258—PART 258—CRITERIA FOR MUNICIPAL SOLID WASTE LANDFILLS. Promulgated: 56 FR 51016, 10/09/91. U.S. Code: 33 U.S.C. 1345(d) and (e); 42 U.S.C. 6907(a)(3), 6912(a), 6944(a), and 6949a(c).	The provisions of this part establish minimum national criteria under RCRA, as amended, for all municipal solid waste landfill (MSWLF) units and under the CWA, as amended, for MSWLF that are used to dispose of sewage sludge. The criteria ensure the protection of human health and the environment. The practical quantitation limit for naphthalene is 101 mg/L.
40 CFR 261—PART 261—IDENTIFICATION AND LISTING OF HAZARDOUS WASTE, Appendix VIII—Hazardous Constituents. Promulgated: 45 FR 33119, 05/19/80; 53 FR 13388, 04/22/88. U.S. Code: 42 U.S.C. 6905, 6912(a), 6921, 6922, and 6938.	Appendix VIII is a consolidated list of hazardous constituents identified in this part. Solid wastes containing these constituents are subject to notification requirements of RCRA section 3010 and must be disposed of in RCRA-permitted facilities. Naphthalene has a waste number of U165.
40 CFR 302—PART 302—DESIGNATION, REPORTABLE QUANTITIES, AND NOTIFICATION. Promulgated: 50 FR 13474, 04/04/85. U.S. Code: 42 U.S.C. 9602, 9603, and 9604; 33 U.S.C. 1321 and 1361.	Naphthalene is listed as a hazardous substance with an RQ of 100 lb (45.4 kg).

Regulatory action	Effect of regulation or other comments
40 CFR 372—PART 372—TOXIC CHEMICAL RELEASE REPORTING: COMMUNITY RIGHT-TO-KNOW. Promulgated: 53 FR 4525, 02/16/88. U.S. Code: 42 U.S.C. 11013 and 11028.	This part sets forth requirements for the submission of information relating to the release of toxic chemicals under section 313 of Title III of SARA (1986). Information collected under this part is intended to inform the general public and the communities surrounding covered facilities about releases of toxic chemicals, to assist research, and to aid in the development of regulations, guidelines, and standards. See section 372.65 for chemicals and chemical categories to which this part applies.
40 CFR 401—PART 401—GENERAL PROVISIONS. Promulgated: 39 FR 4532, 02/01/74, as amended at 47 FR 24537, 06/04/82. U.S. Code: 33 U.S.C. 1251 *et seq.*	Regulations promulgated prescribe effluent limitations guidelines for existing sources, standards of performance for new sources, and pretreatment standards for new and existing sources. Naphthalene is considered a toxic pollutant.
40 CFR 704—PART 704—REPORTING AND RECORDKEEPING REQUIREMENTS. Promulgated: 49 FR 33653, 08/24/84; U.S. Code: 15 U.S.C. 2607(a).	This part specifies reporting and recordkeeping procedures under section 8(a) of TSCA for manufacturers, importers, and processors of chemical substances and mixtures such as naphthalene.
40 CFR 716—PART 716—HEALTH AND SAFETY DATA REPORTING. Promulgated: 51 FR 32726, 09/15/86. U.S. Code: 15 U.S.C. 2607(d).	The provisions of this part require the submission of lists and copies of health and safety studies on chemical substances and mixtures selected for priority consideration for testing rules under section 4(a) of TSCA and on naphthalene.

Source: The regulations in this table have been updated through the 2001 Code of Federal Regulations 40 CFR, 1 July 2001.

Table 2-6. OSHA regulations

Regulatory action	Effect of regulation or other comments
29 CFR 1910.1000—Sec. 1910.1000 Air contaminants. Promulgated: 58 FR 40191, 07/27/93. U.S. Code: 5 U.S.C. 553.	OSHA sets the PEL at 10 ppm (50 mg/m^3) as an 8-h TWA.
29 CFR 1915.1000—Sec. 1915.1000 Air contaminants. Promulgated: 61 FR 31430, 06/20/96.	OSHA sets the PEL at 10 ppm (50 mg/m^3) as an 8-h TWA for shipyards.

Source: The regulations in this table have been updated through the 2001 Code of Federal Regulations 29 CFR, 1 July 2001.

3 Human Cancer Studies

Two case-series of cancer occurring in individuals exposed to naphthalene have been reported: laryngeal and other cancers occurring in naphthalene-exposed workers in Germany and colorectal carcinoma occurring among individuals in Africa who had used a naphthalene compound for medicinal reasons. Wolf (1976, 1978, as cited in NTP 1992, 2000) reported a cluster of cancer including four cases of laryngeal and one case each of gastric and colon cancer occurring in 6 of 15 naphthalene distillation plant workers in East Germany (now part of the Federal Republic of Germany). Ajao *et al*. (1988) reported that of 23 cases of colorectal carcinoma diagnosed between June 1982 and 1984, 11 were in patients under 30 years of age. None of the early-onset cases were regarded as cases of familial polyposis. Half of the patients with early-onset colorectal cancer reported consumption of kafura, which is a naphthalene compound used to treat anorectal problems; the other half did not recall whether they had been treated with this compound as a child.

The available data are insufficient for evaluation of the carcinogenicity of naphthalene in humans.

4 Studies of Cancer in Experimental Animals

The NTP conducted two-year inhalation-exposure studies of the carcinogenicity of naphthalene in B6C3F$_1$ mice (NTP 1992, see Appendix A) and F344/N rats (NTP 2000, see Appendix B). Other relevant studies include short-term carcinogenicity tests (Tsuda *et al.* 1980, Adkins *et al.* 1986), an intraperitoneal injection study in mice (LaVoie *et al.* 1988), and a chronic feeding study in rats (Schmahl 1955, cited in NTP 2000). These data are reviewed in this section. The International Agency for Research on Cancer (IARC) reviewed naphthalene in February 2002 and concluded that naphthalene was possibly carcinogenic to humans based on sufficient evidence of carcinogenicity in experimental animals (Group 2B) (IARC 2002).

4.1 Studies in mice

4.1.1 NTP carcinogenicity bioassay

The NTP selected naphthalene for study because there was inadequate information for regulatory decisions and there was potential for chronic human exposure. This study (NTP 1992) also was published by Abdo *et al.* (1992). The purity of the naphthalene used in this study was greater than 99%; samples contained 0.23% water and 0.15% total impurities (NTP 1992).

Groups of 10- to 11-week-old B6C3F$_1$ mice (75 of each sex) were exposed to naphthalene by inhalation at a concentration of 0, 10, or 30 ppm (0, 50, or 150 mg/m^3), six hours/day, five days/week, for 104 weeks (NTP 1992). Two additional groups of mice (75 of each sex) were exposed to naphthalene at 30 ppm, to ensure that a sufficient number of high-dose animals survived to the end of the study. The original study design called for interim sacrifices at 14 days and 3, 6, 12, and 18 months for hematology evaluations. However, the 3-, 6-, 12-, and 18-month interim evaluations were cancelled because of high mortality in the male control group attributed to fighting-related wounds in the group housed mice.

To avoid condensation in the exposure chambers, the high concentration was approximately half of the saturation concentration for naphthalene vapor at 20°C. The low concentration was the ACGIH occupational threshold limit value. The available data indicate that occupational exposure to naphthalene vapor generally is much lower than the threshold limit value (< 0.001 to about 0.2 ppm); however, concentrations ranging from 0.3 to 220 ppm were reported in an industrial area described as “naphthalene melt present” (see Section 2.7).

Naphthalene vapor was generated by direct sublimation from a 500-mL flask and was delivered with nitrogen through metering valves. Average exposure-chamber concentrations were maintained within 20% of the target concentrations throughout the study period, except that during week 4, the average concentration in the low-dose chamber was only 5.5 ppm.

Mice were housed five per cage, with water available *ad libitum* and feed available *ad libitum* except during exposure periods. All animals were observed twice daily and were weighed at the beginning of the study, weekly for the first 13 weeks, and monthly

thereafter. Hematology parameters were measured for up to five mice of each sex from each chamber 14 days after study initiation. All animals were necropsied, and all organs and tissues were examined for grossly visible lesions. A complete histopathologic examination was performed on all control and high-dose animals and on all animals dying or killed moribund before 21 months. For the low-dose group, histopathologic examination was limited to the lungs and nasal cavity.

Naphthalene exposure did not significantly affect survival of female mice. However, in male mice, survival at the end of the study was 37%, 75%, and 89% in the control, low-dose, and high-dose groups, respectively, and increased survival was significantly associated with increasing exposure. Low survival in the control group was attributed to increased fighting in this group (see Appendix A, pp. A-25 and A-26, Table 3 and Figure 3 in NTP 1992). Nonetheless, more than 50% of the male control group survived to week 92, leaving a sufficient number for evaluation of carcinogenicity. Mean body weights of exposed female mice were slightly lower than, but within 10% of, the mean weight of the controls throughout the study. Mean body weights were slightly lower in exposed male mice than in controls for the first 18 months (see Appendix A, pp. A-23 to A-24, Figure 2 in NTP 1992). No clinical findings were attributed to naphthalene exposure.

Naphthalene exposure did not significantly increase the incidences of neoplasms in male mice. The incidences of pulmonary alveolar/bronchiolar adenoma, carcinoma, and alveolar/bronchiolar adenoma or carcinoma combined were similar in exposed and control male mice after adjustment for survival differences (Table 4-1). Tumor incidences generally were within the historical control ranges.

Increased incidences of some lung neoplasms, primarily alveolar/bronchiolar adenoma, were observed in high-dose female mice and attributed to naphthalene exposure (Table 4-1). Papillary adenoma of the nose was observed in two low-dose female mice, but not attributed to naphthalene exposure.

Alveolar/bronchiolar adenoma and carcinoma form a morphologic continuum. Adenomas were locally compressive nodular masses consisting of cords of well-differentiated epithelial cells, whereas carcinomas were composed of ribbons and/or coalescing sheets of smaller, more anaplastic cells, which sometimes extended into adjacent parenchyma (NTP 1992).

In addition to the neoplastic lesions, naphthalene exposure increased the incidences and severity of chronic inflammation of the nose and lungs, metaplasia of the olfactory epithelium, and hyperplasia of the respiratory epithelium in the nose (Table 4-2). The more advanced inflammatory lesions were called “granulomatous inflammation” and were characterized by cellular infiltrates with large, foamy macrophages, sometimes accompanied by multinucleated giant cells. Although the severity of the non-neoplastic lesions generally was minimal to mild, they were considered features of an overall inflammatory response that was directly related to naphthalene exposure.

Table 4-1. Incidences of lung tumors in B6C3F$_1$ mice following inhalation exposure to naphthalene for two years

Sex	Conc. (ppm)	N	Lung tumor incidence (%)[a]		
			Adenoma	Carcinoma	Combined
Male	0	70			7 (25.7)
	10	69			17 (31.9)
	30	135			31 (26.0)
	Trend				NS
	HC[b]	478			94 (10–30)
Female	0	69			5 (8.3)
	10	65			2 (3.5)
	30	135			29 (26.5)**
	Trend				$P \leq 0.001$
	HC[b]	466			39 (0–12)

Source: NTP 1992.

**$P \leq 0.01$, NS = not significant (logistic regression test).

[a]Kaplan-Meier estimated tumor incidence at the end of the study after adjustment for intercurrent mortality.

[b]Historical control incidence from all NTP inhalation studies (range %).

Table 4-2. Incidences of non-neoplastic lesions in B6C3F$_1$ mice following inhalation exposure to naphthalene for two years

Sex	Conc. (ppm)	N	Incidence (%)				
			Nose			Lung	
			Chronic inflammation	Metaplasia olfactory epithelium	Hyperplasia respiratory epithelium	Chronic inflammation	Granulomatous inflammation
Male	0	70	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	10	69	67 (97)***	66 (96)***	66 (96)***	21 (30)***	19 (28)***
	30	135	133 (99)***	134 (99)***	134 (99)***	56 (41)***	15 (11)*
	Trend		$P \leq 0.001$	$P \leq 0.001$	$P \leq 0.001$	$P \leq 0.001$	NS
Female	0	69	1 (1)	0 (0)	0 (0)	3 (4)	0 (0)
	10	65	65 (100)***	65 (100)***	65 (100)***	13 (20)**	38 (58)***
	30	135	135 (100)***	135 (100)***	135 (100)***	52 (39)***	42 (31)***
	Trend		$P \leq 0.001$	$P \leq 0.001$	$P \leq 0.001$	$P \leq 0.001$	$P = 0.004$

Source: NTP 1992.

*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$ (logistic regression test).

4.1.2 Strain A mouse lung tumor bioassay

The strain A mouse lung tumor bioassay was investigated as a short-term *in vivo* model for predicting the carcinogenicity of chemicals administered by inhalation (Adkins *et al.* 1986). These mice are highly susceptible to lung cancer and have been used extensively in bioassays for assessing the carcinogenic activity of many chemicals (Stoner *et al.* 1993). Groups of 30 female Strain A/J mice (six to eight weeks old) were exposed to reagent-grade naphthalene (98% to 99% purity) at a concentration of 0, 10, or 30 ppm, six hours/day, five days/week, for six months. The positive-control group of 20 mice received a single i.p. injection of urethane. Food and water were available *ad libitum* during nonexposure periods. The animals were sacrificed after six months, the lungs were removed and fixed for 24 hours, lung nodules were counted by three or more technicians, and sections of lungs with nodules were histopathologically examined. The mean numbers of adenomas per mouse were compared with a one-way analysis of variance (Kruskal-Wallis test).

Survival was not affected by naphthalene exposure. Of the 30 animals in each group, 29 survived in the chamber-control and high-dose groups and 27 in the low-dose group. All mice in the positive control group survived to the end of the study and developed adenomas, an average of 28.9 per mouse. The incidence of alveolar adenoma was slightly higher in the low-dose (29%) and high-dose (30%) groups than in the chamber controls (21%); however, the differences were not statistically significant. The average number of tumors per mouse, 0.21, 0.35, and 0.37 in the control, low-dose, and high-dose groups, respectively, did not differ significantly. The number of tumors per tumor-bearing lung was significantly greater ($P < 0.05$) in the naphthalene-exposed mice than in the chamber controls; however, the number of tumors per tumor-bearing lung was significantly lower

in the chamber controls than in historical controls of this mouse strain (Adkins *et al.* 1986).

4.2 Studies in rats

4.2.1 NTP carcinogenicity bioassay

NTP selected naphthalene for study in rats because of its carcinogenicity in mice (see Section 4.1.1) and because previous studies in rats had not used inhalation exposure and were inadequate. The naphthalene used in this study had total impurities of 0.6% (NTP 2000).

Groups of six-week-old F344/N rats (49 of each sex) were exposed to naphthalene by inhalation at a concentration of 0, 10, 30, or 60 ppm, six hours/day, five days/week, for 105 weeks, resulting in estimated daily doses of 0, 3.6 to 3.9, 10.7 to 11.4, or 20.1 to 20.6 mg/kg body weight (b.w.). The highest exposure level was the highest concentration that can be generated without condensation. For evaluation of toxicokinetic parameters, additional groups of rats (9 of each sex) were exposed to naphthalene at a concentration of 10, 30, or 60 ppm for up to 18 months. The animals were housed individually; water was available *ad libitum,* and feed was available *ad libitum* except during exposure periods. All animals were observed twice daily. Clinical findings were recorded every four weeks to week 92 and every two weeks thereafter. Animals were weighed at the beginning of the study, every four weeks from week 4 to week 92, and every two weeks thereafter. Complete necropsies and microscopic examinations were performed on all core study animals. All tissues and organs were examined for grossly visible lesions, and all major tissues were fixed and preserved for microscopic examination. Blood samples for toxicokinetic evaluation were drawn after 2 weeks and 3, 6, 12, and 18 months. The samples were collected from three males and three females per group at six time points after exposure (0 to 480 minutes for the 10-ppm group, 0 to 720 minutes for the 30-ppm group, and 0 to 960 minutes for the 60-ppm group) and analyzed for naphthalene concentrations.

Naphthalene vapor was generated from a 2-L glass reaction flask surrounded by a heated mantle. Heated nitrogen metered into the flask carried the vaporized naphthalene out of the generator, and the vapor was transported to the exposure room through a heated Teflon line. The vapor was diluted with heated air filtered through a high-efficiency particulate air filter and charcoal before entering a distribution manifold. Average concentrations were maintained within 1% of the target concentrations throughout the study period.

There were no significant differences in survival rates between exposed and control male or female rats (see Appendix B, pp. B-33 and B-34, Table 3 and Figure 2 in NTP 2000). Throughout the study, mean body weights were lower in exposed males than in controls, whereas mean body weights of exposed and control females were generally similar (see Appendix B, pp. B-35 to B-37, Figure 2 and Tables 4 and 5 in NTP 2000). There were no clinical findings related to naphthalene exposure.

Rats exposed to naphthalene had increased incidences of several neoplasms and nonneoplastic lesions of the nose. Lesions occurred in all three levels of the nasal cavity

that are routinely examined in NTP studies. Malignant nasal neoplasms frequently blocked the nasal passages or destroyed the normal architecture of the nasal turbinates and occasionally invaded the brain. Nasal neoplasms included neuroblastoma of the olfactory epithelium and adenoma of the respiratory epithelium (Table 4-3).

Neuroblastoma incidence showed a dose-related trend in both male and female rats, and the incidence in female rats at 60 ppm was significantly higher than that in chamber controls. Nasal adenoma incidence also showed a dose-related trend in male rats and was significantly increased in all exposed groups. The incidence of nasal adenoma was slightly higher in female rats at 30 or 60 ppm than in controls, but the differences were not significant. These types of nasal tumors are extremely rare in F344 rats. Neither neuroblastomas nor nasal adenomas were observed in any animals in historical control groups.

The neuroblastomas were variably sized unilateral or bilateral invasive masses that arose in Level III of the nasal cavity and extended into Levels I and II. Other masses extended along the mucosa and replaced the epithelium of the turbinates and nasal septum. The morphology of the neoplasms varied. Component neoplastic cells were round, polygonal, or spindle-shaped and arranged in variably sized irregular islands, cords, and rosettes separated by fibrovascular stroma. In other masses, component cells were arranged in a glandular pattern. Some cells had scant eosinophilic to amphophilic cytoplasm with pale oval to polygonal vesicular nuclei and prominent central nucleoli; others had abundant cytoplasm and elongate, intensely basophilic nuclei. Small nests of neoplastic cells were present in the lamina propria of the turbinates and nasal septum and in olfactory nerve bundles. A few neoplasms had focal irregular areas of squamous metaplasia, sometimes extensive, with formation of keratin pearls. Variably sized focal areas of coagulative necrosis also were observed in most neoplasms. Mitotic figures were abundant. Neoplams that invaded the cribriform plate extended into the olfactory lobes of the brain (NTP 2000).

Adenomas arose from the respiratory and transitional epithelia of Levels I and II of the nasal cavity along the medial or lateral aspects or tips of the nasoturbinates or the lateral wall. They were irregular exophytic, polypoid, pedunculated or broad-based sessile masses that varied in size and sometimes partially occluded the nasal passages. Component neoplastic cells were well differentiated, simple to cuboidal to columnar and arranged primarily as variably sized glands surrounded by scant fibrovascular stroma with few focal solid areas of cells. In some masses, the epithelium appeared to be pseudostratified. The glands often were variably distended by luminal accumulations of proteinaceous secretory material and cellular debris. A few adenomas were composed of less well differentiated cells that were squamoid in morphology; these cells were large, round to polygonal, with scant to moderate amounts of eosinophilic cytoplasm and large round to oval nuclei containing one or two prominent nucleoli (NTP 2000).

Table 4-3. Nasal tumor incidences in F344/N rats following inhalation exposure to naphthalene for two years

Sex	Conc. (ppm)	N	Tumor incidence (%)[a]

			Adenoma (respiratory epithelium)	Neuroblastoma (olfactory epithelium)	Brain metastasis
Male	0	49			0
	10	49			0
	30	48			0
	60	48			2
	Trend				
	HC[b]	299			
Female	0	49			0
	10	49			1
	30	49			0
	60	49			4
	Trend				
	HC[b]	299			

Source: NTP 2000.

*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$ (poly-3 test).

[a]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.

[b]Historical control incidences based on all NTP inhalation studies that used the new NTP 2000 diet. Incidences from studies using the former NIH-07 diet were 0/1,048 males and 0/1,044 females.

Unlike mice (see Section 4.1.1), rats did not show increased incidences of lung neoplasms. Alveolar/bronchiolar adenoma or carcinoma combined occurred in 2/49, 3/49, 1/48, and 0/49 male rats exposed to naphthalene at 0, 10, 30, or 60 ppm, respectively. The only lung neoplasm observed in female rats was in the control group. The only effects in rat lung that may have been related to naphthalene exposure were minimal chronic inflammation (males) and alveolar epithelial hyperplasia (females).

Several non-neoplastic lesions of the nose were significantly more common in exposed rats than in chamber controls (see Appendix B, pp. B-39, Table 6 in NTP 2000). These included epithelial and goblet-cell hyperplasia, squamous metaplasia, and hyaline degeneration of the respiratory epithelium; atrophy, atypical (basal-cell) hyperplasia, inflammation, and hyaline degeneration of the olfactory epithelium; and hyperplasia and squamous metaplasia of the Bowman's glands in the olfactory region of the nose. Based on a severity scale with 1 = minimal, 2 = mild, 3 = moderate, and 4 = marked, the severity of olfactory epithelial and glandular lesions increased in a dose-related manner from mild (1.9 to 2.2) to moderate (2.9 to 3.5) severity. Most of these lesions are commonly observed in inhalation studies with chemical irritants and are adaptive responses. Ward *et al.* (1993) reviewed 19 inhalation bioassays conducted by the NTP and reported that nasal lesions were reported for 5/5 nasal carcinogens and for 12/14 nasal noncarcinogens. They concluded that while cell proliferation may be a factor in multistage carcinogenesis, the association between cell proliferation and carcinogenesis is not always demonstrable.

Olfactory epithelial atypical (basal-cell) hyperplasia was not observed in the control groups, nor were similar lesions reported in other NTP inhalation studies. This lesion occurred in 88% to 98% of the exposed rats and increased in severity with dose. The cells involved in olfactory epithelial atypical hyperplasia and focal areas of intraepithelial hyperplasia or dysplasia appeared to form a continuum with the neuroblastoma. Respiratory epithelial adenoma was not clearly associated with any of the non-neoplastic lesions. Lung lesions that may have been related to naphthalene exposure included chronic inflammation (males) and alveolar epithelial hyperplasia (females) (Table 4-4).

Table 4-4. Incidences of selected non-neoplastic lesions in F344 rats following inhalation exposure to naphthalene for two years

			Incidence (%)		
			Nose	Lung	
Sex	Conc. (ppm)	N	Atypical hyperplasia olfactory epithelium	Chronic inflammation	Alveolar epithelial hyperplasia
Male	0	49	0 (0)	2 (4)	23 (47)
	10	49	48 (98)**	13 (27)*	12 (24)
	30	48	45 (94)**	6 (13)	9 (19)
	60	48	46 (96)**	15 (31)*[a]	16 (33)
Female	0	49	0 (0)	16 (33)	4 (8)
	10	49	48 (98)**	15 (31)	11 (22)*
	30	49	48 (98)**	19 (39)	11 (22)*
	60	49	43 (88)**	22 (45)	9 (18)

Source: NTP 2000.

*$P \leq 0.05$, **$P \leq 0.01$ (poly-3 test)

[a]49 rats examined.

4.2.2 Other studies

Tumors were not observed in rats orally administered naphthalene once daily, six days/week, for 700 days (Schmahl 1955, cited in NTP 2000). The total dose administered was 10 g, or about 41 mg/kg b.w. per day. No controls were reported, and only 28 rats were used. In the same study, rats given 820 mg of naphthalene subcutaneously or intraperitoneally over a 40-week period did not develop tumors.

Tsuda *et al.* (1980) investigated the induction of resistant hepatocytes as a possible short-term *in vivo* test for carcinogenicity by testing 21 carcinogenic chemicals and 7 noncarcinogenic analogs. Naphthalene was included as one of the noncarcinogenic analogs. Following exposure of rats to liver carcinogens, a small number of hepatocytes become resistant to the inhibitory effect of 2-acetylaminofluorene on cell proliferation stimulated by partial hepatectomy or administration of carbon tetrachloride. These resistant hepatocytes, detected by staining for gamma-glutamyl transpeptidase (γ-GT), are presumed to be preneoplastic lesions that can form hepatocytic nodules through clonal expansion. Twelve hours after partial hepatectomy, 8 rats were administered

naphthalene intragastrically at a dose of 100 mg/kg. A control group of 17 rats was administered the solvent vehicle (corn oil). The animals were fed a basal diet containing 0.02% 2-acetylaminofluorene from week 2 to week 4 after hepatectomy and were given carbon tetrachloride intragastrically at a dose of 2.0 mL/kg at the start of week 3. The animals were returned to the basal diet without 2-acetylaminofluorene after week 4, and were sacrificed at week 5. Rats given naphthalene did not differ significantly from controls in the number, area, or size of foci of resistant hepatocytes.

Groups of 31 male and 16 female CD1 mice were injected i.p. with a 0.05 M solution of naphthalene in dimethylsulfoxide (DMSO) at 1, 8, and 15 days of age (for a total dose of about 1.75 µmol) and examined at 52 weeks. Tumor incidence was not significantly higher than in control mice (21 of each sex) injected with DMSO alone (LaVoie *et al.* 1988).

4.3 Summary

The NTP published two-year carcinogenicity studies of naphthalene administered by inhalation to $B6C3F_1$ mice (NTP 1992) and F344/N rats (NTP 2000). These studies showed no evidence of carcinogenic activity of naphthalene in male $B6C3F_1$ mice; some evidence of carcinogenic activity in female $B6C3F_1$ mice, based on increased incidence of pulmonary alveolar/bronchiolar adenoma; and clear evidence of carcinogenic activity in male and female F344/N rats, based on increased incidences of respiratory epithelial adenoma and olfactory epithelial neuroblastoma of the nose.

Other studies have shown weak to no evidence that naphthalene is carcinogenic in experimental animals. The strain A mouse lung tumor bioassay showed a slight, but not statistically significant, increase in alveolar adenoma in female mice exposed to naphthalene; however, the number of tumors per tumor-bearing lung was increased ($P < 0.05$). There was no evidence that naphthalene was carcinogenic in rats by routes of administration other than inhalation; however, the numbers of animals in these experiments were small, and there were no controls. In a short-term assay, rats given a partial hepatectomy followed by a single intragastric exposure to naphthalene, two weeks of exposure to 2-acetylaminofluorene in the diet, and a single intragastric dose of carbon tetrachloride did not develop increased numbers of resistant hepatocytes (as indicated by γ-GT activity).

5 Genotoxicity

The available literature on the genotoxicity of naphthalene (Sections 5.1–5.4) and naphthalene-related compounds (Section 5.5) is summarized below.

5.1 Prokaryotic systems

5.1.1 Reverse mutation in Salmonella typhimurium

In studies with *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, naphthalene at exposure levels of 0.3 to 100 μg/plate did not induce reverse mutation, with or without induced rat liver S9 metabolic activation (Mortelmans *et al.* 1986, NTP 1992, 2000). Negative results also were reported in the following studies, with and without S9 metabolic activation: McCann *et al*. (1975) (10 to 1,000 μg/plate), Kaden *et al*. (1979) (2 mM), Florin *et al*. (1980) (0.03 to 30 μmol/plate), Seixas *et al*. (1982) (1.6 mM), Connor *et al*. (1985) (100 to 2,000 μg/plate), Sakai *et al*. (1985) (5 to 250 μg/plate), Bos *et al*. (1988) (1 to 50 μg/plate), and Kangsadalampai *et al*. (1997) (12.64 to 50.56 μg/plate).

5.1.2 Mutation in Escherichia coli

Naphthalene was not genotoxic in the SOS-chromotest with *Escherichia coli* strain PQ37 at concentrations of 0.156 to 10.0 μg/plate, either with or without induced rat liver S9 (Mersch-Sundermann *et al.* 1992, 1993). Naphthalene also did not induce mutations in *Escherichia coli* strains WP2 and WP100 at a concentration of 2000 μg/plate, either with or without metabolic activation (Mamber *et al.* 1983), or in lysogenic strain GY5027 and indicator strain GY4015, with metabolic activation (Mamber *et al*. 1984).

5.2 Eukaryotic systems

5.2.1 Mutagenicity in Drosophila melanogaster

Naphthalene tested at concentrations of 1 to 10 mM in the wing somatic mutation and recombination test in *Drosophila melanogaster* induced mutations in flies from both the standard cross and the high-bioactivation cross (the progeny of which have increased sensitivity to promutagens and procarcinogens). Naphthalene increased the frequency of wing spots in the progeny of both crosses; however, the mutagenic effect was greater in the bioactivated flies (Delgado-Rodriguez *et al.* 1995).

5.3 Mammalian systems

5.3.1 In vitro *assays*

5.3.1.1 Mutation in human B-lymphoblastoid cells

Sasaki *et al.* (1997) and Grosovsky *et al*. (1999) evaluated naphthalene for genotoxicity in the human B-lymphoblastoid cell line MCL-5 at the heterozygous thymidine kinase (*TK*) locus and the hemizygous hypoxanthine phosphoribosyl transferase (*HPRT*) locus. Exposure to naphthalene at 40 μg/mL (0.31 μmol/mL) did not significantly increase mutation frequency at the *TK* locus, but resulted in small increases in mutation frequency at the *HPRT* locus. Because the increases in *TK* and *HPRT* mutation frequency did not differ significantly, and because the types of mutations recoverable at *HPRT* are expected

to be a subset of those recoverable at *TK*, the authors suggested that the small increase in *HPRT* mutations might not be biologically significant.

5.3.1.2 Cell transformation

The cell-transforming ability of naphthalene has been investigated in several cellular systems, including rat embryo cells infected with the Rauscher leukemia virus (0.1 and 0.5 μg/mL) (Freeman *et al.* 1973), mouse embryo cells infected with the AKR leukemia virus (0.1 to 5.0 μg/mL) (Rhim *et al.* 1974), and mouse mammary gland cells (0.001 to 1.0 μg/mL) (Tonelli *et al.* 1979). Naphthalene exposure did not increase the incidence of cell transformation in any of these studies.

5.3.1.3 Cytogenetic effects

Naphthalene at concentrations of 30 to 67.5 μg/mL increased the incidence of chromosomal aberrations in Chinese hamster ovary (CHO) cells, but only in the presence of rat liver S9 metabolic activation (NTP 1992, 2000).

Sasaki *et al*. (1997) and Grosovsky *et al*. (1999) evaluated naphthalene (0.23 μmol/mL) in the CREST modified micronucleus test, which distinguishes chromosomal loss from chromosomal breakage. Exposure to naphthalene (0.23 μmol/mL) resulted in statistically significant increases in CREST-negative micronuclei, indictating chromosomal breakage, but did not in CREST-positive micronuclei (which indicate chromosomal loss) or in total micronuclei.

5.3.1.4 Sister-chromatid exchange

Naphthalene at concentrations of 2.7 to 90 μg/mL induced sister-chromatid exchange in CHO cells, both with and without rat liver S9 metabolic activation NTP (1992, 2000). However, naphthalene at 100 μM did not induce sister-chromatid exchange in human peripheral lymphocytes (Tingle *et al.* 1993).

5.3.1.5 DNA damage/repair test

Naphthalene at concentrations of 0.03 to 3 mM did not induce DNA single-strand breaks in rat hepatocytes (Sina *et al.* 1983).

5.3.2 In vivo *assays*

5.3.2.1 Oxidative stress and DNA damage

Oral administration of naphthalene to Sprague-Dawley rats (120 mg/kg b.w. per day for 120 consecutive days) resulted in oxidative stress (increased lipid peroxidation) and DNA breakage in liver and brain tissue (Bagchi *et al.* 1998). In comparison with results from concurrent controls, liver and brain samples from rats exposed to naphthalene showed 1.2- to 1.4-fold increases in lipid peroxidation and 1.1- to 1.9-fold increases in DNA fragmentation; all increases were noted between treatment days 45 and 105. The authors concluded that these tissue-damaging effects may contribute to the toxicity and carcinogenicity of naphthalene.

A single oral dose of 1,100 mg of naphthalene to female Sprague-Dawley rats resulted in oxidative stress and DNA breakage (Vuchetich *et al.* 1996). In comparison with results

from concurrent controls, naphthalene administration increased lipid peroxidation in liver and brain mitochondria and microsomes 2.0- to 2.2-fold at 24 hours. Treatment with vitamin E succinate (VES) three days before and four days after administration of naphthalene protected rats from oxidative stress and reduced DNA breakage in hepatic tissue. VES treatment reduced lipid peroxidation at 24 hours by 16% to 25% in liver and brain mitochondria and microsomes. Naphthalene administration increased DNA single-strand breaks in liver and brain tissue 3.0- and 2.4-fold, respectively, at 24 hours. Following coadministration of naphthalene and VES, single-strand breaks in liver samples were increased only 1.6-fold at 24 hours, but the frequency of single-strand breaks in brain samples was not reduced.

5.4 Other tests

Naphthalene was tested for its ability to induce micronucleus formation in the erythrocytes of *Pleurodeles waltl* (salamander) larvae exposed *in vivo* (Djomo *et al.* 1995). Naphthalene was genotoxic at 0.50 ppm (the highest concentration tested), weakly genotoxic 0.25 ppm, and not genotoxic at 0.125 ppm (the lowest concentration).

5.5 Naphthalene-related compounds

1,4-Naphthalene diamine, 1-naphthol, 1-methylnaphthalene, 2-methylnaphthalene, and 1,4-dimethylnaphthalene did not induce reverse mutation in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 (McCann *et al.* 1975, Florin *et al.* 1980, Mortelmans *et al.* 1986). However, 1-methylnaphthalene induced mutations in *Salmonella typhimurium* strain TM677 (Kaden *et al.* 1979) and ambient air extracts from an environmental chamber in which naphthalene was reacted with nitrogen-containing reagents induced mutations in *Salmonella typhimurium* strain TA 98 (Arey *et al.* 1992). In *Escherichia coli* strain PQ37, 2,7-dinitronaphthalene and 2-nitronaphthalene were mutagenic; 1-amino-4-nitronaphthalene gave marginally positive results; and 1-nitronaphthalene, 1,5-dinitronaphthalene, and 2-methyl-1-nitronaphthalene were not mutagenic (Mersch-Sundermann *et al.* 1993). Both 1-nitronaphthalene and 1,5-dinitronaphthalene were genotoxic in the wing somatic mutation and recombination test in *Drosophila melanogaster* (Delgado-Rodriguez *et al.* 1995).

In the human B-lymphoblastoid cell line MCL-5, 2-nitronaphthalene significantly increased the mutation frequency at the *TK* locus, but not at the *HPRT* locus. No increases in mutation frequency were observed with 1-nitronaphthalene, 1-hydroxy-2-nitronaphthalene, 2-hydroxy-1-nitronaphthalene, or 1,4-naphthoquinone. In tests of the same five chemicals in the CREST modified micronucleus test, 1,4-naphthoquinone significantly increased the frequency of CREST-positive micronuclei and total micronuclei, but the other four chemicals gave negative results (Sasaki *et al.* 1997, Grosovsky *et al.* 1999). 1-Naphthylamine and 2-naphthylamine induced transformation of mouse mammary gland cells (Tonelli *et al.* 1979).

5.6 Summary

Table 5-1 summarizes the data on naphthalene genotoxicity. The majority of tests have not shown naphthalene to be mutagenic *in vitro*. Naphthalene did not induce reverse mutation in *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, TA1537, TM677, UTH8413, or UTH8414 or in *Escherichia coli* strains PQ37, WP2, WP100,

GY5027, or GY4015. It also did not induce cell transformation in mouse mammary gland cells or rat or mouse embryo cells, mutations or micronuclei in human B-lymphoblastoid MCL-5 cells, or DNA strand breaks in rat hepatocytes. However, naphthalene induced chromosomal aberrations and sister-chromatid exchange in CHO cells, micronuclei indicating chromosomal breakage in human lymphoblastoid cells, and oxidative stress and DNA damage in Sprague-Dawley rats. In non-mammalian test systems, naphthalene induced mutations in *Drosophila melanogaster* and micronuclei in *Pleurodeles waltl* larvae.

Table 5-1. Comparative summary of genetic effects of naphthalene exposure

Test system	End Point	Results[a]		Reference
		without metabolic activation	with metabolic activation	
S. typhimurium TA98, TA100, TA 1535, TA1537	reverse mutation	–	–	McCann *et al*. 1975, Florin *et al*. 1980, Mortelmans *et al*. 1986, NTP 1992, 2000
S. typhimurium TA97, TA98, TA100	reverse mutation	–	–	Sakai *et al.* 1985
S. typhimurium TA98, TA100	reverse mutation	–	–	Bos *et al.* 1988, Kangsadalampai *et al.* 1997
S. typhimurium TA98, TA100, UTH8413, UTH8414	reverse mutation	–	–	Connor *et al*. 1985
S. typhimurium TA1537	reverse mutation	–	–	Seixas *et al*. 1982
S. typhimurium TM677	forward mutation	NR	–	Kaden *et al*. 1979, Seixas *et al*. 1982
Escherichia coli PQ37	DNA damage (β-galactosidase activity)	–	–	Mersch-Sundermann *et al.* 1992, 1993
Escherichia coli WP2, WP100	DNA damage (rec assay)	–	–	Mamber *et al*. 1983
Escherichia coli GY5027, GY4015	prophage induction	NR	–	Mamber *et al.* 1984
Drosophila melanogaster	mutation and mitotic recombination	+	+	Delgado-Rodriguez *et al.* 1995
Pleurodeles waltl larvae	micronuclei	+	NA	Djomo *et al*. 1995
Mouse mammary gland cells	cell transformation	–	NA	Tonelli *et al*. 1979
Rat embryo cells	cell transformation	–	NA	Freeman *et al*. 1973
Mouse embryo cells	cell transformation	–	NA	Rhim *et al*. 1974

Test system	End Point	Results[a]		Reference
		without metabolic activation	with metabolic activation	
Rat hepatocytes	DNA strand breaks	–	NA	Sina *et al*. 1983
CHO cells	chromosomal aberrations	–	+	NTP 1992, 2000
CHO cells	sister-chromatid exchange	+	+	NTP 1992, 2000
Human peripheral lymphocytes	sister-chromatid exchange	NR	–	Tingle *et al*. 1993
Human B-lymphoblastoid MCL-5 cells	mutation at *TK* and *HPRT* loci	–	NA	Sasaki *et al*. 1997, Grosovsky *et al*. 1999
Human B-lymphoblastoid MCL-5 cells	micronuclei		NA	Sasaki *et al*. 1997, Grosovsky *et al*. 1999
Sprague-Dawley rats	DNA fragmentation and breakage	+	NA	Vuchetich *et al*. 1996, Bagchi *et al*. 1998

[a]+ = positive result, – = negative result, NR = not reported, NA = not applicable.

*Positive for CREST-negative micronuclei (chromosomal breakage).

**Negative for CREST-positive micronuclei (chromosomal loss) and total micronuclei.

6 Other Relevant Data

Naphthalene is rapidly absorbed and metabolized when inhaled or administered dermally or orally. In its reports of mouse and rat carcinogenicity bioassays, the NTP also reviewed the literature pertaining to naphthalene's absorption, distribution, metabolism, excretion, and mechanism of action (NTP 1992, 2000) and developed a model to estimate its uptake, distribution, and metabolism in rats and mice, using chronic toxicity data from the two-year studies and data from single six-hour inhalation exposures. The NTP did not conduct or review any subchronic studies of naphthalene or propose a mechanism of action for the carcinogenicity of naphthalene in animals or humans (NTP 1992, 2000). In its recent review of naphthalene, IARC (2002) also reviewed data relevant to naphthalene's absorption, distribution, metabolism, and mechanism of action; naphthalene was classified as *possibly carcinogenic to humans* (Group 2B).

6.1 Mammalian absorption, distribution, and excretion

The NTP (2000) reviewed the literature pertaining to the absorption of naphthalene. Orally administered naphthalene is readily absorbed by a variety of animal species, including rats (strain not specified), laying pullets, swine, and dairy cattle. Once absorbed, naphthalene is metabolized quickly regardless of the route of administration. After dermal or i.p. administration, naphthalene is absorbed, metabolized, and excreted primarily in the urine.

6.1.1 Human studies

Limited information was found pertaining to the absorption, distribution, or excretion of naphthalene by humans. The NTP (2000) literature review reported that absorption of naphthalene has been demonstrated by observation of signs and symptoms of toxicity in infants accidentally exposed to naphthalene vapors in clothes. Transplacental transport of naphthalene and/or its metabolites is evidenced by hemolytic anemia in newborns whose mothers ingested naphthalene during the last trimester of pregnancy. A study conducted by the Centers for Disease Control and Prevention found 1-naphthol in 86% of 983 urine samples from human participants, with an average concentration of 17 μg/L; 2-naphthol was detected in 81% of 977 samples, with an average concentration of 7.8 μg/L. Although 1-naphthol may be produced by the cleavage of carbaryl, a 1-naphthyl-*N*-methylcarbamate insecticide, as well as from the oxidation of naphthalene, the study investigators concluded that these results reflected naphthalene exposure.

Studies by Bieniek (1994, 1997) on industrial workers exposed to naphthalene provided additional evidence for absorption of naphthalene by humans. Urine samples were collected from workers exposed to naphthalene and other phenolic compounds through distillation of naphthalene oil or employment in a coke plant, and personal air samples were collected during the workday. The highest values (7.48 ± 2.187 mg/L; geometric mean ± geometric standard deviation; N = 75) for urinary 1-naphthol, a urinary metabolite of naphthalene, were observed for naphthalene oil distillation operators. The urinary excretion of 1-naphthol varied for cokers at two different coke plants based on the technology used in the plants. In a coke plant using older technology the urinary 1-naphthol values were 4.86 ± 2.465 mg/L (N = 57), while in a plant using more modern

technology the mean excretion was 0.89 ± 1.783 mg/L (N = 66). Subjects who were not occupationally exposed to naphthalene had urinary 1-naphthol levels of 0.13 ± 1.868 mg/L (N = 24). Urinary 1-naphthol concentrations of coke oven workers were highly correlated (r = 0.80) with naphthalene concentration in breathing zone air (personal air samples) (Bieniek 1994). In a later report, Bieniek (1997) identified 1-naphthol, 2-naphthol, and 1,4-naphthoquinone in urine samples collected from coke plant-workers exposed to naphthalene as tar distillation process operators or naphthalene oil distillation operators. Significant correlations were reported for urinary 1-naphthol in breathing-zone air for both tar distillation operators ($r^2 = 0.46$, $P < 0.001$; N = 69) and naphthalene oil distillation operators ($r^2 = 0.55$, $P < 0.001$; N = 33). Correlation coefficients were slightly lower for urinary 2-naphthol; however, they were still highly significant ($P < 0.001$) for both work categories. The author concluded that urinary 1-naphthol and 2-naphthol seemed to be useful biomarkers in assessing exposure of coke plant workers to naphthalene.

Kanikkannan *et al.* (2001a, 2001b) investigated the percutaneous permeation of JP-8 (2001a) or JP-8+100 (2001b) jet fuels across human skin *in vitro*. JP-8, which is the major jet fuel used by the U.S. Army and Air Force, is a kerosene-based petroleum distillate with variable composition from batch to batch; however, its general composition is 18% aromatic hydrocarbons with the remainder as aliphatic hydrocarbons (C_8 to C_{17}). The JP-8+100 jet fuel is a recently introduced product used by the U.S. Air Force and consists of JP-8 plus three performance additives (butylated hydroxytoluene antioxidant, metal deactivator MDA, and 8Q405 detergent/dispersant). The concentration of naphthalene was 0.26% (w/w) in the batches of both JP-8 and JP-8+100 tested by Kanikkannan *et al.* (2001a, 2001b). The steady state flux value for naphthalene across human skin was significantly ($P < 0.01$, Student's t-test) higher (0.451 ± 0.022 μg/cm^2 per hour) than the corresponding value for pig skin (See Section 6.1.2, below) (Kanikkannan *et al.* 2001a). The authors concluded that naphthalene permeated through human skin without any apparent lag time.

6.1.2 Animal studies

Chen and Dorough (1979) found that within 48 hours of an i.p. injection of 100 mg/kg of [^{14}C]naphthalene in female Sprague-Dawley rats, 23% to 41% of the label was excreted in the urine and 5% to 10% in the bile. Of the label excreted in the urine, 5% to 20% was unconjugated, and 80% to 95% was sulfate, glucuronide, and mercapturic acid conjugates.

At 48 hours after dermal administration of 43 μg of [^{14}C]naphthalene to Sprague-Dawley rats, 70% of the label had been excreted in the urine, 14% in the expired air, and 4% in the feces (Turkall *et al.* 1994). Metabolites identified in the urine were 2,7- and 1,2-dihydroxynaphthalene, 1,2-naphthoquinone, and 1- and 2-naphthol. Less than 0.5% of the parent compound was excreted in the urine. The plasma half-life was 2.1 hours for the absorption phase and 12 hours for the elimination phase.

After a single 20-mg/kg i.p. dose of [ring-U-^{3}H]naphthalene was administered to male Wistar rats, more than 88% of the compound was excreted in urine and feces within 72 hours. No significant naphthalene deposits formed in the tissues, and only minor amounts

(5% of the dose) remained in the muscles at 72 hours after administration (Kilanowicz *et al.* 1999). Four naphthalene urinary metabolites (see Figure 1-2) were identified by GC/MS; the primary urinary metabolites were 1- and 2-naphthol (approximately 33% and 9%, respectively). Unchanged parent compound accounted for approximately 46% of total urinary excretion. Radioactivity in plasma reached a maximum at 2 hours after administration, with biphasic half-lives for clearance of 0.8 h (phase I) and 99 h (phase II). The half-life of radioactive naphthalene in erythrocytes was approximately 9 hours (monophasic). The authors reported that one hour after administration, the tritium concentrations were highest in fat, liver, and kidneys; however, no data were provided. Total balance data for 24, 48, and 72 hours after administration are shown in Table 6-1. Quantitative evaluation at 72 hours showed approximately 46% of the administered naphthalene was eliminated as unchanged parent compound, while approximately 48% of the metabolites contained oxygen obtained through ring hydroxylation, hydration, and glutathione conjugation. No explanation was given for the much higher percentage (46%) of unmetabolized naphthalene excreted in the urine by Wistar rats in this study compared to the earlier study by Turkall *et al.* (1994) (see above) in which less than 0.5% of dermally administered naphthalene was excreted unchanged in the urine of Sprague-Dawley rats.

The penetration of naphthalene from complex mixtures across skin preparations *in vitro* also has been studied. Sartorelli *et al.* (1999) applied a mixture of 13 polycyclic aromatic hydrocarbons (PAHs), including naphthalene, in solution in either a lubricating oil or acetone (with artificial sweat) to full-thickness skin prepared from the abdomen of monkeys (*Cercopithecus aetiops*). They reported that the passage of naphthalene across the skin preparations was significantly slower when the PAHs were applied in the oil matrix compared to the acetone solution with artificial sweat. Steady state flux rates for naphthalene were the highest among the 13 PAHs; 0.2740 ± 0.2189 nmol/cm^2 per hour for lubricating oil medium and 1.0107 ± 0.3981 nmol/cm^2 per hour for acetone with artificial sweat. Naphthalene also had the shortest lag time for absorption from either vehicle, 4.86 ± 7.99 hour from lubricating oil and 1.18 ± 0.01 hour from acetone with artificial sweat.

Kanikkannan *et al.* (2001a, 2001b) (see Section 6.1.1, above) also investigated the percutaneous permeation of JP-8 (2001a) or JP-8+100 (2001b) jet fuels across pig ear skin *in vitro*. The permeation of [^{14}C]naphthalene tracer across pig skin *in vitro* was slightly, but significantly ($P < 0.05$, Student's t-test), lower for JP-8 than for JP-8+100 (steady state flux values of 0.376 ± 0.017 and 0.419 ± 0.033 μg/cm^2 per hour, respectively) (Kanikkannan *et al.* 2001b). The authors concluded that naphthalene permeated through pig ear skin without any apparent lag time.

6.1.3 Pharmacokinetic modeling

The NTP developed a physiologically based pharmacokinetic (PBPK) model to estimate the uptake, distribution, and metabolism of naphthalene in rats and mice (NTP 2000, Willems *et al.* 2001) based on data obtained from the NTP chronic toxicity studies of naphthalene and from single six-hour inhalation exposures to the same concentrations used in the chronic studies (NTP 1992, 2000 [TR Appendix D], Abdo *et al.* 1992). A diffusion-limited PBPK model was used to predict naphthalene metabolism and tissue

distribution in F344/N rats and B6C3F$_1$ mice exposed to naphthalene at concentrations of 0, 10, or 30 ppm for mice and 0, 10, 30, or 60 ppm for rats, six hours/day, five days/week, for 104 to 105 weeks. Whole-blood samples from rats were analyzed for naphthalene at 2 weeks and 3, 6, 12, and 18 months (see Appendix B, Tables D1 and D2 and Figures D2 and D3, pp. B-150 to B-155). Additional groups of rats and mice were evaluated after single six-hour inhalation exposures to naphthalene at the same concentrations used in the two-year studies (see Appendix B, Table D3 and Figure D4, pp. B-154 and B-156).

Table 6-1. Total balance of [ring-U-^{3}H]naphthalene in 6 male rats following a single 20-mg/kg i.p. dose (mean ± SEM)

	Percent of administered dose		
Medium	24 h	48 h	72 h
Urine	55.53 ± 7.35	64.40 ± 10.15	68.15 ± 8.79
Feces	10.42 ± 2.68	17.20 ± 1.55	19.75 ± 1.17
Erythrocytes + plasma[a]	0.74 ± 0.08	0.65 ± 0.04	0.65 ± 0.06
Fat tissue[b]	0.40 ± 0.06	0.12 ± 0.05	0.10 ± 0.05
Muscle[c]	1.67 ± 0.37	1.85 ± 0.19	5.00 ± 0.37
Liver	0.40 ± 0.02	0.47 ± 0.05	0.28 ± 0.04
Total	69.16	84.69	93.93

Source: Kilanowicz *et al.* 1999.

[a]Estimate based on 7 mL blood/100 g rat body weight.

[b]Estimate based on 12% of the total body weight as fat.

[c]Estimate based on 40% of the total body weight as muscle.

Data from the NTP PBPK model indicate rapid absorption of naphthalene, with metabolism of almost all of the naphthalene absorbed by rats and mice, estimates from the model are 88% to 96% metabolized by rats and 96% to 98% metabolized by mice (Willems *et al.* 2001). These high rates of metabolism indicate that once naphthalene is absorbed into the general circulation, very little parent compound is eliminated via exhalation. The species difference observed for absorption may be attributed to the greater metabolic capacity of mice. Total naphthalene metabolized (i.e., the internalized dose) was nearly equal for the mice exposed at 10 ppm and the rats exposed at 60 ppm, a finding that may be attributed to the higher ventilation rates and greater naphthalene metabolism in mice.

The NTP (2000) PBPK results revealed several gender- and species-related differences. Metabolic capacity in the lungs of rats and mice was similar between the sexes. The lung metabolic saturation level was equal in male and female rats; however, in mice, metabolic saturation occurred at lower naphthalene blood concentrations in females than in males. The liver metabolic pathway represented by the Michaelis-Menten equation

predicted equivalent metabolic capacity and saturation level in male and female rats. However, both the metabolic capacity and saturation level were lower in female mice than in male mice. The second liver metabolic pathway, characterized by a Hill equation with a Hill exponent of 2, also predicted similar metabolic capacity and saturation level in male and female rats. In mice, the Hill interpretation predicted equivalent metabolic capacity in males and females, but a lower saturation level in females.

In the lungs, the estimated steady-state concentration of naphthalene was fairly similar in rats and mice exposed to equivalent concentrations; however, the rates of metabolism and the cumulative metabolism in the lung were significantly greater in mice than in rats. Increased rates of metabolism were not proportional to increased exposure, suggesting metabolic saturation in the lung; further, saturation was more apparent in the rat than in the mouse. Metabolism was higher in mouse than rat liver, but the species difference was less pronounced than in the lung. Metabolic saturation was evident only in the liver of rats exposed to naphthalene at 60 ppm. In both species, 65% to 75% of the metabolic clearance occurred during the six-hour exposure. However, metabolic clearance in rats exposed at 60 ppm accounted for only about half of the total inhaled dose; this finding was attributed to metabolic saturation in the liver, which resulted in greater storage of parent compound in the fat in this exposure group (NTP 2000).

6.2 Metabolism

6.2.1 Human studies

Little information was found regarding the metabolism of naphthalene in humans. The NTP (2000) reported that glutathione conjugation of naphthalene metabolites plays an important role in naphthalene's elimination in rodents but not in primates, including humans. The Agency for Toxic Substances and Disease Registry (1995) reviewed the literature and reported that naphthol (isomer not specified) was detected in the urine of patients four days after naphthalene ingestion; naphthalene was not identified in the urine after day five. 1-Naphthol, 2-naphthol, 1,2-naphthoquinone, and 1,4-naphthoquinone were identified in the urine of an 18-month-old child approximately nine days after exposure. All of these metabolites except 1,4-naphthoquinone still were present at day 13. The route of exposure could not be established conclusively (ATSDR 1995).

Metabolism of naphthalene by human microsomal preparations *in vitro* has been reported. Microsomal fractions from human lung tissue in the presence of glutathione and glutathione transferases metabolized naphthalene to naphthalene dihydrodiol and three glutathione conjugates (Buckpitt and Bahnson 1986). Human liver microsomes isolated from six histologically normal livers produced 1,2-dihydro-1,2-naphthalenediol (naphthalene-1,2-dihydrodiol), the principal stable metabolite, and 1-naphthol. For human and induced mouse microsomes, the ratios of *trans*-1,2-dihydrodiol to 1-naphthol were 8.6 and 0.4, respectively. Naphthalene-1,2-dihydrodiol was the product of sequential 1,2-epoxidation and hydrolysis, whereas 1-naphthol was formed by spontaneous re-arrangement of the 1,2-epoxide. More than one cytochrome P450 enzyme mediated the metabolism of naphthalene, as demonstrated by its incomplete inhibition by a series of selective P450 inhibitors (Tingle *et al.* 1993). Lanza *et al.* (1999) characterized the epoxidation of naphthalene by recombinant CYP2F1 expressed in lymphoblastoid cells to evaluate the hypothesis that this human enzyme could bioactivate naphthalene.

The CYP2F1 enzyme is the human ortholog of the mouse enzyme CYP2F2 (see Section 6.3.2.3 below). The metabolites of naphthalene formed by incubation of microsomes prepared from human lymphoblastoid cells expressing CYP2F1 were identified as glutathione conjugates separated by HPLC. Glutathione conjugates 1 and 3 are formed from naphthalene 1*S*,2*R*-oxide, and conjugate 2 is formed from the naphthalene 1*R*,2*S*-oxide enantiomer (see Figure 6-1). Quantities of conjugates 1 and 3 were >3-fold and 4-fold greater, respectively, than that of conjugate 2. Thus, the authors concluded that the human enzyme (CYP2F1) might predominantly form a different enantiomeric epoxide of naphthalene compared to the mouse (CYP2F2) enzyme.

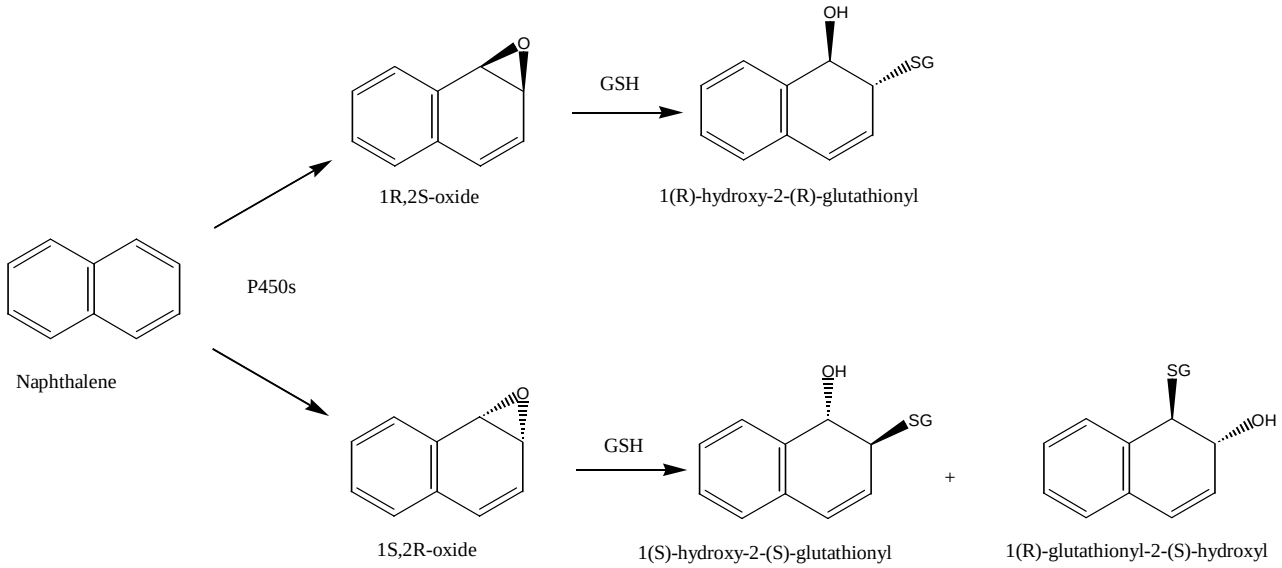
Polymorphisms of human cytochromes P450 and other metabolic enzymes have been studied for their involvement in naphthalene metabolism as measured by urinary excretion of 1-naphthol and 2-naphthol. Yang *et al.* (1999) observed a relationship between urinary naphthalene metabolites and polymorphisms of CYP1A1, CYP2E1, or glutathione S-transferase M1 (GSTM1) in a Japanese population. No differences in metabolism were reported for CYP1A1 exon 7 polymorphism; however, smokers with a C to T transition (c2) in one (c1/c2) or both (c2/c2) alleles of the 5'-flanking region of the CYP2E1 gene had higher concentrations of urinary 2-naphthol than did individuals with two wild-type (c1/c1) alleles. Genetic deficiency of GSTM1 was also correlated with higher concentrations of urinary 1-naphthol and 2-naphthol compared to individuals expressing normal levels of the enzyme.

Nan *et al.* (2001) carried out a similar study of excretion of the naphthalene metabolite 2-naphthol among Korean coke oven workers and university students in which they compared the effects of occupation, lifestyle, and genetic polymorphisms of CYP1A1, CYP2E1, GSTM1, and glutathione S-transferase θ1 (GSTT1). Urinary 2-naphthol concentrations were significantly higher in the coke oven workers compared to the students. Among coke oven workers, CYP2E1 and GSTM1 were significant factors in the univariate analysis of urinary 2-naphthol levels. GSTM1 and smoking were significant factors for the university students. The authors concluded that CYP2E1 and GSTM1 are important enzymes in the metabolism of naphthalene.

6.2.2 Animal studies

The metabolism of naphthalene is complex, with formation of multiple oxygenated metabolites and their stereoisomers (see Appendix B, p. B-16 and Figure 1, p. B-18). In a review of the literature, the NTP (2000) reported the first step in naphthalene metabolism to be the formation of naphthalene-1,2-oxide (Figure 6-1) by oxygen and the NADPH-dependent microsomal monooxygenase system, with subsequent conversion to the trans-1,2-diol and other products (Figure 6-2). The two stereoisomers of naphthalene epoxide (naphathalene-1,2-oxide) are shown in Figure 6-1. The stereoselective epoxidation of naphthalene in various animal species and tissues has been proposed as an important factor in the acute toxicity of naphthalene (Buckpitt *et al.* 1987). Buckpitt and Franklin (1989) reported a strong correlation between the rates of formation of 1*R*-2*S*-naphthalene oxide in various tissues and tissue-selective toxicity. They further stated that microsomal metabolism of naphthalene in rabbit and rat liver results in the formation of 1,2-dihydro-1,2-naphthalenediol (dihydrodiol) and 1-naphthol. The 1,2-epoxide is an obligate intermediate in the *in vitro* formation of 1-naphthol, dihydrodiol (Figure 6-2), and

glutathione (GSH) conjugates (Figure 6-1). These reactive epoxide intermediates may undergo further metabolism by three major metabolic pathways, including hydration by epoxide hydrolases, conjugation by glutathione transferases (GSTs), and, in *in vitro* systems, spontaneous rearrangement of the epoxide to 1-naphthol (95%) and 2-naphthol (5%). Four diastereomeric GSH conjugates are possible from naphthalene-1,2-oxides, and three have been isolated and identified, including 1-(R)-hydroxy-2-(R)-glutathionyl, 1-(S)-hydroxy-2-(S)-glutathionyl, and 1-(R)-glutathionyl-2(S)-hydroxyl (Buckpitt *et al.* 1987) (Figure 6-1).



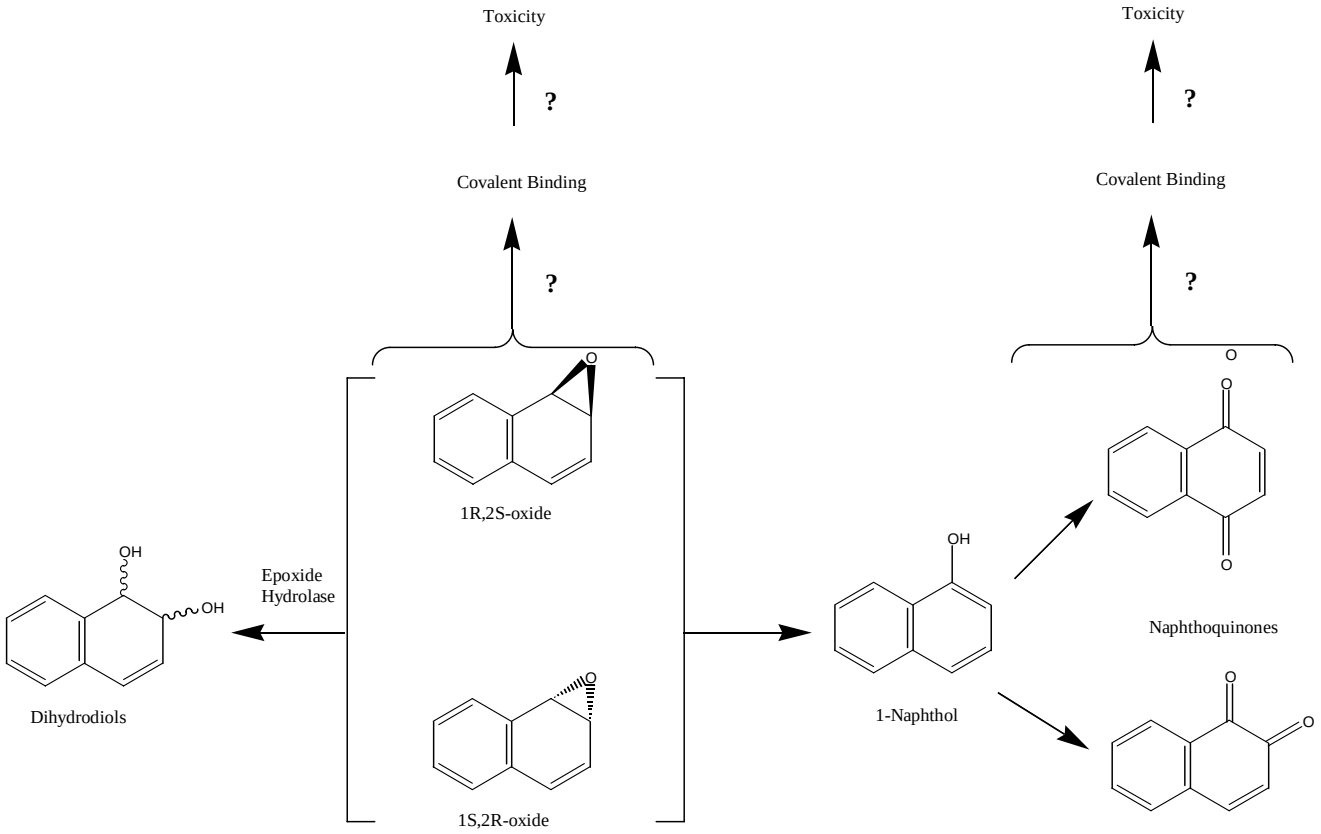
Adapted from Shultz *et al.* 1999.

Figure 6-1. Metabolism of naphthalene to reactive epoxides and their GSH conjugates

Using microdissected airways, Buckpitt *et al.* (1985) found that the rate of naphthalene metabolism was higher in mice than in rats or hamsters. Additionally, metabolism in the distal airways was higher than in the trachea in all three species. At all airway levels, mouse and hamster postmitochondrial supernatants, prepared from the dissected airways, metabolized naphthalene oxide to diol and three GSH conjugates at rates 8- to 10-fold higher than rat tissues.

The rate of naphthalene metabolism by microsomal preparations from rat, hamster, and monkey lungs was considerably lower than that by similar preparations from mouse lung (12%, 37%, and 1% of the rate in mouse lung microsomes, respectively) (Buckpitt *et al.* 1992). The mouse lung microsomal preparation favored the formation of the 1*R*,2*S*-epoxide over the 1*S*,2*R*-epoxide, whereas in the nonsensitive species (rats and hamsters), the 1*S*,2*R*-oxide dominated (Buckpitt *et al.* 1992). Supporting evidence for the preferential metabolism through the 1*R*,2*S*-epoxide pathway in mice *in vivo* was reported by Pakenham *et al.* (2002). They showed that the ratio of urinary mercapturates derived from the 1*R*,2*S*-epoxide to those formed from the 1*S*,2*R*-epoxide was 1:1 or greater in

Swiss-Webster mice (ratios as high as 6:1 were observed at low concentrations of inhaled naphthalene), while the ratio was less than 1:1 at all doses in Sprague-Dawley rats. Lanza *et al.* (1999) reported that the human CYP2F1 also predominantly formed naphthalene 1*S*,2*R*-oxide, the opposite enantiomeric epoxide from the mouse enzyme. Buckpitt *et al* (1992) pointed out that the rate of metabolism by primate lung microsomal enzymes was similar to that of human lung enzymes. Thus, the rate of metabolism in mouse lung microsomes is approximately 10-times greater than for rat lung and 100 times greater than for human. They concluded that the correlation between the rate and stereochemistry of the epoxidation of naphthalene and the toxicity of naphthalene in rodents may suggest that the airways of primates could be less susceptible to naphthalene injury if cytotoxicity is dependent on the stereochemistry and rate of epoxidation.



Adapted from Chichester *et al.* 1994.

Figure 6-2. Potential reactive intermediates in naphthalene metabolism

Buckpitt *et al.* (1992) also compared the rates of metabolism of naphthalene by three different segments of nasal mucosa from mouse, rat, and hamster. Tissue was sampled from lateral wall, septum, and olfactory regions and homogenized. Postmitochondrial supernatants were prepared and used to assess metabolism of naphthalene to the diol and three different glutathione conjugates. The highest rate of metabolism was observed with mouse olfactory epithelium; rat olfactory epithelium metabolized naphthalene at approximately half the rate of the mouse tissue; and the metabolic rate of hamster

olfactory epithelium was less than 5% of that of the mouse. The other two nasal regions, i.e., the lateral and septal regions, had metabolic rates that were less than half those of the olfactory region. The glutathione conjugates were formed predominantly from 1*R*,2*S*-naphthalene oxide in all three species. In rat olfactory epithelium the ratio of conjugate 2 (derived from 1*R*,2*S*-naphthalene oxide) to conjugates 1 and 3 (derived from 1*S*,2*R*-naphthalene oxide) was greater than 36.Although considerable interspecies similarities in naphthalene metabolites have been observed, some notable exceptions exist, as shown in Table 6-2. 1,2-Dihydroxynaphthalene is formed only in guinea pigs, and no glucuronides were detected in that species. GSH conjugation of naphthalene metabolites plays an important role in the elimination pathway in rodents but not in primates, including humans. It was reported by the NTP (2000) that single gavage doses of naphthalene at 30, 75, or 200 mg/kg b.w. administered to male Wistar rats resulted in a dose-related increase in thioether excretion in the urine. In contrast, this increase was not seen in similarly treated male or female chimpanzees. Based on the spectrum of naphthalene metabolites found in mammals, a metabolic pathway was proposed (NTP 2000, Figure 1, p. B-18).

Table 6-2. Metabolites of naphthalene identified in the urine of various species[a]

Metabolite	Rat	Mouse	Rabbit	Guinea Pig
1-Naphthol	+	+	+	+
1-Naphthyl sulfate	+	+	+	+
1-Naphthyl glucuronide	+	+	+	–
2-Naphthol	+	+	+	+
1,2-Dihydroxynaphthalene	–	–	–	+
1,2-Dihydro-1,2-dihydroxynaphthalene	+	+	+	+
1,2-Dihydro-2-hydroxy-1-naphthyl glucuronide	+	–	+	–
1-Naphthyl mercapturic acid	+	+	+	+

Source: NTP 2000.

[a]+ = metabolite present; – = metabolite not present.

Naphthalene or naphthol metabolites injected i.p. into male C57BL/6 and DBA/2 mice were bioactivated by cytochrome P450 to an electrophilic epoxide intermediate, which was further metabolized to naphthoquinones and possibly to a free-radical intermediate (Wells *et al.* 1989).

Naphthalene was shown to be bioactivated in a dose-dependent manner by a reconstituted cytochrome P450 system *in vitro* (Doherty *et al.* 1985) to electrophilic intermediates (naphthalene-1,2-oxides), which were metabolized to naphthoquinones (1,2-naphthoquinone and 1,4-naphthoquinone) and possibly to free-radical intermediates (Figure 6-2); *in vivo*, these metabolites subsequently deplete GSH and become covalently bound to tissue macromolecules (Buckpitt and Warren 1983, Warren *et al.* 1982).

Microsomal preparations from liver, lungs, and kidneys are able to transform naphthalene metabolites. Human liver microsomal preparations metabolized naphthalene to 1-naphthol and naphthalene 1,2-dihydrodiol (Tingle *et al.* 1993) (Figure 6-2). Similar preparations from either human (Buckpitt and Bahnson 1986) or mouse (Buckpitt *et al.* 1984) lung tissue metabolized naphthalene to 1,2-naphthalenediol and three different glutathione conjugates, which were identified as trans-1*S*-hydroxy-2*S*-glutathionyl-1,2-dihydronaphthalene; trans-1*R*-hydroxy-2*R*-glutathionyl-1,2-dihydronaphthalene, and trans-1*R*-glutathionyl-2*R*-hydroxy-1,2-dihydronaphthalene (Buonarati *et al.* 1990) (Figure 6-1). Lung, liver, and kidney microsomal preparations from rats, mice, or hamsters converted naphthalene to these conjugates in the presence of GSH and GSTs (Buckpitt *et al.* 1987). It should be noted that the rate and stereoselectivity of naphthalene metabolism in human lung microsomes differs substantially from that in mouse lung microsomes. In human lung microsomes, metabolism by the intermediate epoxide pathway occurred at less than 3% of the rate in mouse lung microsomes (Buckpitt and Bahnson 1986). In a recent *in vitro* study with mouse lung Clara cells exposed to naphthalene, Zheng *et al*. (1997) found 1,2-naphthoquinone to be covalently bound to the cysteine residues of proteins.

Naphthalene is excreted primarily in the urine (Figure 6-1) as unchanged parent compound or as glutathione, cysteine, glucuronic acid, and sulfate conjugates (see Section 1.3) (Kilanowicz *et al.* 1999).

Intraperitoneal injection of naphthalene to male rats resulted in urinary excretion of 20% to 30% of the administered dose in the first 24 hours and 3% to 11% in the second 24 hours (Horning *et al.* 1980). Acidic conjugates accounted for 80% to 95% of the total metabolites excreted. Enzymatic hydrolysis to neutral metabolites revealed that 20% to 40% of the total radioactivity represented glucuronides and sulfates, indicating that the remainder of the acidic conjugates included thioethers. Two methylthio metabolites, 1α,4β-di(methylthio)-2β,3α-dihydroxynaphthalene and 1α-methylthio-2β,3α,4β-trihydroxynaphthalene, were identified, and the authors stated that numerous other methylthio metabolites were formed. The initial reaction of naphthalene metabolism results in the production of an epoxide intermediate, which undergoes oxidation to form di-, tri-, and tetra-hydroxylated compounds. The major metabolites identified include the neutral forms and acidic (glucuronide and sulfate) conjugates of the following: 1-naphthol, 2-naphthol, 1,2-naphthalenediol, 1,2-dihydro-1,2-naphthalenediol (cis and trans), 1,4-dihydro-1,4-naphthalenediol (cis and trans), and 1,1-, 2,7-, and 2,6-naphthalenediol (ATSDR 1995).

Intraperitoneal injection of naphthalene to male mice resulted in urinary excretion of 65% of the administered dose in the first 24 hours and 3% in the second 24 hours. As in rats, the majority of the excreted metabolites (96%) were conjugates. The major naphthalene metabolites recovered after hydrolysis were 1-naphthol, *trans*-1,2-dihydrodiol, *trans*-1-hydroxy-2-methylthio-1,2-dihydronaphthalene, 1-methylthionaphthalene, and 2-naphthol. Seven sulfur-containing derivatives were identified as (1-hydroxy-1,2-dihydro-2-naphthalenylthio)acetic acid, 2-hydroxy-3-(1-hydroxy-1,2-dihydro-2-naphthalenylthio)propanoic acid, 1,2,3-trihydroxy-1,2,3,4-tetrahydro-4-naphthalenylthio)acetic acid, 1-(naphthalenylthio)acetic acid, 2-hydroxy-3-(1-naphthalenylthio)propanoic acid, *N*-acetyl-*S*-(1-naphthalenyl)-L-cysteine, and the major

sulfur-containing derivative *N*-acetyl-*S*-(1-hydroxy-1,2-dihydro-2-naphthalenyl)-L-cysteine. The sulfur metabolites were produced by conjugation with glutathione followed by removal of the glycyl and glutamyl moieties and modification of cysteine. Nine methylthio derivatives, including 1-methylnaphthalene and *trans*-1-hydroxy-2-methylthio-1,2-dihydronaphthalene, also were identified in the urine. Compared to rats administered equivalent doses, mice excreted more naphthalene as bivalent sulfur metabolites than as glucuronides of hydroxylated naphthalene (Stillwell *et al.* 1982).

6.3 Toxicity

6.3.1 Human studies

The NTP report (2000) provided a detailed account of naphthalene's toxicity to humans. Naphthalene inhalation causes headache, confusion, eye irritation, nausea, and profuse perspiration, with vomiting, optic neuritis, hematuria, and edema. Toxicity and death have been reported in newborn infants exposed to naphthalene vapors in clothes. Naphthalene ingestion results in abdominal pain, nausea, vomiting, diarrhea, darkening of the urine, irritation of the bladder, jaundice, anemia, and hypothermia. Cataract formation, retinal hemorrhage, chorioretinitis, optic nerve atrophy, and blindness have been reported in humans exposed to naphthalene. Naphthalene poisoning has produced hemolytic anemia in children and adults. Santucci and Shah (2000) reviewed the hospital charts of 24 children identified with hemolysis and subsequently diagnosed as glucose-6-phosphate dehydrogenase deficient. They reported that 14 of the 24 children had naphthalene-associated hemolysis while the remaining 10 had infection-associated hemolysis.

6.3.2 Animal studies

The NTP (2000) reported the median lethal doses (LD_{50}s) for rats and mice (strains not specified), respectively, to be 1,110 to 9,430 and 350 to 710 mg/kg b.w. for oral administration, 2,500 and 969 mg/kg b.w. for dermal administration, and 1,000 and 350 mg/kg b.w. for i.p. administration (NTP 2000). These values suggest that mice are more sensitive than rats to the acute effects of naphthalene. The reported median lethal concentration for rats exposed to naphthalene vapors for eight hours was 500 mg/m^3 (NTP 2000). The major sites affected by naphthalene toxicity are the hematologic and pulmonary systems and the eye.

6.3.2.1 Hematological effects

The NTP (2000) literature review reported several hematological effects of naphthalene exposure. In CD-1 mice administered naphthalene at 267 mg/kg b.w. per day by gavage for 14 days, hematologic parameters were only slightly altered; no hemolytic anemia was observed. However, dogs receiving naphthalene mixed in feed for a dose of 263 or 1,525 mg/kg b.w. per day for seven days developed hemolytic anemia, suggesting that mice are less sensitive than dogs to the hemolytic effects of naphthalene.

6.3.2.2 Ocular effects

In its review, the NTP (2000) reported several ocular effects of naphthalene exposure. Lens opacity was reported in black-hooded and brown Norway rats given naphthalene at 700 or 5,000 mg/kg b.w. per day for 79 to 102 days. Cataracts involving the whole lens

occurred in pigmented and albino rabbits within two weeks of daily oral administration of naphthalene at 1 g/kg b.w.; the incidence was greater in the albino strain. van Heyningen and Pirie (1967) reported that tissues of the eye in rabbits enzymatically converted naphthalene metabolites, including 1,2-dihydro-1,2-dihydroxynaphthalene, 2-hydroxy-1-naphthyl sulfate, and (1,2-dihydro-2-hydroxy-1-naphthyl glucosid)uronic acid, to 1,2-dihydroxynaphthalene. They concluded that 1,2-dihydroxynaphthalene was the primary toxic agent in cataractogenesis through its autoxidation to 1,2-naphthoquinone, which bound to lens proteins. A study with male C57BL/6J mice suggested that naphthalene cataractogenesis requires P450 (CYP1A and CYP2A) bioactivation to a reactive metabolite (possibly a naphthoquinone), a free-radical derivative, or a combination of both (Wells *et al.* 1989). In these studies, pretreatment of mice with the cytochrome P450 inhibitor SKF-525A or the spin-trapping agent α-phenyl-*N*-butylnitrone in addition to treatment with vitamin E or caffeic acid inhibited naphthalene cataractogenicity. L-Cysteine prodrugs also were effective in preventing naphthalene-induced cataracts in mice, apparently by maintaining hepatic glutathione concentrations (Rathbun *et al.* 1996).

6.3.2.3 Respiratory tract toxicity

The respiratory tract has been identified as a site of naphthalene toxicity in rats and mice. In mice, lung injury induced by naphthalene exposure is related to the degree of monooxygenase activity, covalent binding to tissue macromolecules, depletion of the pulmonary GSH stores, and stereoselectivity of the metabolism (Plopper *et al.* 1992, Chichester *et al.* 1994, Cho *et al.* 1994, Höke and Zellerhoff 1998). The relative quantities of reactive naphthalene metabolites covalently bound intra- or extra-cellularly may depend on the saturation of intracellular detoxification pathways, such as hydrolysis or enzymatic attack (Richieri and Buckpitt 1987).

Intraperitoneal administration of naphthalene produced a highly organ-selective and species-selective lesion of the pulmonary bronchiolar epithelium in mice, but no such lesion was detected in rats or hamsters. In its review, the NTP reported that a single i.p. injection of naphthalene at 0.05 mmol/kg b.w. (6 mg/kg) or 2 mmol/kg b.w. (256 mg/kg) induced necrosis of the bronchial/bronchiolar epithelium in C57BL/6J mice (NTP 2000). This lesion was reversible, and regeneration occurred after seven days. Necrosis of the bronchial epithelial (Clara) cells occurred in the lungs of C57BL/6J mice given a single i.p. injection of naphthalene at 125 or 250 mg/kg b.w. An i.p. injection of 400 or 600 mg/kg in Swiss TO mice damaged the Clara cells of the lung and proximal tubule epithelial cells of the kidney. In contrast, an i.p. injection of 1,600 mg/kg in Wistar-derived rats did not produce any damage in the lung or the kidney. The NTP review stated that these results suggested that rats were less susceptible to naphthalene toxicity than mice and that the difference in susceptibility was due to differences in the rate of naphthalene metabolism.

Plopper *et al.* (1992) studied histopathologic changes in the respiratory tract 24 hours after parenteral administration of a single oral dose of naphthalene to Swiss Webster mice (0 to 400 mg/kg b.w.), Sprague-Dawley rats (0 to 1,600 mg/kg b.w.), and Golden Syrian hamsters (0 to 800 mg/kg b.w.). Naphthalene injury (swelling, vacuolization, exfoliation, or necrosis) to the tracheobronchial epithelium in the mice was specific to Clara cells.

Dose-related injury occurred in the terminal bronchioles and involved proximal airways, at dose levels well below the mouse LD_{50}. Clara cells in the rat were refractory to injury, and proximal airways were more susceptible than distal airways in the hamster. Naphthalene was cytotoxic to the olfactory epithelium in rats and mice, with the effect seen at a higher dose in mice (400 mg/kg b.w.) than in rats (200 mg/kg b.w.). Recent studies with adult and neonatal Swiss Webster mice showed that Clara cells in the neonates were more susceptible than adult cells to injury following exposure to naphthalene *in vivo* (Fanucchi *et al.* 1997).

Buckpitt *et al.* (1985) reported that immunohistochemical analysis of airway explants from a sensitive species (mice) and nonsensitive species (rats and hamsters) indicated that the cells from mice contain a unique P450 (CYP2F, a family of microsomal enzymes uniquely expressed in the lung and olfactory mucosal cells (Lakritz *et al.* 1996, Shultz *et al.* 1999)). This enzyme catalyzes the high degree of metabolic stereoselectivity to 1*R*-2*S*-naphthalene oxide over the 1*S*-2*R*-oxide (66:1 enantiomeric ratio) in mice (Shultz *et al.* 1999). The orthologs of CYP2F2 in the rat and primate are designated CYP2F4 and CYP2F1, respectively (Shultz *et al.* 2001). The rates of metabolism of naphthalene by the enzymes of these species differ widely.

Severe bronchiolar epithelial cell necrosis in male Swiss Webster mice was observed following a single i.p. dose of 200 mg/kg of [^{14}C]naphthalene; however, no renal or hepatic necrosis was noted (Warren *et al.* 1982). Pretreatment with piperonyl butoxide substantially reduced the bronchiolar necrosis, confirming the involvement of cytochrome P450–mediated metabolism in the lung. Reactive metabolites bound covalently to tissue macromolecules in a dose-dependent manner, and binding was consistently increased in tissues with higher cytochrome P450 levels, such as lung, liver, and kidney. Covalent binding in the liver, a non-target tissue, was as high as binding in the lung; binding demonstrated a significant dose threshold that depended on the substantial depletion of tissue GSH reserves. Pretreatment of mice with diethyl maleate resulted in a dose-related reduction in GSH levels and increased severity of the lung lesion, indicating pulmonary metabolic activation and production of reactive naphthalene metabolites. GSH levels and covalent binding were inversely related, suggesting that GSH is important in the detoxification of reactive naphthalene metabolites. West *et al*. (2000) found GSH to be critical in the development of resistance to naphthalene injury by murine Clara cells.

6.3.2.4 Other Effects

The NTP Technical Report (2000) reported additional effects observed following naphthalene exposure. In rats, a single i.p. injection of naphthalene (1 g/kg b.w.) caused accumulation of ammonia in the brain, which correlated positively with naphthalene's lethality. Naphthalene inhibited aryl hydrocarbon hydroxylase activity in liver homogenates and microsomal preparations obtained from rats receiving 40-mg/kg i.p. injections for three days. A single 250-mg/kg i.p. dose of naphthalene to C57BL/6J mice depressed microsomal monooxygenase enzyme activity in the lung by 30% to 70%; enzyme activity was not affected in the liver.

Evaluation of the cytotoxicity of naphthalene and several of its metabolites to human liver microsomes indicated that the parent compound and the alcohol (1-naphthol) were

metabolized to species that were cytotoxic to resting human mononuclear leukocytes (Wilson *et al.* 1996). 1-Naphthol was more cytotoxic than naphthalene, and the dihydrodiol was not cytotoxic. The cytotoxicity of naphthalene and 1-naphthol increased with increasing concentration of liver microsomal protein, but 1-naphthol was activated at a much lower microsomal protein concentration than the parent compound.

6.4 Potential mechanisms of carcinogenicity

In two-year inhalation exposure studies (NTP 1992, 2000, Abdo *et al.* 1992), exposure to naphthalene induced lung neoplasms in female $B6C3F_1$ mice and nasal tumors in male and female F344/N rats (see Section 4.1). The difference in the sites of neoplasms between rats and mice may be related to differences in the anatomy of the nasal passages and in the metabolic activation of naphthalene in these two species. The NTP (2000) discussed the difference in the sites of neoplasms in rats and mice and the potential explanations for these differences. The distribution of potential carcinogens to the nasal passages of different species may contribute to this effect and the rates of production and clearance of carcinogenic metabolites of naphthalene may be different. Based on this discussion, the NTP speculated that activation and deactivation of naphthalene and accumulation of carcinogenic metabolite(s) could be greater in the nasal passages of rats than of mice; conversely the metabolism and accumulation of carcinogenic metabolites may be greater in the lungs of mice than of rats (see pp. B-47 to B-50). The high rate of metabolism of naphthalene in mouse lung and in rat nasal epithelium could be related to site-specific tissue damage. However, others have suggested that the presence of unique cytochromes P450 in the rat nasal mucosa could play a protective role in reducing the lung toxicity of some inhaled chemicals (Thornton-Manning and Dahl 1997).

6.4.1 Pulmonary carcinogenesis

The NTP report provided information on the metabolism and tissue distribution of inhaled naphthalene but did not propose a potential mechanism of naphthalene carcinogenicity. The results from the NTP PBPK model indicate that tissue dosimetry alone does not explain naphthalene's carcinogenic potential in the mouse lung but not in the rat lung (NTP 2000). The higher rate of metabolism in the mouse lung may be a contributing factor; however, because the model does not include detoxification rates for potentially carcinogenic naphthalene metabolites, lung concentrations of reactive intermediates cannot be compared with the naphthalene exposure concentrations. If detoxification processes are faster in mice than in rats, then rates of metabolic activation alone cannot reliably predict lung cancer risk. Naphthalene oxide is the primary metabolite formed by cytochrome P450–mediated oxidation of naphthalene. However, the metabolism of naphthalene oxide was not included in the NTP PBPK model. Further, no data were available on the blood concentrations of 1*R*-2*S*- and 1*S*-2*R*-naphthalene oxide.

Vuchetich *et al.* (1996) and Bagchi *et al.* (1998) proposed that oxidative stress induced by chronic oral administration of naphthalene to rats (see Section 5.3.2) could damage tissue and might contribute to the toxicity and carcinogenicity of this compound. The possible role of oxidative stress in naphthalene toxicity was supported by the ability of an antioxidant, vitamin E succinate, to significantly decrease the urinary excretion of lipid metabolites in rats exposed to naphthalene. The authors proposed that quinone

derivatives of naphthalene, including 1,2- and 1,4-naphthoquinone, could undergo redox cycling reactions that would produce reactive oxygen species and semiquinone anion radicals. Their reports, however, did not directly establish a role for these potentially reactive naphthalene metabolites in naphthalene toxicity. Thus, the chemical mechanism underlying any potential carcinogenicity of naphthalene remains to be elucidated.

6.4.1.1 Species differences in sensitivity to epoxides

Mice appear to be more susceptible to lung neoplasm induction by epoxides and epoxide-forming chemicals than are rats (Melnick and Huff 1993). Melnick and Sills (2001) reviewed the carcinogenicity of the epoxide-forming chemicals 1,3-butadiene, isoprene, and chloroprene in rats and mice together with that of the epoxide, ethylene oxide. The authors reported that all four chemicals induced lung tumors in mice, but only chloroprene induced lung tumors in rats (female). The NTP (2000) stated that if naphthalene oxide is the sole agent responsible for induction of lung neoplasms in mice exposed to naphthalene, then the species difference in response in the lung may be attributed to a combination of higher rates of naphthalene oxide production in the mouse lung and, possibly, a greater susceptibility of the mouse lung to epoxide-induced carcinogenesis.

The metabolism of chemicals to epoxides may differ among species, among strains, and even within the airways of individual animals. Forkert *et al.* (2001) demonstrated that CYP2E1-mediated formation of 1,1-dichloroethylene epoxide and other reactive intermediates and the severity of lung cytotoxicity both were correlated with the levels of CYP2E1 in the lungs of three strains of mice. The species difference in bioactivation and detoxication of 1,3-butadiene between B6C3F_1 mice and Sprague-Dawley rats has also been proposed to result from differences in cytochrome P450 activity. Buckpitt *et al.* (1995) examined the metabolism of naphthalene by specific subcompartments of microdissected airways from mice, rats, and hamsters. They reported that naphthalene was converted to its epoxide more rapidly in the tracheobronchial airways of susceptible than nonsusceptible species, and that expression of the cytochrome P450 isoform CYP2F2 correlated with the conversion rates.

6.4.1.2 Toxicity of naphthalene to Clara cells of the lung in mice and rats

Gram (1997) reviewed a number of studies on species differences in naphthalene lung toxicity. Clara cells in the lungs of mice were much more susceptible to naphthalene toxicity than were these cells in the lungs of rats or hamsters. In one study, naphthalene (i.p. administration) caused Clara cell toxicity at 50 mg/kg in mice, minor alterations in Clara cells at 800 mg/kg in hamsters, and no effects in rats at naphthalene levels up to 1,600 mg/kg. West *et al*. (2001) reported injury in Clara cells in the lungs from inhalation exposures as low as 2 ppm in mice, while no toxicity was reported in rats at concentrations as high as 110 ppm.

Several studies have examined the pattern of events in the Clara cells that result in naphthalene-induced acute toxicity. One study showed a pattern of focal swelling of the Clara cells, followed by changes in Clara cell ultrastructure, including the rearrangement of cytoskeletal filaments, and finally, loss of cell membrane permeability (Van Winkle *et al.* 1999). Another study showed that glutathione levels decreased by 50% in the first

hour after the injection of 200 mg/kg naphthalene in mice. This decrease occurred before the first signs of cellular damage (cytoplasmic vacuolization), showing that the loss of intracellular glutathione is an early event that precedes signs of cellular damage (Plopper *et al.* 2001). It has been proposed that the species differences in Clara cell toxicity (see above) are caused by naphthalene's ability to deplete glutathione more severely in the mouse lung, as compared to the hamster or rat (Gram 1997).

Naphthalene administration (i.p. injection of 200 mg/kg b.w.) to Swiss Webster mice resulted in exfoliation of Clara cells two days after treatment (Van Winkle *et al.* 1995). Over the same time course that the volume fraction of Clara cells decreased, i.e., one to two days after exposure to naphthalene, the proliferation of cells in the epithelium and interstitium also was increasing. Cell proliferation was maximal at two days post treatment, and Clara cell differentiation markers had returned to control levels by day five. Stripp *et al*. (1995) also followed the re-expression of Clara cell differentiation markers in FVB/n mice injected i.p. with naphthalene. They observed a re-population of the naphthalene-injured airways by proliferation of immature epithelial cells preferentially at airway bifurcations.

6.4.1.3 Pulmonary neuroendocrine cell hyperplasia after naphthalene toxicity

Pulmonary airway epithelial cells include a population of pulmonary neuroendocrine cells (PNECs) that are scattered throughout the bronchial tree and are able to secrete a variety of neuropeptides. Since these cells and the peptides that they secrete may play a role in fetal lung development, some researchers have postulated a role for these cells in recovery from chemical toxicity and have examined their number and activity in lungs of mice expose to naphthalene. Stevens *et al.* (1997) and Peake *et al.* (2000) reported that PNEC hyperplasia could be detected in the lungs of FVB/n mice five days after i.p. injection with 300 mg/kg b.w. naphthalene in corn oil. Increased cell proliferation was involved in this hyperplasia as evidenced by an increase in [^{3}H]thymidine labeling in clusters of PNECs in neuroepithelial bodies (NEBs). The number of PNECs per mm^2 was threefold higher in the lungs of naphthalene-treated mice compared to those of control mice. The authors concluded that proliferation of PNECs could play a key role in the renewal of airway epithelial cells after toxic injury by naphthalene. They also suggested that further research is needed to elucidate the relationship between injury and repair of Clara cells and the proliferation of PNECs after toxic injury.

6.4.2 Nasal and olfactory epithelia as targets for chemical carcinogenesis

Pino *et al.* (1999) were able to identify only one report of a spontaneous tumor of the olfactory epithelium in rodents. However, they questioned the spontaneous nature of even that one tumor since it was observed in a "low-dose group of rats in a two-year carcinogenicity feeding study, in which no carcinogenicity for rats had been determined (Shibuya *et al.* 1996)." [The agent being tested was not identified by Shibuya *et al.* (1996).] Brown *et al.* (1991) reviewed the literature on proliferative lesions of the rodent nasal cavity. The authors drew several general conclusions from their review, including a statement that rodents rarely develop spontaneous nasal tumors. The conclusions that specifically related to nasal tumors of rats are summarized here. They found that rats were more susceptible than were mice to the induction of nasal cavity epithelial tumors. Olfactory epithelial tumors were described as almost uniformly malignant and invasive.

Additionally, they pointed out that tumors of the olfactory region induced by exposure to chemicals could result from targeting to the region even with systemic administration; thus, an inhalational route of exposure was not required to demonstrate carcinogenesis.

6.4.2.1 Metabolism of naphthalene by nasal epithelia

The metabolism of naphthalene by nasal mucosal postmitochondrial supernatants varied among species (mouse, rat, and hamster) (see Section 6.2.2, above), but the relative rates of metabolism did not correlate well with the relative sensitivity of the olfactory mucosa of these species to naphthalene toxicity (Buckpitt *et al.* 1992). The rat nasal mucosal tissue was the most sensitive to the toxic effects of naphthalene and was the only site of tumors in rats in the two-year bioassay (NTP 2000 and Section 4.2, above). The highest rate of metabolism, however, was two-fold higher for mouse olfactory epithelium compared to that of the rat. Buckpitt *et al.* (1992) suggested that the differences in sensitivity of rat versus mouse or hamster olfactory epithelium could be related to other metabolic factors such as epoxide hydrolase or glutathione S-transferase activity or the levels of reduce glutathione in tissues of the different species.

6.4.2.2 Chemicals that produce nasal tumors

Haseman and Hailey (1997) summarized the incidence of nasal tumors in almost 500 rodent carcinogenicity studies included in the NTP database at that time. They identified 12 chemicals that produced nasal tumors in rats (Table 6-3). Two additional chemicals that were subjects of two-year NTP bioassay studies subsequent to their review also are included in Table 6-3.

6.4.2.3 Species differences in nasal anatomy

Although the nasal airways of animals and humans have some similarities, the anatomy of the upper respiratory tract differs among species of experimental animals used to study carcinogenicity and between these animal species and humans. Reznik (1990) and Ménache *et al*. (1997) reviewed the comparative anatomy, physiology, and function of the upper airways of humans and of laboratory animals used in toxicology studies. One important distinction is that the human nose has a relatively simple structure necessary to perform its primary function of breathing (respiration) while other mammals tend to have more complex noses to support their primary function of olfaction. However, even within species of laboratory animals, differences in the ratio of respiratory to olfactory epithelium exist. For example, the respiratory epithelium per unit volume of nasal cavity is greater in mice than in rats.

The differences in anatomy may affect the way in which inhaled materials distribute to and are cleared from specific cell types of the nasal cavities. Morgan and Monticello (1990) reviewed the literature on the site specificity of nasal lesions induced by inhalational exposure of laboratory animals to a variety of chemicals. They concluded that the combined effects of airflow and tissue sensitivity determined the nature and distribution of nasal lesions resulting from a number of xenobiotic chemicals. The differences in nasal anatomy have been invoked in discussions of differential toxicity of chemicals across species; however, these differences may be more relevant to risk assessments than to the potential carcinogenicity of chemicals such as naphthalene.

Table 6-3. Chemicals that produced nasal tumors in rats in NTP/NCI studies (Adapted from Haseman and Hailey 1997).

Chemical	Technical report	Nasal carcinogenicity	Sex	Route of administration	Tumor types
Allyl glycidol ether	376	E	M	Inhalation	(a)
		–	F		Adenoma
p-Cresidine	142	+	M	Feed	Neuroblastoma
		+	F		Neuroblastoma
1,2-Dibromo-3-chloropropane	206	+	M	Inhalation	(b)
		+	F		(b)
1,2-Dibromethane	210	+	M	Inhalation	(b)
		+	F		(b)
2,3-Dibromo-1-propanol	400	+	M	Skin paint	Adenoma
		+	F		Adenoma
Dimethylvinyl chloride	316	+	M	Gavage	(c)
		+	F		(c)
1,4-Dioxane	080	+	M	Water	(c)
		+	F		(c)
1,2-Epoxybutane	329	+	M	Inhalation	Adenoma
		E	F		Adenoma
Furfuryl alcohol[1]	482	+	M	Inhalation	(d)
		E	F		Adenoma
Iodinated glycerol	340	E	M	Gavage	Adenoma
		-	F		Adenoma
Pentachlorophenol, purified[2]	483	+	M	Feed	Carcinoma
		-	F		-
Procarbazine	019	+	M	IP injection	Neuroblastoma
		+	F		Neuroblastoma
Propylene oxide	267	+	M	Inhalation	Adenoma
		+	F		Adenoma
2,6-Xylidine	278	+	M	Feed	(b)
		+	F		(b)

E = Equivocal evidence; - negative; + positive
(a)- One papillary adenoma, one squamous cell carcinonma, one poorly differentiated adenocarcinomas
(b)- Carcinoma, squamous cell papilloma, squamous cell carcinoma, adenoma, adenocarcinoma, adenomatous polyp, or carcinosarcoma
(c)- Carcinoma, squamous cell carcinoma, or adenocarcinoma
(d)- Two adenomas, one carcinoma, and three squamous cell carcinomas
[1]NTP Technical Report 482 (1999a).
[2]NTP Technical Report 483 (1999b).

6.4.2.4 Epithelial cell proliferation in nasal carcinogenesis

An increase in the rate of cell proliferation has been considered to be a contributing factor in carcinogenesis as it may provide an increased opportunity for somatic mutations during DNA replication. Formaldehyde, which is a weakly genotoxic carcinogen, has been studied extensively, and its effect on target cell proliferation correlates with tumor incidence (Monticello and Morgan 1997). The same authors, however, have pointed out that other inhaled irritant gases that induce severe nasal cytotoxicity and other non-neoplastic effects do not induce nasal tumors with chronic exposure (Monticello *et al.* 1993). Ward *et al.* (1993) reviewed the association between cell proliferation and nasal carcinogenesis in rodent bioassays sponsored by the NTP. They reported that of the 19 chemicals that were subjects of inhalation bioassays, 5 were nasal carcinogens, while the remaining 14 were nasal noncarcinogens. When the nasal lesions observed with the 19 chemicals were summarized by species/sex experiments, nasal lesions, including epithelial hyperplasia and metaplasia, were present in 15/15 experiments with the 5 nasal carcinogens, while 36/59 experiments with nasal noncarcinogens were positive for these lesions. The authors of this review concluded that, "Although cell proliferation may contribute to multistage carcinogenesis, cell proliferation is not necessarily a tumor promoter or cocarcinogen."

6.5 Naphthalene-related compounds

No data on the carcinogenicity of the naphthalene metabolites 1-naphthol, 2-naphthol, 1,2-naphthoquinone, and 1,4-naphthoquinone (see Figures 1-2, 1-3, and 6-2) were found. The carcinogenicity of a number of naphthalene-related compounds has been reviewed by the NTP and IARC. Table 6-4 summarizes the data available on the carcinogenicity and mutagenicity of these naphthalene derivatives; studies of the mutagenicity of naphthalene-related compounds are discussed in Section 5.5.

Table 6-4. Carcinogenicity and mutagenicity data for some naphthalene derivatives

Naphthalene derivative	CAS#	Carcinogenicity and mutagenicity data
1-Naphthylamine	134–32–7	IARC (1987) Group 3 (not classifiable as to its carcinogenicity to humans). Positive for mutation in *Salmonella typhimurium*, chromosomal aberrations in cultured rodent cells, and aneuploidy in yeast; negative for mutation in *Drosophila*, cell transformation in Syrian hamster embryo cells, and mutation in yeast (IARC 1987).
2-Naphthylamine	91–59–8	*Known to be a human carcinogen* (NTP 2001) based on sufficient evidence in humans (bladder cancer in workers) and in animals (urinary bladder carcinoma in hamsters, dogs, nonhuman primates, and mice). Positive for mutation in *Salmonella typhimurium*, *Drosophila*, and Chinese hamster ovary cells (HSDB 2002b).
N-phenyl-2-naphthylamine	135–88–6	No evidence in male or female F344 rats or male B6C3F_1 mice. Equivocal evidence in B6C3F_1 female mice (two rare kidney neoplasms) (NTP 1988). Weakly positive for sister-chromatid exchange and chromosomal aberrations in Chinese hamster ovary cells; negative for mutation in *Salmonella typhimurium* (NTP 2002a).
1,5-Naphthalenediamine	2243–62–1	Negative in male F344 rats and positive in female F344 rats (clitoral and uterine neoplasms). Positive in male B6C3F_1 mice (thyroid neoplasms) and female B6C3F_1 mice (thyroid, liver, and lung neoplasms) (NTP 1978a). IARC (1982) Group 3 (not classifiable as to its carcinogenicity to humans). Positive for mutation in *Salmonella typhimurium* (IARC 1982).
1-Nitronaphthalene	86–57–7	Negative in male and female F344 rats and male and female B6C3F_1 mice (NTP 1978b). IARC (1981) Group 3 (not classifiable as to its carcinogenicity to humans). Positive for mutation in *Salmonella typhimurium;* weakly positive for chromosomal aberrations and negative for sister chromatid exchange *in vitro* (cell line not specified);and negative for sex-linked recessive lethal/reciprocal translocation in *Drosophila* (NTP 2002b).

6.6 Summary

Naphthalene is rapidly absorbed and metabolized when inhaled or administered dermally or orally to animals. Naphthalene is excreted in the urine as the unchanged parent compound, as metabolites (including 1-naphthol, 2-naphthol, naphthoquinones, and dihydroxynapthalenes), or as glutathione (GSH), cysteine, glucuronic acid, and sulfate conjugates. Urinary naphthalene metabolites found in workers at a coke plant correlated significantly with naphthalene concentrations in personal air samples, indicating that naphthalene is absorbed in humans. The first step in the metabolism of naphthalene is the formation of naphthalene-1,2-oxide by cytochrome P450 in the presence of NADPH, which is converted to the trans-1,2-diol and other products. The electrophilic naphthalene intermediates are metabolized to naphthoquinones and possibly to free-radical intermediates; *in vivo*, these metabolites contribute to depletion of GSH, and excess metabolites may bind covalently to tissue macromolecules. Higher rates of metabolism in microdissected airways have been reported to occur in mice than in rats or hamsters.

The toxicity of naphthalene is manifested primarily in the hematologic system in humans and dogs (hemolytic anemia), the pulmonary system in rodents (lung injury) and the eye in humans and rodents (lens opacity and cataracts).

Naphthalene induced lung neoplasia in female B6C3F$_1$ mice and nasal tumors in male and female F344/N rats. The mechanism of action has not been elucidated. Toxicity of naphthalene to lung and other tissues has been attributed to formation of the 1*R*,2*S*-naphthalene oxide; a strong correlation has been reported between the rates of formation of 1*R*,2*S*-naphthalene oxide in various tissues and tissue-selective toxicity. Naphthalene-induced oxidative damage and DNA breakage, which have been observed in rat liver and brain tissue, may contribute to the toxicity and carcinogenicity of naphthalene. Mice appear to be more susceptible to induction of lung neoplasia by epoxides and epoxide-forming chemicals than are rats. Differences between rats and mice in the metabolism of naphthalene by nasal epithelia and in nasal anatomy may contribute to the species differences in susceptibility to these tumors.

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Appendix A: NTP (1992). Toxicology and Carcinogenesis Studies of Naphthalene (CAS No. 91-20-3) in B6C3F$_1$ Mice (Inhalation Studies). TR 410. PP A-1 – A-173.

Appendix B: NTP (2000). Toxicology and Carcinogenesis Studies of Naphthalene (CAS No. 91-20-3) in F344/N Rats (Inhalation Studies). TR 500. PP B-1 – B-177.