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TOXICOLOGICAL REVIEW

OF

2-METHYLNAPHTHALENE

(CAS No. 91-57-6)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

April 2003

NOTICE

This document is an **external peer review draft**. It has not been formally released by the U.S. Environmental Protection Agency and should not at this stage be construed to represent Agency position on this chemical. It is being circulated for review of its technical accuracy and science policy implications.

U.S. Environmental Protection Agency
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FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to 2-methylnaphthalene. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of 2-methylnaphthalene.

In Section 6, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose response. Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at 301-345-2870.

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This document and summary information on IRIS have received peer review both by EPA scientists and by independent scientists external to EPA. Subsequent to external review and incorporation of comments, this assessment has undergone an Agency-wide review process whereby the IRIS Program Director has achieved a consensus approval among the Office of Research and Development; Office of Air and Radiation; Office of Prevention, Pesticides, and Toxic Substances; Office of Solid Waste and Emergency Response; Office of Water; Office of Policy, Economics, and Innovation; Office of Children's Health Protection; Office of Environmental Information; and the Regional Offices.

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1. INTRODUCTION

This document presents background and justification for the hazard and dose-response assessment summaries in EPA's Integrated Risk Information System (IRIS). IRIS Summaries may include an oral reference dose (RfD), inhalation reference concentration (RfC) and a carcinogenicity assessment.

The RfD and RfC provide quantitative information for noncancer dose-response assessments. The RfD is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis but may not exist for other toxic effects such as some carcinogenic responses. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer effects during a lifetime. The inhalation RfC is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). It is generally expressed in units of mg/m^3.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral exposure and inhalation exposure. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways. The *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg/day. The *unit risk* is the quantitative estimate in terms of either risk per µg/L drinking water or risk per $\mu g/m^3$ air breathed. Another form in which risk is presented is a drinking water or air concentration providing cancer risks of 1 in 10,000; 1 in 100,000; or 1 in 1,000,000.

Development of these hazard identification and dose-response assessments for 2-methylnaphthalene has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines that were used in the development of this assessment may include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), *Draft Revised Guidelines for Carcinogen Assessment* (U.S. EPA, 1999), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), (proposed) *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998b, 2000a), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000b), *Benchmark Dose Technical*

Guidance Document (U.S. EPA, 2000c) and *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA 2000d).

The literature search strategy employed for this compound was based on the CASRNs for 2-methylnaphthalene (91-57-6) and methylnaphthalene (1321-94-4), and at least one common name. At a minimum, the following databases were searched: RTECS, HSDB, TSCATS, CCRIS, GENE-TOX, DART/ETIC, EMIC, TOXLINE, CANCERLIT, MEDLINE, and Current Contents. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through March, 2003.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

2-Methylnaphthalene (CASRN 91-57-6) is a polycyclic aromatic hydrocarbon (PAH), consisting of two-fused aromatic rings with a methyl group attached on one of the rings at the number two carbon (Figure 1). Synonyms include β-methylnaphthalene. Some physical and chemical properties are shown below (ATSDR, 1995; CRC, 1990).

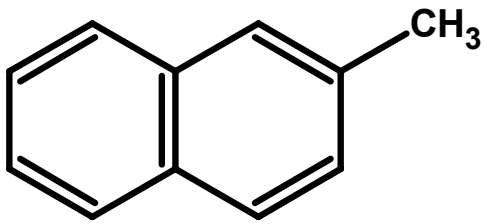

Figure 1: 2-Methylnaphthalene

Chemical Formula:	$C_{11}H_{10}$
Molecular Weight:	142.20 g/mol
Melting Point:	34.6 °C
Boiling Point:	241 °C
Density:	1.0058 g/mL (at 20 °C)
Water Solubility:	24.6 mg/L (at 25 °C)
Log K_{ow}:	3.86
Log K_{oc}:	3.39
Vapor Pressure:	0.068 mmHg at 20 °C
Henry's Law Constant:	4.99×10^{-4} atm-m^3/mol

2-Methylnaphthalene is a natural component of crude oil and coal, and is found in pyrolysis and combustion products such as cigarette and wood smoke, emissions from

combustion engines, asphalt, coal tar residues, and used oils (ATSDR, 1995; HSDB, 2002; Warshawsky, 2001). Methylnaphthalene (CASRN 1321-94-4) refers to a mixture of approximately two-thirds 2-methylnaphthalene and one-third 1-methylnaphthalene (CASRN 90-12-0). Methylnaphthalene is manufactured from coal tar by extraction of heteroaromatics and phenols. Distillation of methylnaphthalene removes 1-methylnaphthalene, leaving 2-methyl-naphthalene. Mixtures containing 2-methylnaphthalene are used in the formulation of alkyl-naphthalenesulfonates (used for detergents and textile wetting agents), chlorinated naphthalenes, and hydronaphthalenes (used as solvents). Pure 2-methylnaphthalene is a component used in the manufacture of vitamin K and the insecticide carbaryl (1-naphthyl-N-methylcarbamate) (HSDB, 2002).

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

No studies are available regarding the toxicokinetics of 2-methylnaphthalene in humans by any route of exposure.

The available animal data indicate that 2-methylnaphthalene is absorbed rapidly following ingestion (approximately 80% within 24 hours). Once absorbed, it is widely distributed among tissues, reaching peak concentrations in less than 6 hours. It is quickly metabolized by the liver, lungs, and other tissues. 2-Methylnaphthalene is rapidly excreted (55-90% within 48 hours), primarily as urinary metabolites.

3.1. ABSORPTION

Quantitative evidence of the rapid and extensive absorption of 2-methylnaphthalene is provided by a study of guinea pigs orally exposed to 2-methylnaphthalene (Teshima et al., 1983).

Teshima et al. (1983) orally administered 10 mg/kg of 2-[^{3}H]methylnaphthalene in olive oil to male Hartley guinea pigs. Groups of 3 animals were sacrificed at 3, 6, 24, and 48 hours after exposure and radioactivity was measured in various organs and tissues (Table 1). The amount of the radiolabel detected outside the gastrointestinal tract (i.e., internal organs, blood, and urine together) provides an estimate of absorbed material, whereas radiolabel found in the gastrointestinal contents and feces provides an estimate of 2-methylnaphthalene that is not absorbed. These data indicate that at least 25-72% of the administered dose was absorbed by 3 hours, 44-80% by 6 hours, and 80-86% by 24 hours. These percentages may underestimate the actual amounts absorbed since there may be significant enterohepatic cycling (as discussed in Section 3.4).

Although no quantitative studies are available regarding the rate or extent of 2-methylnaphthalene absorption by the respiratory tract or skin, findings of systemic toxicity following exposure by these routes provide qualitative evidence of absorption. Inhalation

exposure to concentrations ≥352 mg/m^3 of 2-methylnaphthalene for 4 hours induced a delayed pain response in Wistar rats, indicating that some absorption may have occurred (Korsak et al., 1998). Dermal exposure of B6C3F1 mice to 119 mg/kg of a mixture of 2-methylnaphthalene and 1-methylnaphthalene in an approximate 2:1 ratio twice weekly for 30 or 61 weeks (equivalent to 35 mg/kg-day) induced pulmonary toxicity in virtually all exposed mice (Emi and Konishi, 1985; Murata et al., 1992). By comparison, oral exposure of B6C3F1 mice to 52.3 mg/kg of 2-methylnaphthalene for 81 weeks led to the development of pulmonary toxicity in approximately half of the exposed mice (Murata et al., 1997). Given that 2-methylnaphthalene is extensively absorbed following oral exposure (Teshima et al., 1983), these results indicate that considerable dermal absorption of 2-methylnaphthalene occurred.

Table 1. Distribution of Radioactivity in Guinea Pigs after Oral Administration of 2-[^{3}H]Methylnaphthalene (adapted from Teshima et al., 1983)

Tissue	3 hours	6 hours	24 hours	48 hours
μg of ^{3}H/g wet tissue				
Gallbladder	20.2	15.7	0.4	0.04
Kidney	5.6	7.6	0.3	0.1
Liver	1.7	2.7	0.2	0.1
Blood	0.8	0.7	0.1	0.1
Lung	0.7	0.8	0.1	0.1
Others (combined)	0.8	1.1	0.2	0.1
Percent of total administered dose				
Internal organs	1.4	2.1	0.1	0.1
Blood	0.6	0.5	0.1	0.1
Gastrointestinal contents	27.9	20.2	3.1	1.0
Urine	23.1	41.3	78.6	72.2
Feces	0	0	10.8	11.9
Total recovery	53	64.2	92.7	85.2

3.2. DISTRIBUTION

Following oral administration, 2-methylnaphthalene is absorbed from the gastrointestinal tract into the portal circulation and transported to the liver, where it undergoes oxidative metabolism to form more polar metabolites. These metabolites are then transported via systemic circulation to the various organs and tissues, including the kidney. Excretion occurs primarily in the urine. No human distribution data are available, but two animal studies that measured distribution of radioactivity following acute oral (Teshima et al., 1983) and injection dosing (Griffin et al., 1982) were identified. No distribution studies following inhalation or dermal exposure are available.

Teshima et al (1983) orally administered single doses of 10 mg/kg 2-[^{3}H]methyl-naphthalene to male Hartley guinea pigs (3/group) and observed peak tissue concentrations of

radiolabel by 3 hours in the blood and gallbladder, and at 6 hours in all other tissues measured, as shown in Table 1. The detection of a relatively high concentration of radiolabel in the gallbladder at 3 hours suggests that liver concentrations may have actually peaked before 3 hours. Teshima et al. (1983) reported a clearance half-life of 10.4 hours from the blood, but did not specify the details of their calculations.

Griffin et al. (1982) administered single intraperitoneal injections of 400 mg/kg of [^{14}C]-2-methylnaphthalene to male C57BL/6J mice. Groups of 4 control and 4 exposed mice were sacrificed at 0.5, 1, 3, 6, 12, and 24 hours after injection for measurement of radioactivity in fat, kidney, liver, and lung. Blood 2-methylnaphthalene concentrations decreased with a reported elimination half-life of 3 hours, indicative of rapid distribution to other tissues or elimination from the body. Peak tissue concentrations of 2-methylnaphthalene equivalents (nmol/mg wet weight) were attained about 1 hour after injection in the liver, 2 hours after injection in the fat, and 4 hours after injection in the kidney and the lung (Griffin et al., 1982). Peak concentrations were highest in fat (13 nmol/mg), followed by lower concentrations in liver (3.5 nmol/mg), kidney (2.9 nmol/kg), and lung (0.7 nmol/kg). These results indicate that 2-methylnaphthalene did not preferentially accumulate in the lung although the lung was the only site of toxicity. Histological examination found that the single 400-mg/kg dose of 2-methylnaphthalene induced bronchiolar necrosis (minimal to prominent sloughing of lining cells in the bronchiolar lumen as revealed by light microscopy) in all exposed mice (Griffin et al., 1982). No lesions were found in the liver or kidney of exposed mice at any time point. Consistent with the attainment of peak lung tissue concentration at 4 hours after injection and the consequent development of these lesions, none were evident until 8 hours after injection. These authors also evaluated distribution by measurement of irreversible binding of [^{14}C]-2-methylnaphthalene to various tissues over a dose (0, 50, 100, 300, and 500 mg/kg; intraperitoneal injection) and time course (1, 2, 4, 8, 12, and 24 hrs). Irreversible binding of 2-methylnaphthalene metabolites was maximal in lung, liver, and kidney tissues at 8 hours post administration. This binding was dose dependent in all tissues between 50-500 mg/kg and concentrations of bound radioactivity were higher in the liver and kidney than in the lung (the only tissue where lesions were found).

In addition, Griffin et al. (1982) evaluated the influence of changes in metabolism on distribution. Groups of mice (5/group) were treated with inhibitors (piperonyl butoxide or SKF525-A) or inducers (phenobarbital or 3-methylcholanthrene) of cytochrome P450 (CYP) enzymes, or with diethylmaleate to deplete tissue levels of glutathione prior to treatment with 2-methylnaphthalene (50-500 mg/kg) for the measurement of irreversible tissue binding. The CYP enzyme inhibitor piperonyl butoxide led to significantly decreased irreversible binding in the liver, lung, and kidney (by approximately 70, 40, and 50%, respectively). Administration of the CYP enzyme inducer phenobarbital led to significantly reduced irreversible binding in the lung (by approximately 30%) and reduced (not statistically significant) irreversible binding in the liver (by approximately 50%). Depletion of reduced glutathione by treatment with diethylmaleate led to significantly reduced irreversible binding in the kidney and lung (by approximately 40 and 30%, respectively).

3.3. METABOLISM

The proposed metabolic pathway for 2-methylnaphthalene in mammals is shown in Figure 2. This pathway has been elucidated by identification of urinary metabolites eliminated from laboratory animals following acute exposure, by studies measuring the effects of enzyme modulators on the toxic and biochemical changes caused by 2-methylnaphthalene exposure in mice, and by *in vitro* analyses of purified enzyme preparations (microsomal fractions and recombinant enzymes) from liver, lung, and kidney tissues.

CYP enzymes catalyze the first competing steps, which involve oxidation at the methyl group (the predominant path) or oxidation at several positions on the rings (Figure 2). Approximately 50-80% of 2-methylnaphthalene is oxidized at the 2-methyl group to produce 2-hydroxymethylnaphthalene (Breger et al., 1983; Melancon et al., 1982; Teshima et al., 1983). 2-Hydroxymethylnaphthalene is further oxidized to 2-naphthoic acid (the carboxylic acid derivative) (Grimes and Young, 1956; Melancon et al., 1982; Teshima et al., 1983), either directly or through the intermediate, 2-naphthaldehyde. 2-Naphthaldehyde has been detected only following *in vitro* incubation of 2-methylnaphthalene with recombinant mouse CYPIIF2 (Schultz et al., 2001). 2-Naphthoic acid may be conjugated with glycine or with glucuronic acid. These two reactions can be catalyzed by amino acid transferase (i.e., ATP-dependent acid: CoA ligase and N-acyltransferase) and uridine diphosphate glucuronosyltranferase, respectively (Parkinson, 2001). The conjugation of 2-naphthoic acid with glycine forms 2-naphthuric acid, the most prevalent metabolite of 2-methylnaphthalene detected in urine (Grimes and Young, 1956; Melancon et al., 1982; Teshima et al., 1983).

Approximately 15-20% of 2-methylnaphthalene undergoes ring epoxidation at the 3,4-, 5,6-, or 7,8- positions (Breger et al., 1983; Melancon et al., 1985). These reactions are catalyzed by CYP enzymes, including CYPIA and CYPIB. The epoxides themselves have not been isolated, but are proposed intermediates, based on observed metabolites. These epoxides are thought either to be further oxidized by epoxide hydrolase to produce dihydrodiols (the 3,4-dihydrodiol, 5,6-dihydrodiol, or 7,8-dihydrodiol of 2-methylnaphthalene), or conjugated with glutathione (Griffin et al., 1982; Melancon et al., 1985). Glutathione conjugation can be catalyzed by isozymes from the large family of glutathione S-transferases or can proceed spontaneously (Parkinson, 2001). The hydroxy-glutathionyl-dihydro-2-methylnaphthalenes were detected after incubation of 2-methylnaphthalene with hepatic microsomes from Swiss-Webster mice or with isolated recombinant mouse CYPIIF2 enzyme and glutathione S-transferase (Schultz et al., 2001). Figure 2 shows six hydroxy glutathionyl 2-methylnaphthalenes; two are formed for each of the epoxide intermediates (3,4-, 5,6-, and 7,8-epoxides), and each can exist in two enantiomeric forms not shown in Figure 2 (Schultz et al., 2001).

Figure 2 also shows 3 other minor metabolites formed via the 7,8-epoxide pathway. 1-Glutathionyl-7-methylnaphthalene was identified in the urine of guinea pigs and by *in vitro* experiments with guinea pig microsomes (Teshima et al., 1983). 7-Methyl-1-naphthol and 7-

methyl-2-naphthol were identified in the urine of 4 species (rats, mice, guinea pigs, and rabbits) following oral exposure (Grimes and Young, 1956).

The mammalian metabolism of 2-methylnaphthalene has been analyzed in two quantitative experiments (Melancon et al., 1982; Teshima et al., 1983). Melancon et al. (1982) administered single subcutaneous injections of 0.3 mg/kg of 2-methyl [8-^{14}C]naphthalene to 4 female Sprague-Dawley rats. In collected urine, 3-5% of the administered dose was unchanged 2-methylnaphthalene, 30-35% was naphthuric acid, 6-8% were other conjugates of naphthoic acid, 6-8% were dihydrodiols of 2-methylnaphthalene, 4-8% were other nonconjugated metabolites, and 36-45% were other high-polarity unidentified metabolites. Teshima et al. (1983) administered single oral doses of 10 mg/kg of 2-[^{3}H]methylnaphthalene to male Hartley guinea pigs (3/group). At 24 hours, 78.6% of the total administered dose had been excreted in urine as metabolites. Sixty-one percent of radioactivity in urine was accounted for by 2-naphthuric acid, 11% by glucuronide conjugates of 2-naphthoic acid, 4% by unconjugated 2-naphthoic acid, 10% by S-(7-methyl-1-naphthyl)cysteine, and at least 8% by metabolites of 7-methyl-1-naphthol. Additionally, unquantified glutathione conjugates were detected in the livers of treated guinea pigs (Teshima et al., 1983). Taken together, these reports indicate that metabolism of 2-methylnaphthalene is rapid (at least 52% in rats within 3 days and at least 80% in guinea pigs within 1 day) and that 80-85% of the metabolism of 2-methylnaphthalene occurs via oxidation of the 2-methyl group, with ring epoxidation accounting for only 15-20%.

Standard assays in microsomal preparations (from male Sprague-Dawley rat liver, C57Bl/J6 mouse liver and lung, and Swiss-Webster mouse liver, lung, and kidney tissues) clearly demonstrate that the initial steps of 2-methylnaphthalene metabolism are mediated by CYP enzymes (Breger et al., 1981; Griffin et al., 1982; Melancon et al., 1985). These experiments showed that catalysis of 2-methylnaphthalene metabolism to either dihydrodiols (the ring epoxidation pathway) or 2-hydroxymethylnaphthalene (the alkyl-group oxidation pathway) required the cofactor NADPH and was inhibited by heat denaturation or carbon monoxide. Other studies that measured covalent binding of 2-methyl[8-^{14}C]naphthalene to liver, lung, and kidney microsomal proteins of male Swiss-Webster mice (Buckpitt et al., 1986) or liver slices of male ddY mice (Honda et al., 1990) observed a similar dependence of binding on CYP activity (i.e., inhibited by cold temperature, nitrogen atmosphere, piperonyl butoxide, and SKF 525A).

Microsomal studies with inducers and inhibitors of CYP activity have likewise demonstrated the importance of CYP enzymes in 2-methylnaphthalene metabolism, but have not provided clear mechanistic information. For example, pretreatment of male Sprague-Dawley rats (prior to microsomal preparation) with the CYP enzyme inducer β-naphthoflavone increased the overall rates of metabolism 4-fold, but the CYP enzyme inducer phenobarbital increased production of only 1 of the 3 dihydrodiol isomers (also 4-fold; specific isomer not determined) (Breger et al., 1981; Melancon et al., 1985). Pretreatment of mice (before microsome collection)

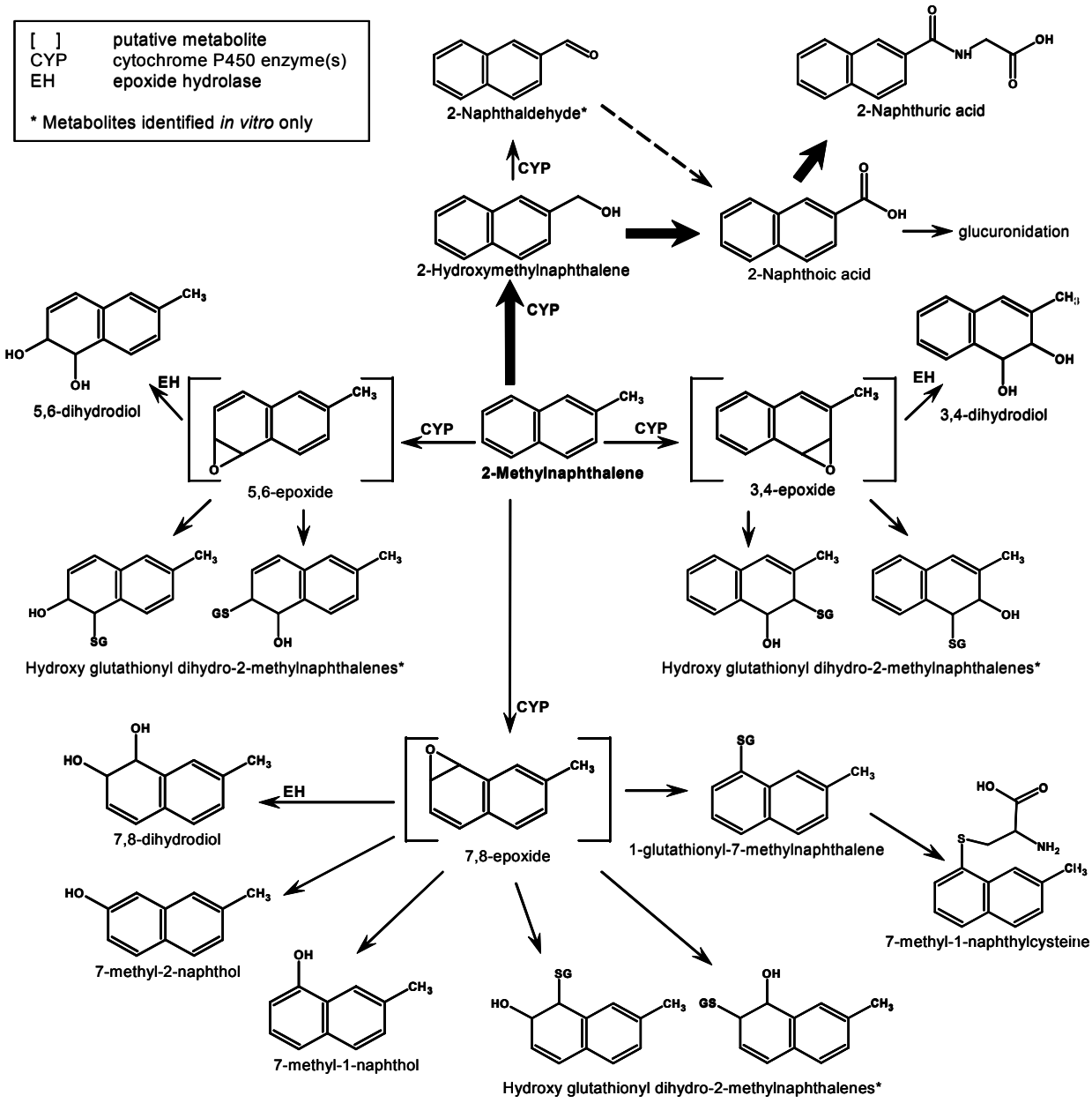

Figure 2: Metabolism of 2-methylnaphthalene (adapted from Buckpitt and Franklin, 1989; Shultz et al., 2001; Teshima et al., 1983).

with 3-methylcholanthrene (an inducer of CYPIA) reduced the pulmonary (but not hepatic) formation of one dihydrodiol isomer by half (Griffin et al., 1982). Phenobarbital increased the hepatic formation of a different isomer 3-fold, while neither piperonyl butoxide (a mixed monoxygenase inhibitor) nor diethylmaleate (depletes glutathione) had significant effects on metabolite formation (Griffin et al., 1982). Conversely, no significant changes in the metabolism of 2-methylnaphthalene could be induced in lung and liver microsomes of DBA/2J mice by pretreatment with 3-methylcholanthrene, piperonyl butoxide or diethylmaleate, although phenobarbital did increase the formation of one of the dihydrodiols (> 4-fold) without decreasing formation of the other two (Griffin et al., 1983). Taken together, these data suggest that different isozymes are responsible for different steps in the metabolism of 2-methylnaphthalene, and that they likely exhibit tissue- and strain-specificity.

Experiments that tested the effects of CYP enzyme inducers and inhibitors on the distribution and toxicity of 2-methylnaphthalene in mice (Griffin et al., 1982, 1983) provided suggestive evidence that CYP enzymes might metabolically activate 2-methylnaphthalene to one (or more) derivatives with higher toxicity; however, the identities of these putative metabolites are unknown. These studies are further discussed in detail in Section 4.4.3.

Titration experiments in rat hepatic microsomes demonstrated that epoxide hydrolase was rate-limiting for the formation of dihydrodiols (Melancon et al., 1985). Inhibitors of epoxide hydrolase (cyclohexane oxide and trichloropropylene oxide) fully inhibited the pulmonary and hepatic formation of all 3 dihydrodiols from 2-methylnaphthalene in mouse liver and lung microsomes (Griffin et al., 1982).

Animal studies provide evidence that glutathione conjugation is an important detoxification pathway. Griffin et al (1982) assessed reduced glutathione levels following intraperitoneal exposure of male C57BL/6J mice (4/group) to 400 mg/kg 2-methylnaphthalene at 0.5, 1, 3, 6, 12, 18, and 24 hours post injection. Compared with controls, exposed mice showed statistically significantly decreased levels of glutathione in the liver (32-37% decreased) at 3 and 6 hours after injection with 2-methylnaphthalene. Glutathione levels in the liver at other time points, and in the lung and kidney at all time points, were not decreased in exposed mice compared with controls. These results indicate that this dose of 2-methylnaphthalene led to a short-lived depletion of glutathione tissue levels only in the liver. Because glutathione does not conjugate directly with 2-methylnaphthalene, it is hypothesized that glutathione binds to a more reactive metabolite.

Other studies have also observed decreased glutathione tissue or intracellular levels in response to exposure to high acute doses of 2-methylnaphthalene, demonstrative of glutathione conjugation (Griffin et al., 1982, 1983; Honda et al., 1990). Similarly, glutathione depletion (by 35% compared to controls) was detected in primary cultures of female Sprague-Dawley rat hepatocytes treated with 1 mM of 2-methylnaphthalene (Zhao and Ramos, 1998).

Although many PAHs induce the activity of enzymes that metabolize them, no enzyme induction by 2-methylnaphthalene has been reported. Fabacher and Hodgson (1977) found no

changes in parameters of enzyme activity in the livers of male inbred North Carolina Department of Health strain mice (4/group) given intraperitoneal injections of 100 mg/kg of 2-methylnaphthalene daily for 3 days. Endpoints measured included O- or N-demethylation of p-nitroanisole and aminopyrene; metabolism of benzphetamine and piperonyl butoxide, pyridine and n-octylamine; microsomal protein levels; or carbon monoxide spectra. Chaloupka et al. (1995) measured hepatic and pulmonary microsomal ethoxyresorufin O-deethylase activity (EROD) and hepatic methoxyresorufin O-deethylase (MROD) levels in male B6C3F1 mice (≥ 4/group) given intraperitoneal injections of a mixture of 2-ring PAHs containing 23.2% 2-methylnaphthalene (as well as 23.8% naphthalene, 13.3% 1-methylnaphthalene, and 0.22% indan). MROD is a measure of CYPIA2, and EROD measures CYPIA1 and IA2 enzyme activity. Doses of the mixture containing 300 mg/kg of 2-methylnaphthalene did not induce lung microsomal EROD activity or hepatic MROD activity, and hepatic EROD was only minimally induced by doses containing 150 and 300 mg/kg of 2-methylnaphthalene (2.4- and 6-fold, respectively).

Important differences exist in the metabolism of 2-methylnaphthalene and naphthalene (ATSDR, 1995; Buckpitt et al., 1986, 1989; NTP, 2000). CYP enzymes catalyze the initial metabolic step for both compounds, but ring epoxidation is the only initial reaction for naphthalene, whereas, for 2-methylnaphthalene, alkyl-group oxidation is the principal initial reaction and ring epoxidation is a minor metabolic fate.

No studies evaluating the metabolism of 1-methylnaphthalene in humans or animals are available. Metabolism of this chemical may follow a similar pathway as that described here for 2-methylnaphthalene (i.e., side chain oxidation) since these chemicals are structurally related to each other. However, no studies providing evidence for this common pathway of metabolism were found.

3.4. ELIMINATION AND EXCRETION

No human data are available regarding the elimination or excretion of 2-methylnaphthalene. The limited animal data available indicate that absorbed 2-methyl-naphthalene is rapidly eliminated (approximately 55-90% within 2 days). Approximately 85% of the administered dose is eliminated in urine, and 11-14% in feces (Melancon et al., 1982; Teshima et al., 1983). No studies are available describing elimination of 2-methylnaphthalene by exhalation or other routes.

Table 1 shows the percent of urinary and fecal elimination of an oral dose of 10 mg/kg of 2-[^{3}H]methylnaphthalene from guinea pigs (Teshima et al., 1983). Despite the high initial levels of radioactivity detected in the gall bladder, urinary excretion exceeded fecal excretion by 7-fold, suggesting significant re-absorption of radioactivity from bile in the intestinal tract back into the body (i.e., enterohepatic cycling).

Female Sprague-Dawley rats (4/group) given subcutaneous injections of 0.3 mg/kg of 2-methyl [8-^{14}C]naphthalene eliminated 54.8% of the administered dose in urine within 3 days (Griffin et al., 1982).

Grimes and Young (1956) reported that urinary excretion was qualitatively similar among rabbits, guinea pigs, and mice given 2-methylnaphthalene by gavage or by intraperitoneal injection, but did not provide quantitative details.

3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC (PBTK) MODELING

No human or animal PBTK models were identified for 2-methylnaphthalene.

PBTK rat and mouse models have been developed for naphthalene (Ghanem and Shuler, 2000; NTP, 2000; Quick and Shuler 1999; Sweeney et al., 1996; Willems et al., 2001). These models were designed for oral, inhalation, intraperitoneal, and intravenous exposure, based on diffusion rates and tissue partitioning coefficients, as well as *in vivo* data for distribution, metabolism, and toxicity. The models assume that naphthalene is metabolized only in the liver and lungs to naphthalene oxide (the 1,2-epoxide of naphthalene); naphthalene oxide is assumed to be metabolized only in the liver and lungs by epoxide hydrolase (to dihydrodiols) or glutathione transferase (to glutathione conjugates).

The PBTK models for naphthalene in rodents are inadequate for predicting the toxicokinetics of 2-methylnaphthalene. An integral feature of the naphthalene models is the metabolism of naphthalene exclusively to naphthalene oxide. In contrast, only 15-20% of 2-methylnaphthalene undergoes ring epoxide formation, and 3 different isomers are produced (Melancon et al., 1982; Teshima et al., 1983). Therefore, the models for naphthalene would not adequately predict the toxicokinetics of 80-85% of the metabolites of 2-methylnaphthalene.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS—EPIDEMIOLOGY AND CASE REPORTS

No epidemiology studies or case reports are available which examined the potential effects of human exposure to 2-methylnaphthalene by any route of exposure.

4.2. PRECHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

4.2.1. Oral Exposure

4.2.1.1. Prechronic Toxicity

Fitzhugh and Buschke (1949) evaluated the ability of 2-methylnaphthalene to induce cataract formation in rats. No cataracts were found in a group of 5 weanling F344 rats fed a diet of 2% 2-methylnaphthalene (equivalent to 2000 mg/kg-day[1]) for at least 2 months, but cataracts were detected in rats fed an equivalent concentration of naphthalene. Evaluation of this study is limited by the lack of experimental details. In this study, 2000 mg/kg-day was an apparent NOAEL for cataract formation.

Murata et al. (1997) conducted a 13-week range-finding study exposing B6C3F1 mice (10/sex/group) to diets containing 0, 0.0163, 0.049, 0.147, 0.44, or 1.33% of 2-methyl-naphthalene for 13 weeks. Estimated doses for males were: 0, 29.4, 88.4, 265, 794, or 2400 mg/kg-day; for females estimated doses were: 0, 31.8, 95.6, 287, 859, or 2600 mg/kg-day[2]. Approximate average doses (across sexes) were: 0, 31, 92, 276, 827, or 2500 mg/kg-day. The 0.147% 2-methylnaphthalene diet reduced weight gain in both sexes by 20-21%, while the 0.44 and 1.33% 2-methylnaphthalene diets reduced weight gain by 30-38% in both sexes. The authors attributed these effects to food refusal. Only mice in the 0.44 and 1.33% dose groups were examined histologically, and no exposure related adverse effects were identified in any organ. Evaluation of these data is limited by inadequate reporting of study results. In this study, 92 mg/kg-day and 276 mg/kg-day (averaged between sexes) are the NOAEL and LOAEL, respectively for reduced weight gain.

4.2.1.2. Chronic Toxicity

Murata et al. (1997) fed B6C3F1 mice (50/sex/group) diets of 0, 0.075, or 0.15% of 2-methylnaphthalene for 81 weeks. The average intakes were reported as 0, 54.3 or 113.8 mg/kg-day for males and 0, 50.3, or 107.6 mg/kg-day for females. Mice were monitored daily for clinical signs of toxicity. For the first 16 weeks, food consumption and body weight were measured weekly, and then every other week thereafter. Blood was collected at sacrifice for leukocyte classification and comprehensive biochemical analyses. Organ weights were measured for the brain, heart, kidney, liver, individual lobes of the lung, pancreas, salivary glands, spleen, and testis. Histopathology was performed for these tissues and the adrenals, bone (sternal, vertebral, and rib), eye, harderian glands, mammary gland, ovary, seminal vesicle, skeletal muscle, skin, small and large intestine, spinal cord, stomach, trachea, uterus, and vagina. Quantitative differences between groups were statistically analyzed using Fisher's exact test and analysis of variance (ANOVA) with a multiple comparison post-test by Dunnett; $p \leq 0.05\%$ was used as the threshold for statistical significance.

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[1]A daily dose of approximately 2000 mg/kg is calculated, assuming an average body weight of 0.18 kg for subchronically exposed F344 rats and an average daily food intake of 0.018 kg/day (U.S. EPA, 1988). Calculations: 2% in the diet = 20,000 mg/kg of food. 20,000 mg/kg of food x 0.018 kg of food/day' ÷ 0.18 kg of body weight = 2000 mg/kg-day of 2-methylnaphthalene.

[2]Daily doses were calculated assuming average body weights of 0.0316 and 0.0246 kg for male and female B6C3F1 mice, and average daily food consumptions of 0.0057 and 0.0048 kg/day for males and females, respectively (U.S. EPA, 1988). Sample calculation: 0.0162% = 163 mg/kg of food. 163 mg/kg of food x 0.0057 kg of food /day ÷ 0.0316 kg of body weight = 29.4 mg/kg-day of 2-methylnaphthalene.

Both 2-methylnaphthalene and 1-methylnaphthalene were tested simultaneously under the same experimental conditions and protocols (Murata et al., 1993, 1997)[3]. A shared group of control mice (50 males and 50 females) was used for both of these studies. All dose groups for both 1- and 2-methylnaphthalene, as well as the controls, were housed in the same room. Quantitative details regarding the control animals, as well as, some of the methodology utilized for the analysis of non-neoplastic endpoints, and the qualitative description of these endpoints (for both studies) were provided in Murata et al (1993) paper, but were omitted from the later paper (Murata et al, 1997).

Survival and food consumption were not affected by exposure to 2-methylnaphthalene at 0.075 or 0.15% dietary levels for 81 weeks (Murata et al., 1997). Body weight data were presented graphically as mean growth curves for males and females in the control and exposed groups. Group means and standard deviations were not presented. The study report specified that the reduction in final mean body weight was statistically significant for the high-dose male group. The mean final body weights for the male and female high-dose groups were reported to be reduced by 7.5 and 4.5%, respectively, compared with controls. Because the magnitudes were less than 10% compared with controls, the decreased body weight was not considered to be a biologically significant effect.

As shown in Table 2, dietary exposure to 2-methylnaphthalene was associated with a statistically significant increased incidence of pulmonary alveolar proteinosis in male and female mice in both exposure groups, compared with controls (Murata et al., 1997). Both 2-methylnaphthalene-exposed males and females showed a statistically significant ($p<0.05$) trend in increased incidences of pulmonary alveolar proteinosis (Cochran-Armitage trend tests performed by Syracuse Research Corporation). Pulmonary alveolar proteinosis was characterized by the accumulation of phospholipids in the alveolar lumens. Upon gross inspection, white protuberant nodules approximately 1–5 mm in diameter were visible (Murata et al., 1993). Histologically, there was visible filling of alveolar lumens with cholesterol crystals, foamy cells, and an amorphous acidophilic material (Murata et al., 1993). No prominent fibrosis, edema, alveolitis, or lipidosis were seen in alveolar walls or in epithelial cells. No evidence of bronchiolar Clara cell necrosis or sloughing was observed (Murata et al., 1997). No histopathological evidence of non-neoplastic effects were reported for any other tissue.

[3]Mice exposed to 0.075% or 0.15% 1-methylnaphthalene showed increased incidences for pulmonary alveolar proteinosis in males and females and for total lung tumors in males only (Murata et al., 1993). Daily doses calculated from reported total intakes were 75.1 and 143.7 mg/kg-day of 1-methylnaphthalene for females and 71.6 and 140.2 mg/kg-day for males. For male mice exposed to dietary concentrations of 0.075% or 0.15% 1-methylnaphthalene, incidences were 13/50 and 15/50 for total lung tumors, and 23/50 and 19/50 for pulmonary alveolar proteinosis (Murata et al., 1993). For female mice, respective incidences were 2/50 and 5/49 for total lung tumors, and 23/50 and 17/49 for pulmonary alveolar proteinosis. No other exposure-related adverse effects were observed in any other organs or tissues.

Table 2. Incidence of Pulmonary Alveolar Proteinosis in B6C3F1 Mice Fed 2-Methylnaphthalene for 81 Weeks (from Murata et al., 1997)

	Female			Male		
Dose (% diet) Dose (mg/kg-day)	0 0	0.075 50.3	0.15 107.6	0 0	0.075 54.3	0.15 113.8
Pulmonary alveolar proteinosis	5/50	27/49*	22/49*	4/49	21/49*	23/49*
Lung adenoma Lung adenocarcinoma Total lung tumors	4/50 1/50 5/50	4/49 0/49 4/49	5/48 1/48 6/48	2/49 0/49 2/49	9/49* 1/49 10/49*	5/49 1/49 6/49

* Statistically significant by Fisher's exact test ($p<0.05$).

The authors indicated that the control non-zero incidence of pulmonary alveolar proteinosis (9/99) for both males and females was unusual because pulmonary alveolar proteinosis has not appeared spontaneously in more than 5000 B6C3F1 mice housed in the same room in the past. The appearance of pulmonary alveolar proteinosis in the control mice was similar to but was less pronounced than that seen in 2-methylnaphthalene exposed mice. Murata et al. (1997) speculated that the increased incidence of pulmonary alveolar proteinosis in controls may have resulted from inhalation of volatilized 2-methylnaphthalene or 1-methylnaphthalene due to insufficient room ventilation. The control incidences of pulmonary alveolar proteinosis might have been elevated by exposure to either or both methylnaphthalene isomers.

In humans, pulmonary alveolar proteinosis has been associated with increased serum lactate dehydrogenase (LDH) (Goldstein et al., 1998; Wang et al., 1997). However, no changes in serum LDH were reported in mice exposed to 2-methylnaphthalene (Murata et al., 1997).

The authors also reported other statistically significant differences between control and 2-methylnaphthalene exposure groups, but no data were provided regarding the magnitude or exposure levels at which they occurred. Serum neutral fat levels were elevated in exposed males and females, and relative and absolute brain and kidney weights were increased among exposed males. In exposed females, counts of stab and segmented form neutrophils were significantly decreased, and lymphocyte counts were increased compared to controls (Murata et al., 1997). The biological significance of these differences is unclear, due to the lack of reported data (i.e., response magnitude and exposure level).

Table 2 also shows the incidence of lung adenomas, lung adenocarcinomas, and total lung tumors (i.e., adenomas plus adenocarcinomas) in mice exposed to 2-methylnaphthalene. The male high dose group (54.3 mg/kg-day) had a statistically significant increased incidence of lung adenomas and total lung tumors compared with controls. Tumor incidences in the higher dose group were not statistically significantly increased. Analysis of the male total lung tumor data by the Cochran-Armitage trend test at the $p \leq 0.05$ level did not find a statistically significant trend with increasing dose (performed by Syracuse Research Corporation). This study provides only limited evidence of a carcinogenic response in male mice to 2-

methylnaphthalene in the diet. No significant elevations in tumor incidence were observed for exposed male mice at other (non-lung) sites or in exposed female mice at any site. It is uncertain if the total lung tumor incidences cited in the study report refer to the number of lung-tumor bearing mice or to the number of lung tumors found in a group, since the study authors noted that the lung tumors were mostly single.

4.2.2. Inhalation Exposure

No studies are available in which health effects were evaluated in animals following prechronic or chronic inhalation exposure to 2-methylnaphthalene.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

No studies are available regarding the effects of 2-methylnaphthalene on reproduction or development in humans or animals via any route of exposure.

4.4. OTHER STUDIES

4.4.1. Acute Toxicity Data

No acute oral toxicity studies were identified for 2-methylnaphthalene.

There are two acute inhalation studies with 2-methylnaphthalene: one examining neurobehavior in rats and sensory/respiratory irritation in mice (Korsak et al., 1998) and one examining hematologic endpoints in dogs (Lorber et al., 1972).

Korsak et al. (1998) evaluated acute neurotoxicity in rats and sensory/respiratory irritation in mice immediately following whole-body exposure to 2-methylnaphthalene. Male Wistar rats were placed on a hot-plate (54.5°C) to measure latency of paw-lick response immediately after exposure to 0, 229, 352, or 525 mg/m^3 of 2-methylnaphthalene for 4 hours (20, 10, 10, and 20 rats/group, respectively). The Kruskal-Wallis statistical test was used to evaluate pain sensitivity, with $p \leq 0.05$ considered significant. Mean latencies (measured in seconds) to the paw lick response were 10.5 ± 2.6, 13.9 ± 3.3, 25.7 ± 6.3, and 33.3 ± 19.9 for the control through high-dose groups, respectively. The mean latencies in the 2 highest groups were statistically significantly higher than the control mean, indicating a decreased sensitivity to pain. Defining latency elongation ≥60 seconds as a 100% decrease in pain sensitivity, exposure to the low- through high-dose groups decreased pain sensitivity by 6.8, 30.7, and 46.0%, respectively. Rotarod performance (the trained ability to maintain balance on a rotating rod for 2 minutes) was tested in groups of 10 rats immediately after cessation of exposure to the same concentrations used in the pain sensitivity test. No failures occurred in the control, low-, or mid-concentration groups. In the high concentration group, only 1/10 rats failed to stay on the rod. Thus, no significant effect on rotarod performance was observed.

To assess sensory/respiratory irritation of 2-methylnaphthalene, male Balb/C mice (8-10/group) were exposed to 0, 28, 58, 125, or 349 mg/m^3 of 2-methylnaphthalene for 6 minutes,

and respiratory rates were measured before, during, and for 12 minutes after exposure (Korsak et al., 1998). Respiratory rate decreased most rapidly in the first 2 minutes of exposure. Immediately after 6 minutes of exposure, respiratory rates were decreased by approximately 8, 30, 70, and 80% at the low through high concentrations, respectively, but returned to 75-95% of normal within 12 minutes after cessation of exposure. The calculated concentration depressing respiratory rate in mice by 50% (RD_{50}) was 67 mg/m^3 (95% upper confidence interval of 81 mg/m^3). The authors considered irritation to be the cause of these respiratory changes.

Lorber (1972) did not observe hemotoxicity in dogs following acute whole-body exposure to 2-methylnaphthalene. Based on an unpublished observation, the author hypothesized that exposure to a specific commercial pesticide had affected blood counts in intact and splenectomized dogs. A 3% mixture of methylated naphthalenes had been used to dissolve some components of the pesticide. Therefore, on 4 consecutive days, a pesticide fogger was used to bathe dogs (4-6 intact dogs and 4-12 splenectomized dogs/group) in a mist of 1 liter of kerosene containing 2-methylnaphthalene or practical-grade 2-methylnaphthalene for four 5-minute periods, with pauses (7-10 minutes) during which the mist settled. The strains and genders of the dogs were not reported. The amounts of 2-methylnaphthalene fogged could not be determined from the information provided[4]; therefore, no accurate exposure concentration could be estimated[5]. Blood was collected prior to first exposure, prior to last exposure, and 7 and 10 days after first exposure. Iliac bone marrow aspirates were collected under anesthesia before and after exposure. Endpoints measured were mean levels of leukocytes, reticulocytes, platelets, and red blood cell survival. Post-exposure values were compared to pre-exposure values using student's t test at the $p \leq 0.05$ significance level. No statistically significant differences were observed for any of the endpoints evaluated. Because exposure levels experienced by the dogs could not be reliably estimated, the study does not identify a reliable inhalation NOAEL for hematologic effects from acute exposure to 2-methylnaphthalene.

Although no acute oral or inhalation studies have evaluated the effects of 2-methylnaphthalene on lung histopathology, supporting data that the lung is a target of 2-methylnaphthalene has been provided by acute injection studies. In mice, histological changes and sloughing of Clara cells (a type of non-ciliated cell that lines the bronchioles of the lungs)

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[4]Lorber et al. (1972) reported that dogs were fogged with one liter of refined, deodorized kerosene either by itself or containing one of the chemicals in amounts similar to what might be found in liter or gallon quantities of commercial insecticides. The latter will be termed simulated gallons. 2-Methylnaphthalene and practical-grade 2-methylnaphthalene were mixed in 1 liter volumes of kerosene in a concentration similar to the three per cent mixture often employed commercially. The proportion of 2-methylnaphthalene in the mixture was not reported. Therefore, a liter would have had some quantity less than 30 g of 2-methylnaphthalene. Given that 1 gallon = 4.545 liters, a simulated gallon would have had an approximate quantity less than 100 g of 2-methylnaphthalene.

[5]Lorber et al. (1972) reported that dogs were exposed in cages as far as possible from the fogger, in a 10x9x8 foot room. Given that 1 foot = 0.3048 meters, this volume was approximately 20 m^3. Homogenous dispersion of 30 or 100 g into 20 m^3 would have produced atmospheres of 1000 or 5000 mg/m^3 of 2-methylnaphthalene for 41-50 minutes/day for 4 days. Inhaled concentrations were likely to have been substantially less because the amounts of 2-methylnaphthalene in the test solutions were less than 30 or 100 g, as discussed in footnote 3. Additionally, the rapid settling of the fogged mixtures would have resulted in substantially reduced inhaled concentrations.

have been reported at doses as low as 100 mg/kg (Buckpitt et al., 1986; Griffin et al., 1981, 1982, 1983; Honda et al., 1990; Rasmussen et al., 1986). Higher doses of 2-methylnaphthalene also produced bronchiolar and pulmonary necrosis.

Griffin et al. (1981) administered single intraperitoneal injections of 0, 0.1, 1, 10, 100, 200, 400, 600, 800, or 1,000 mg/kg of 2-methylnaphthalene in corn oil to male C57BL/6J mice (5/group), with sacrifice 24 or 48 hours later. Endpoints measured were survival; liver, kidney, and lung histopathology by light microscopy; and electron microcopy of lung tissue. One death (1/5) was seen at the highest dose. No liver or kidney lesions were detected by light microscopy. No lung toxicity was seen in mice exposed to concentrations up to 10 mg/kg by light or electron microscopy. At 100 mg/kg and above, the incidence and severity of bronchiolar necrosis continued to increase with increasing dose. At 100 mg/kg, pulmonary necrosis was seen in 2/5 mice and was limited to irregularities of the cells lining the bronchioles, with cells present in the lumen. More severe pulmonary necrosis was seen in all mice exposed to doses $\geq$200 mg/kg, with minimal-to-prominent sloughing of the non-ciliated cells (Clara cells) lining the bronchioles. All mice given 1000 mg/kg exhibited complete sloughing of all bronchiolar lining cells. The extent of necrosis was reduced in all treated groups sacrificed 48 hours after dosing compared to those sacrificed 24 hours after dosing. For example, following administration of 200 mg/kg, 5/5 mice showed bronchiolar necrosis at 24 hours, but at 48 hours 3/5 mice showed necrosis.

Griffin et al. (1982) sacrificed male C57BL/6J mice (4-5/group) 1, 2, 4, 8, 12, and 24 hours after administering intraperitoneal injections of 0 or 400 mg/kg of 2-methylnaphthalene. Liver, kidney, and lung tissue were collected for histopathology. No liver or kidney damage was observed. No pulmonary necrosis was seen between 1 and 4 hours. However, by 8 hours all mice exhibited some evidence of necrosis, that ranged from irregularity of the bronchiolar lining with normal areas to prominent sloughing of the bronchiolar lining.

Griffin et al. (1983) examined the pulmonary toxicity of 2-methylnaphthalene in DBA/2J mice, which are considered less responsive to inducers of CYPIA and CYPIB than C57BL/6J mice. Male mice (5/group) were injected intraperitoneally with 0, 0.1, 1, 10, 100, 200, 400, 600, 800, or 1000 mg/kg of 2-methylnaphthalene in corn oil and were sacrificed 24 hours later. Mortality was observed in 2/5 mice in the 1000 mg/kg dose-group. Histopathology of the liver, kidney, and lungs detected no damage to the liver or kidney at any dose, and no pulmonary toxicity was observed at doses up to 10 mg/kg. Slight evidence of pulmonary necrosis was detected in 4/5 mice receiving 100 mg/kg, and severe pulmonary effects were observed in all mice given higher doses. At the 100 mg/kg level, 2 mice showed irregularities of lining cells of one or two bronchioles with cells in the lumen (score of 1+ on a 0, 1+, 2+, 3+, or 4+ severity scale), 2 mice showed minimal sloughing of lining cells into the lumen of some bronchioles (score of 2+), and 1 mouse showed complete sloughing of all bronchiolar lining cells (score of 4+). In the 200-mg/kg group, pulmonary necrosis was scored as 1+ in 2 mice and 2+ in 3 mice. Pulmonary necrosis was scored as 3+ (prominent sloughing of lining cells into lumen) in all mice at 400 mg/kg . All mice at 600 and 800 mg/kg showed complete sloughing of the bronchiolar lining (score of 4+). Mortality was reported for 2/5 mice in the 1000-mg/kg group.

Honda et al. (1990) administered single intraperitoneal injections of 0, 100, 200, 400, or 600 mg/kg of 2-methylnaphthalene to male ddY mice and sacrificed them 24 hours later. No lung damage was seen at 100 or 200 mg/kg. Electron microscopic analysis detected bronchiolar damage at 400 mg/kg and exfoliated Clara cells in the bronchiolar lumen at 600 mg/kg. The numbers of animals per group were not reported. Additional intraperitoneal injection experiments in male ddY mice (3-5/group) observed statistically significant ($p < 0.05$) decreases in pulmonary glutathione levels at 6 and 12 hours post injection with doses as low as 100 mg/kg of 2-methylnaphthalene (20 and 32%, respectively), but plasma glutathione levels were not decreased by doses as high as 400 mg/kg.

Rasmussen et al. (1986) administered single intraperitoneal injections of 0, 1, or 2 mmol/kg of 2-methylnaphthalene (142 or 284 mg/kg) in peanut oil to male Swiss-Webster mice (2/group) with sacrifice at 24 hours, 3 days, 7 days, or 14 days. Lung, liver, and kidney tissue were examined with light microscopy, and lung cells were analyzed by electron microscopy. Lung cell proliferation was measured in the control and 284-mg/kg groups only. Doses of 0.5 and 3 mmol/kg (71 and 427 mg/kg) were also administered, but only electron microscopy results were reported for these mice. Statistical analyses of collected data were not performed. Cytotoxic effects on the epithelium of the lung airways examined by light microscopy were scored on a 0-5 scale: 0, no effect; 1, swelling of Clara cells with occasional sloughed cells in terminal bronchioles; 2, sloughed cells evident in bronchioles, but ciliated cells intact and minimal effects in bronchi and trachea; 3, sloughed Clara cells throughout airways; 4, sloughed Clara cells and ciliated cells in bronchioles with some damage in bronchi and trachea; and 5, sloughed cells throughout all airways, including trachea, leaving large areas of bare basement membrane. Tissue samples were scored without knowledge of the treatment group. Maximal average scores for lung cytotoxic effects were observed 3 days after injection. The maximal average scores were 1.4 and 3.0 for 142- and 284-mg/kg mice, compared with an average score of 0 for control mice. At day 14, cytotoxic effects were still evident and average scores were 1.5 and 2.0 for the 142- and 284-mg/kg mice, compared with 0.4 for control mice.

Electron microscopy of lung tissue collected from exposed mice at 6, 12, or 24 hours after injection showed Clara cell flattening, cytoplasmic vacuolization, loss of smooth endoplasmic reticulum, reduced number of microvilli, prominent ribosomes, and electron-dense mitochondria. Cytoplasmic vacuolization was reported to have occurred in control mice, but not as extensively as in exposed mice. Clara cell ultrastructural changes were reported to have increased in severity with increasing dose, from 71 mg/kg to 427 mg/kg. Airways in mice from the highest dose group (427 mg/kg) were reported to be the most severely affected showing, in addition to Clara cell effects, flattened and vacuolated ciliated cells with dilated cisternae of the granulated endoplasmic reticulum, electron-dense mitochondria, and prominent ribosomes. At 1, 3, and 7 days after injection, cell proliferation indices in bronchiolar epithelial cells from the 284-mg/kg dose group were increased by 3-, 32-, and 3-fold, compared with vehicle control values. Cell proliferation indices in alveolar cells from the 284-mg/kg dose group showed a similar response over time, but were not as greatly increased as in bronchiolar cells. Examination of liver and kidney sections from exposed mice revealed minimal changes in the liver and no changes in the kidney. The study report did not further describe these changes or specify the dose levels at which they occurred.

Buckpitt et al. (1986) administered single doses of 0 or 300 mg/kg of 2-methyl-naphthalene to male Swiss-Webster mice (5/group) by intraperitoneal injection, with sacrifice 24 hours later. Histological examinations identified bronchiolar necrosis in all treated animals, and no lesions among controls. Pulmonary necrosis was considered moderate (bronchiolar epithelial cell swelling, vacuolization, and exfoliation) for 3/5 mice and severe (extensive sloughing in terminal and larger airways with widespread exfoliation) for 2/5 mice. For this study, the only dose tested, 300 mg/kg of 2-methylnaphthalene, is a LOAEL for bronchiolar necrosis in male Swiss Webster mice.

Female Wistar rats (numbers not provided) given single intraperitoneal injections of 0 or 1 mmol/kg (142 mg/kg) of 2-methylnaphthalene showed no evidence of pulmonary necrosis (Dinsdale and Verschoyle, 1987).

4.4.2. Studies with Methylnaphthalene Mixtures

Methylnaphthalene mixtures are used as industrial solvents, coolants, and dye carriers. Methylnaphthalene mixtures are composed of 2-methylnaphthalene and 1-methylnaphthalene in an approximate ratio of 2:1. Animal studies with methylnaphthalene mixtures provide supporting evidence that the lung is a sensitive target organ for 2-methylnaphthalene.

Evidence of lung toxicity was observed in acute oral and dermal lethality testing with a methylnaphthalene mixture (Union Carbide, 1982). Wistar rats (5/females and 3-5 males/group) exposed by gavage to single doses of 4.0 mL/kg (4000 mg/kg)[6] or greater developed dark red and mottled lungs. Female (but not male) rats also exhibited labored breathing. The calculated oral LD_{50} values were 4.29 mL/kg (4200 mg/kg) for males and 3.25 mL/kg (3180 mg/kg) for females. The same report also indicated that female New Zealand white rabbits (4/group) exposed dermally to 8.0 ml/kg (8000 mg/kg) developed dark red lungs and blanched livers. The calculated dermal LD_{50} value for females was 5.38 mL/kg (4660 mg/kg). No signs of toxicity or gross pathology were observed in Wistar rats (5/sex) exposed to a saturated vapor of a methylnaphthalene mixture for 6 hours; the methodology reported was insufficient to estimate the exposure concentration (Union Carbide, 1982). Acute dermal and eye irritation studies with a methylnaphthalene mixture in rabbits found that it was irritating, but not corrosive (Carnegie Mellon, 1974; Union Carbide, 1982). Because these studies were designed to measure lethality, lung pathology in surviving animals was assessed after a 14-day recovery period.

Murata et al. (1992) exposed female B6C3F1 mice (15/group) to 0 or 119 mg/kg of a methylnaphthalene mixture (equivalent to 0 or 34 mg/kg-day) by applying an acetone solution containing 1.2% methylnaphthalene to their backs twice weekly for 30 weeks. Lung tissue samples were analyzed using light and electron microscopy. Exposure to the mixture resulted in a 14% reduction in final body weight (compared to controls) that was not statistically significant.

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[6]Based on a density of 0.978 g/ml for methylnaphthalene (NTP, 2002). Example calculation: 4.0 ml/kg x 0.979 g/ml x 1000 mg/g = 4000 mg/kg.

All mice (15/15) exposed to the methylnaphthalene mixture developed pulmonary alveolar proteinosis. Lung surfaces grossly contained multiple grayish white nodules. Histologically, the alveoli appeared filled with many mononucleated giant cells, cholesterol crystals, and an amorphous eosinophilic myeloid material. The mononucleated giant cells exhibited foamy cytoplasm, which frequently contained lipid droplets and myeloid structures, and some were enlarged. Alveolar walls were partially thickened due to hyperplasia and hypertrophy of Type II pneumocytes or focal hyperplasia of cells resembling Type I pneumocytes in appearance. Focal interstitial fibrosis was seen only in restricted areas. Focal interstitial accumulation of plasma cells was also observed. Ultrastructural analyses verified these observations, and detected numerous necrotic cells in areas of proteinosis. Murata et al. (1992) concluded that the mononucleated giant cells were type II pneumocytes overfilled with myelinoid structures, rather than macrophages that might have engulfed lamellar bodies, and that some of these cells ruptured into the alveolar lumens. The authors reported that a higher dermal dose (238 mg/kg; equivalent to 68 mg/kg-day) than that used in the present study (34 mg/kg-day) induced a 100% incidence of pulmonary alveolar proteinosis in a shorter period (20 weeks), but noted that this was unpublished data (Murata et al., 1992).

Emi and Konishi (1985) painted the shaved backs of female B6C3F1 mice with 0, 29.7, or 118.8 mg/kg of a methylnaphthalene mixture in acetone twice weekly for 61 weeks (equivalent to 0, 8.49 or 33.94 mg/kg-day). The control through high-dose groups contained 4, 11, and 32 mice, respectively. At sacrifice, animals were necropsied, and histology was performed on the skin and principal organs (not identified). Although survival information was not provided, a reported peak in mortality incidence at 38 weeks was attributed to lipid pneumonia. Lipid pneumonia was observed (in animals that died) as early as 10 weeks. The final incidences of lipid pneumonia were 0/4, 3/11, and 31/32 for the control, low, and high dose groups, respectively. Lipid pneumonia was characterized grossly by multiple delocalized white spots and soft clearly demarcated nodules. The predominant histological feature was hypertrophy and hyperplasia of type II pneumocytes in the lung. Additional observations included slight alveolar wall thickening, multinucleated giant cell reaction, and the presence of foamy cells and cholesterol crystals in the alveolar lumen. Evidence of focal alveolar dilation and emphysema were also observed but were considered compensatory reactions.

A subsequent study was performed to analyze the types of lipids present in the lung following exposure to a methylnaphthalene mixture (Taki et al., 1986). Female B6C3F1 mice received doses of 0, 118.8, or 237.6 mg/kg of the methylnaphthalene mixture (3, 8, or 7/group, respectively) in acetone on the shaved skin of their backs twice a week for 50 weeks (equivalent to 0, 33.9 or 67.9 mg/kg-day). Lung tissue was collected at 50 weeks for quantitation of lipid content. Lung histopathology was not reported. Cholesteryl ester was found in the lungs of all exposed animals, but not in controls. Exposure to this mixture also increased lung triglyceride, cholesterol, and phospholipid levels. The most dramatically increased phospholipids were phosphatidylcholine (increased 1.5- to 5-fold in low-dose animals and 3- to 5.7-fold in high-dose animals) and phosphatidylglycerol (increased 1.5- to 5.8-fold in low-dose animals and 3- to 5.8-fold in high-dose animals). The authors considered these changes to be evidence of lipid pneumonia.

T-cell-independent and T-cell-dependent immunity were suppressed in mice injected with a mixture containing 2-methylnaphthalene (Harper et al., 1996). Female B6C3F1 mice (5/group) were given single intraperitoneal injections of 0, 24, 47, 188, or 754 mg/kg of a mixture of 2-ring PAHs (consisting of 38.3% 2-methylnaphthalene, 39.3% naphthalene, 22.0% 1-methylnaphthalene, and 0.36% indan). These mice were then challenged with injections of either T-cell independent or T-cell dependent antigens 2 days later (trinitrophenyl-lipopolysaccharide [TNP] or TNP-haptenated sheep red blood cells, respectively). Mice were sacrificed 2 days after challenge. Levels of serum anti-TNP IgM and the ability of spleen cells to form plaques in the presence of antigen and complement were measured as a determinant of immune function. Decreased plaque formation following T-cell dependent and T-cell independent challenge and increased anti-TNP IgM levels were observed. Similar immunosuppression was observed for a mixture containing the 2-ring PAHs as well as 3-ring and ≥4-ring PAHs. The immunological study was inconclusive regarding the possible effects of 2-methylnaphthalene on the immune system, due to the potentially confounding influence of other chemicals present in the test mixture.

4.4.3. Mode-of-Action Studies

It is unknown whether 2-methylnaphthalene itself or its metabolites are responsible for the development of pulmonary alveolar proteinosis. Several metabolism studies have evaluated the effect of CYP enzyme inducers and inhibitors on 2-methylnaphthalene induced toxicity. For example, Griffin et al (1982) pretreated male C57BL/6J mice with either CYP enzyme inducers or inhibitors prior to intraperitoneal injection with 2-methylnaphthalene (200 or 400 mg/kg) to assess the role of metabolism in 2-methylnaphthalene induced pulmonary toxicity. As described in Section 3.2, exposure to 400 mg/kg of 2-methylnaphthalene alone resulted in the induction of bronchiolar necrosis in all exposed mice compared to controls (Griffin et al., 1982). None of the pretreatments alone nor any of the pretreatments plus 200 mg/kg-day 2-methylnaphthalene resulted in pulmonary toxicity or lethality compared to controls and mice treated with 2-methylnaphthalene alone respectively. Pretreatment with the CYP enzyme inducers phenobarbital and 3-methylcholanthrene appeared to provide some protection from 2-methylnaphthalene (400 mg/kg-day) induced pulmonary toxicity, however the CYP enzyme inhibitors SKF525-A and piperonyl butoxide had no significant effect on 2-methylnaphthalene induced pulmonary toxicity.

In contrast to the effects seen in C57BL/6J mice, pretreatment of male DBA/2J mice with the same CYP enzyme inducers or inhibitors listed above prior to intraperitoneal exposure to 2-methylnaphthalene (as described in 4.4.1.) did not influence the severity of 2-methylnaphthalene-induced bronchiolar lesions (Griffin et al., 1983).

In addition to the role of CYP enzyme activation, studies also suggest that glutathione conjugation of reactive metabolites may play a detoxifying role in response to the acute toxicity of 2-methylnaphthalene. Griffin et al. (1982) reported that pretreatment of male C57BL/6J mice (5/group) with 625 mg/kg diethylmaleate 30 minutes before treatment with 400 mg/kg 2-methylnaphthalene resulted in mortality for 4/5 mice. The surviving mouse exhibited prominent

sloughing of the bronchiolar lining, but a description of lung histopathology was not reported for the nonsurvivors. In contrast, the same dose (400 mg/kg) of 2-methylnaphthalene without glutathione depletion was not fatal, but resulted in the development of bronchiolar necrosis.

No bronchiolar necrosis was observed in male ddY mice given single intraperitoneal injections of 200 mg/kg/ of 2-methylnaphthalene; but pretreatment with diethylmaleate (600 µl/kg) 1 hour prior to injections caused extensive sloughing and exfoliation of bronchiolar epithelial cells in all animals (5/5) (Honda et al., 1990).

4.4.4. Other Cancer Studies

No evidence of co-carcinogenic activity was found in female ICR/Ha Sprague-Dawley mice (30/group) dermally exposed to 0 or 25 µg (32 µg/kg-day of 2-methylnaphthalene) of 2-methylnaphthalene plus 300 ng of benzo[a]pyrene (BaP) in acetone 3 times weekly for 78 weeks (Schmeltz et al., 1978). Both negative (acetone only) and positive (BaP plus 12-o-tetradeconoyl phorbol-13-acetate) controls were included, but 2-methylnaphthalene was not tested alone. Compared to mice treated only with BaP, 2-methylnaphthalene plus BaP increased the time-to-first-tumor (52 versus 58 weeks) and decreased the number of tumor-bearing animals (44% versus 20%). The statistical significance of these findings could not be determined from the data presented. Similar inhibitory effects (compared to BaP alone) were found with mixtures of BaP with naphthalene, 1-methylnaphthalene, 1,2-dimethylnaphthalene, 2-ethylnaphthalene, or the naphthalene-fraction of cigarette smoke.

4.4.5. Genotoxicity Studies

No genotoxicity studies in humans or animals are available. No studies investigating potential germ-line mutations are available. Data from *in vitro* short-term tests provide only limited evidence for genotoxic activity of 2-methylnaphthalene (Florin et al., 1980; Harvey and Halonen, 1968; Hermann, 1981; Kopper Co. Inc., 1982; Kulka et al., 1988; Weis et al., 1998).

No mutagenicity was observed in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 treated with 2-methylnaphthalene (Florin et al., 1980; Hermann, 1981) or methylnaphthalene mixtures (Kopper Co. Inc., 1982), with or without metabolic activation by S9 hepatic microsomal fractions. In these studies, S9 hepatic microsomal fractions were prepared from male Sprague-Dawley, Fischer 344, or Wistar rats induced with either Aroclor 1254 or 3-methylcholanthrene. *In vitro* exposure of human lymphocytes to 2-methylnaphthalene with metabolic activation by S9 produced statistically significant increases in the incidences of sister chromatid exchanges (up to 22%) at all concentrations tested (0.25 to 4 mM) and of chromatid breaks (6.5-fold) only at the highest concentration tested (4 mM) (Kulka et al., 1988). No differences were seen following exposure without metabolic activation. The authors considered the sister chromatid response to be negative because the magnitude of the response was less than a 2-fold increase, and considered the chromatid breaks to be minor because no damage was seen at lower concentrations (up to 2 mM).

In vitro assays in WB-F344 rat liver epithelial cells indicated that 2-methylnaphthalene, as well as naphthalene and 1-methylnaphthalene, inhibited gap junctional intercellular communication (Weis et al., 1998). Inhibition of intracellular communication has been postulated to be an epigenetic mechanism of tumor promotion by preventing intercellular transport of regulatory molecules. The relevance of this finding to human health has not been elucidated.

Harvey and Halonen (1968) showed that 2-methylnaphthalene binds to four nucleic acids (adenosine, thymidine, uridine, and guanosine), as well as 3 structurally analogous compounds (caffeine, tyrptophan and riboflavin) in a silica gel matrix. The physical conditions of the experiment were not provided (e.g., temperature, pH). This experiment provides suggestive evidence that 2-methylnaphthalene may interact with DNA even in the absence of metabolic activation, but more recent corroborating studies are not available.

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE-OF-ACTION—ORAL AND INHALATION

4.5.1. Oral Exposure

There are no studies examining possible associations between acute or repeated oral exposure to 2-methylnaphthalene and noncancer health effects in humans, but a study in mice provides evidence of the development of pulmonary alveolar proteinosis following near-lifetime exposure to 2-methylnaphthalene at dose levels of approximately 50 mg/kg-day (Murata et al., 1997). In this study, male and female B6C3F1 mice were exposed to 0, 0.075%, or 0.15% 2-methylnaphthalene in the diet for 81 weeks. Average daily doses were 0, 54.3 or 113.8 mg/kg-day for males and 0, 50.3 or 107.6 mg/kg-day for females. There was a statistically significant increase in the incidence of pulmonary alveolar proteinosis in both exposure groups compared to controls. Incidences for the control through high-dose groups were 4/49, 21/49, and 23/49 for male mice and 5/50, 27/49, and 22/49 for female mice, respectively. Histological examination of major tissues and organs revealed no other exposure-related non-neoplastic effects at other sites (including the bronchiolar regions of the lung). These findings indicate that the alveolar region of the lung is the critical toxicity target of chronic oral exposure to 2-methylnaphthalene.

In addition to pulmonary alveolar proteinosis, other effects observed in this study included some changes in blood variables (decreased differential counts of neutrophils and increased lymphocytes) and increased serum levels of neutral fat, total lipids, and phospholipids (Murata et al., 1997). The biological significance of the latter two findings is difficult to assess due to the lack of reporting regarding the magnitude of the changes and the dose levels at which they occurred, but the authors proposed that additional research is warranted to determine whether the elevated serum levels of fat may or may not be related to the induction of pulmonary alveolar proteinosis or are a subsequent effect of this condition.

Although the available studies in mice indicate that pulmonary alveolar proteinosis is a potential health hazard from repeated oral exposure to 2-methylnaphthalene, the oral toxicity

data base for 2-methylnaphthalene is sparse (see Table 3). Oral studies in other animal species are restricted to a poorly reported study in rats that found no evidence for cataracts after ≥2 months exposure to 2000 mg/kg-day 2-methylnaphthalene (Fitzhugh and Buschke, 1949). Murata et al. (1997) conducted a preliminary dose-selection study in which B6C3F1 mice (10/sex/group) were fed diets containing 0, 0.0163, 0.049, 0.147, 0.44, or 1.33% 2-methylnaphthalene for 13 weeks. The two highest dose groups were without histologically visible non-neoplastic adverse effects in any organs compared with controls, but showed growth retardation (tissues from mice in the lower dose groups were not evaluated). The finding that pulmonary alveolar proteinosis did not develop in mice after 13 weeks of exposure to dietary concentrations of 0.44% or 1.33%, coupled with the finding that 81 weeks of exposure to 0.075% or 0.147% 2-methylnaphthalene increased the incidence of this effect, suggest that the development of pulmonary alveolar proteinosis requires chronic-duration oral exposure at the dose levels tested. There are no oral exposure studies examining the possible developmental, reproductive, or neurologic toxicity of 2-methylnaphthalene in animals.

Table 3. Oral Toxicity Studies for 2-Methylnaphthalene

Species	Dose/Duration	NOAEL	LOAEL	Effect	Reference
Rat (5 rats of unspecified sex)	≥2 months in diet; 2000 mg/kg-day	2000 mg/kg-day		No cataractogenesis	Fitzhugh and Buschke, 1949
Mouse (10/sex/group)	13 weeks in diet; average doses: 0, 31, 92, 276, 827, or 2500 mg/kg-day	92 mg/kg-day	276 mg/kg-day	Decreased weight gain; no non-neoplastic effects identified histologically in any organs at 827 or 2500 mg/kg-day	Murata et al., 1997
Mouse (50/sex/group)	81 weeks in diet; doses: 0, 54.3, or 113.8 (M); 0, 50.3, or 107.6 (F) mg/kg-day		54.3 (M) 50.3 (F) mg/kg-day	Increased pulmonary alveolar proteinosis at both doses in both sexes	Murata et al., 1997

Additional support that the lung is a critical toxicity target of 2-methylnaphthalene comes from studies of animals exposed to a mixture of methylnaphthalenes. Table 4 summarizes the results from available animal toxicity studies with methylnaphthalene mixtures. The strongest supporting evidence comes from a report that twice weekly application of 119-mg/kg of a mixture of 1- and 2-methylnaphthalene to the skin of B6C3F1 mice for 30 weeks (or 238 mg/kg for 20 weeks) produced a 100% incidence of pulmonary alveolar proteinosis (Murata et al., 1992). In addition, Emi and Konishi (1985) identified lipid pneumonia in 31/32 female B6C3F1 mice exposed to 119 mg/kg doses of a methylnaphthalene mixture applied dermally twice per week for 61 weeks. Lipid pneumonia is a disorder characterized by the alveolar accumulation (without the involvement of epithelial cells) of foamy macrophages that contain lipid droplets in

their cytoplasm. This effect was found in 0/4 control mice and in 3/11 mice treated dermally with 29.7 mg/kg doses of a methylnaphthalene mixture for 61 weeks. A subsequent study reported that 119- or 238-mg/kg doses of methylnaphthalene, applied twice weekly (dermal) to female B6C3F1 mice for 50 weeks, produced changes in lung lipid contents indicative of lipid pneumonia (Taki et al., 1986). There is no conclusive evidence that lipid pneumonia and pulmonary alveolar proteinosis share a common pathogenesis or etiology. These disease conditions are two distinct morphological presentations, but they can appear simultaneously.

Table 4. Toxicity Studies with Mixtures of 2-Methylnaphthalene and 1-Methylnaphthalene

Route	Species	Duration	NOAEL	LOAEL	Effect	Reference
Oral	Rat	Single dose	2,000 mg/kg	4,000 mg/kg	Lung discoloration, labored breathing, and death	Union Carbide, 1982
Inhalation	Rat	6 hours	Substantially saturated vapor		No clinical signs, mortality, or gross lung pathology	Union Carbide, 1982
Dermal	Rabbit	Single dose		4,000 mg/kg 8,000 mg/kg	Death and discolored lung and liver	Union Carbide, 1982
Dermal	Mouse	20 weeks, two times weekly		238 mg/kg per application	Pulmonary alveolar proteinosis	Murata et al., 1992
Dermal	Mouse	30 weeks, two times weekly		119 mg/kg per application	Pulmonary alveolar proteinosis & decreased final body weight	Murata et al., 1992
Dermal	Mouse	50 weeks, two times weekly		119 mg/kg per application	Changes in lung lipids indicative of lipid pneumonia	Taki et al., 1986
Dermal	Mouse	61 weeks, two times weekly		30 or 119 mg/kg per application	Pulmonary lipid pneumonia & decreased survival	Emi and Konishi, 1985

NOAEL = no-observed-adverse-effect level; LOAEL = lowest-observed-adverse-effect level.

The available animal studies indicate that pulmonary alveolar proteinosis is the primary effect seen after administration of 2-methylnaphthalene. Pulmonary alveolar proteinosis is a rare condition in humans that is characterized by the accumulation of surfactant lipids and proteins in the alveoli. Pulmonary alveolar proteinosis develops most commonly between the ages of 20-50 and more often in males then females (3:1, respectively) and in smokers compared to nonsmokers. The main symptom of this condition is dyspnea, at times associated with mild

cough. Altered serum lactate dehydrogenase (LDH) levels are observed in few patients. Patients examined physically may appear normal, but may have minor and nonspecific pulmonary symptoms (i.e., sporadic reduction in diffusing capacity to modest reduction in vital capacity). The majority of cases of pulmonary alveolar proteinosis are diagnosed by the identification of a milky effluent containing large amounts of granular acellular eosinophilic proteinaceous material with abnormal foamy macrophages filled with periodic acid Schiff base (PAS) positive intracellular material in brochoalveolar lavage fluid. Whole lung lavage is the most effective and safest treatment for those who suffer from symptoms due to pulmonary alveolar proteinosis that disrupt their daily activities. Studies indicate that treatment with whole lung lavage may improve symptoms and pulmonary function in the majority of patients with this condition (Shah et al., 2000; Mazzone et al., 2001; Seymour and Presneill, 2002). Persons with pulmonary alveolar proteinosis may require several whole lung lavage treatments, but a small proportion require lavage to maintain functional status or are not responsive. The overall prognosis for pulmonary alveolar proteinosis treated by lavage is excellent, with little individual death (Mazzone et al., 2001). In addition, cases of this condition have been reported to spontaneously resolve (Shah et al., 2000; Mazzone et al., 2001; Seymour and Presneill, 2002). Development of rare secondary infections from organisms such as *Aspergillus* species, *Nocardia* species, and *Mycobacterium* species is the major complication associated with this condition. These secondary infections are less common since the advent of whole lung lavage treatment (Shah et al., 2000; Seymour and Presneill, 2002).

There are two forms of this condition, acquired (primary or secondary) and congenital pulmonary alveolar proteinosis. The majority of cases (estimated to be approximately 80%) of pulmonary alveolar proteinosis occur as the primary acquired disorder of unknown etiology, and are not associated with a familial predisposition. Although uncommon, several underlying conditions such as myelogenous leukemia or exposure to chemicals such as silica or titanium and aluminum dusts can lead to the development of secondary acquired proteinosis. Primary acquired pulmonary alveolar proteinosis is thought to involve the accumulation of surfactant in the alveolar spaces due to altered clearance by dysfunctional macrophages in the alveoli (Seymour and Presneill, 2002; Mazzone et al., 2001; Lee et al., 1997; Wang et al., 1997). Surfactant is synthesized, secreted, and recycled by type II pneumocytes in the alveoli. Surfactant catabolism involves contribution from the type II pneumocytes and macrophages. Studies in humans and knockout mice suggest that clearance of surfactant by macrophages is reduced due to altered activity of the hematopoietic growth factor granulocyte-macrophage colony stimulating factor (GM-CSF) (Shah et al., 2000; Mazzone et al., 2001; Seymour and Presneill, 2002). This growth factor is responsible for transformation of monocytes into mature macrophages in the lungs. These mature macrophages degrade surfactant. Altered activity of this growth factor may be due to the production of a neutralizing antibody to GM-CSF. Inhibition of activity of GM-CSF would lead to immature macrophages and undegraded surfactant and buildup in the lung (Shah et al., 2000; Mazzone et al., 2001; Seymour and Presneill, 2002).

Congenital pulmonary alveolar proteinosis, a rare condition, is an autosomal recessive genetic disorder that may develop at birth or later in life. This form of pulmonary alveolar

proteinosis is primarily believed to be due to a mutation in the surfactant associated protein B (SP-B) gene. In addition, a proportion of infants affected with this form of the disorder are thought to have abnormalities in the receptor for GM-CSF. Infants with congenital pulmonary alveolar proteinosis are affected with severe lung failure shortly after birth and have a poor prognosis for survival (Shah et al., 2000; Seymour and Presneill, 2002). Whole lung lavage (the standard treatment for pulmonary alveolar proteinosis) is difficult to perform on neonates, thus the most promising treatment for infants is lung transplantation (Vaughan and Zimmerman, 2002). Those children that develop pulmonary alveolar proteinosis later in life generally require repeated lavage treatments, but have greater chance of survival (Vaughan and Zimmerman, 2002). In addition, children heterozygous for the mutation in the SP-B gene most likely develop respiratory symptoms later in life and have a more positive prognosis than those that are homozygous recessive for this disorder (Seymour and Presneill, 2002).

The specific molecular mode of action by which 2-methylnaphthalene may cause pulmonary alveolar proteinosis is poorly understood. The suggested mode of action of targeting type II pneumocytes is consistent with what is generally known regarding the etiology of pulmonary alveolar proteinosis in humans. Available evidence in animals supports the hypothesis that type II pneumocytes may be a specific cellular target for the development of 2-methylnaphthalene induced pulmonary alveolar proteinosis. Light microscopic examination of lung tissue from mice that were repeatedly exposed to dermal doses of a methylnaphthalene mixture (119 mg/kg methylnaphthalene mixture twice a week for 30 weeks) showed hyperplasia and hypertrophy of type II pneumocytes in alveolar regions with proteinosis (Murata et al., 1992). Electron microscopic examination showed that alveolar spaces were filled with numerous myelinoid structures resembling lamellar bodies of type II pneumocytes (Murata et al., 1992). Associated with this extracellular material were mononucleated giant cells (balloon cells) containing numerous myelinoid structures, lipid droplets, and electron dense acicular crystals. Murata et al. (1992) hypothesized that, in response to 2-methylnaphthalene, type II pneumocytes produce increased amounts of lamellar bodies due to hyperplasia and hypertrophy, and eventually transform into mononucleated giant cells. The rupture of these cells is hypothesized to lead to the accumulation of the myelinoid structures in the alveolar lumen. No in-depth ultrastructural studies of the pathogenesis of pulmonary alveolar proteinosis from chronic exposure to 2-methylnaphthalene alone were available. However, Murata et al. (1997) suggested that the adverse pulmonary effects detected by light microscopy following chronic oral exposure to 2-methylnaphthalene alone were very similar to those detected following chronic dermal exposure to the methylnaphthalene mixture. These similarities suggest that the mode of action (i.e., specific cellular targeting of type II pneumocytes in the alveolar region of the lung) prompted by observations following exposure to the methylnaphthalene mixture are relevant to 2-methylnaphthalene.

Pulmonary alveolar proteinosis was characterized in mice by an accumulation in the alveolar lumen of foamy cells, cholesterol crystals, and proteinaceous materials rich in lipids (Murata et al., 1992;1993). In the absence of data to indicate otherwise, the pulmonary alveolar proteinosis observed in 2-methylnaphthalene-exposed mice is assumed to be relevant to humans. Although pulmonary function was not measured in mice with 2-methylnaphthalene-induced

pulmonary alveolar proteinosis, human subjects with this condition can display pulmonary function deficits (Lee et al., 1997; Mazzone et al., 2001; Seymour and Presneill, 2002; Shah et al., 2000; Wang et al., 1997).

It is unknown whether 2-methylnaphthalene itself or its metabolites are responsible for the development of pulmonary alveolar proteinosis. The higher incidence of pulmonary alveolar proteinosis in mice exposed dermally to mixtures of 1- and 2-methylnaphthalene described above (Murata et al., 1992), compared with the incidence in mice exposed orally to 2-methylnaphthalene alone at comparable doses (Murata et al., 1997), suggests that first pass hepatic metabolism associated with oral exposure may limit the amount of parent material reaching the lung. Conversely, type II pneumocytes in the alveoli (the possible specific cellular target following oral exposure to 2-methylnaphthalene) are enriched in CYP enzymes (Castranova et al., 1988) and these enzymes are involved in metabolizing 2-methylnaphthalene (see Sections 3.3 and 4.4.2). Thus, taken together these observations suggest that metabolites could possibly play a role in the pathogenesis of pulmonary alveolar proteinosis in the type II pneumocytes. Studies designed to test this hypothesis, however, have not been conducted.

Castranova et al. (1988) noted that two types of cells in the lung exhibit substantial CYP enzyme activity and are expected to metabolize foreign chemicals; the type II pneumocytes in the alveoli and the nonciliated Clara cells lining the bronchioles. Both cell types are secretory, but are located in different parts of the lung (Cho et al., 1995; Junqueira et al., 1995). While chronic exposure of B6C3F1 mice to 2-methylnaphthalene in the diet targeted the type II pneumocytes, inducing pulmonary alveolar proteinosis (Murata et al., 1997), acute intraperitoneal exposure of B6C3F1 mice to 2-methylnaphthalene targeted the Clara cells, inducing bronchiolar necrosis characterized by Clara cell abnormalities, focal or complete sloughing of Clara cells, and complete sloughing of the entire bronchiolar lining (Table 5; Buckpitt et al., 1986; Griffin et al., 1981, 1982, 1983; Honda et al., 1990; Rasmussen et al., 1986). These observations provide indirect evidence that the development of both types of toxic response may involve metabolism of 2-methylnaphthalene.

Table 5. Parenteral (Single Intraperitoneal Injection) Studies of 2-Methylnaphthalene

Species/Strain	NOAEL	LOAEL	Effect	Reference
Rat, Wistar	142 mg/kg		No lung lesions	Dinsdale and Verschoyle, 1987
Mouse, C57BL/6J	10 mg/kg	100 mg/kg	Bronchiolar necrosis	Griffin et al., 1981
Mouse, DBA/2J	10 mg/kg	100 mg/kg	Bronchiolar necrosis	Griffin et al., 1983
Mouse, ddY	200 mg/kg	400 mg/kg	Bronchiolar necrosis	Honda et al., 1990
Mouse, Swiss-Webster		142 mg/kg	Bronchiolar necrosis, bronchiolar epithelial cell proliferation, and minimal liver histopathology	Rasmussen et al., 1986
Mouse, Swiss-Webster		300 mg/kg	Bronchiolar necrosis	Buckpitt et al., 1986
Mouse, C57BL/6J		400 mg/kg	Bronchiolar necrosis	Griffin et al., 1982

NOAEL = no-observed-adverse-effect level; LOAEL = lowest-observed-adverse-effect level.

Studies of the mode of action by which acute intraperitoneal injections of 2-methylnaphthalene cause bronchiolar necrosis in mice indicate an involvement of reactive metabolites produced via CYP enzymes, but the mode of action at the molecular level has not been elucidated, nor has the ultimate toxicant has not been identified.

The mode of action of acute Clara cell toxicity of 2-methylnaphthalene may be similar to that of naphthalene. The mode of action of naphthalene toxicity is hypothesized to involve metabolism by CYPIA1 and other enzymes via ring epoxidation to reactive species such as the 1,2-epoxide and 1,2-quinones (Cho et al., 1995; Greene et al., 2000; Lakritz et al., 1996; Van Winkle et al., 1999). The reactive species then interact with cellular components. The observation that 2-methylnaphthalene is less acutely toxic than naphthalene (Buckpitt and Franklin, 1989; Cho et al., 1995) supports this hypothesis, since only a small fraction of 2-methylnaphthalene (15-20%) undergoes ring epoxidation (Breger et al., 1983; Melancon et al., 1985).

The findings from the mode of action studies of the acute response in mice to intraperitoneal injection with 2-methylnaphthalene support the idea that the lung is a critical toxicity target, but may only be partially related to the pathogenesis of pulmonary alveolar proteinosis from chronic oral or dermal exposure to 2-methylnaphthalene. In mice chronically exposed to 2-methylnaphthalene for 81 weeks, no evidence for exposure-related bronchiolar lesions (Clara cell toxicity) were found (Murata et al., 1993, 1997). This finding may be related to observations suggesting that Clara cells can develop resistance to naphthalene toxicity (Lakritz et al., 1996). Pretreatment of male Swiss-Webster mice with a non-toxic dose (200 mg/kg) of naphthalene for 7 days induced adaptive changes in Clara cells of the bronchiolar

lining, making the lining more resistant to a dose of 300 mg/kg naphthalene compared with mice given only 300 mg/kg naphthalene without pretreatment (Lakritz et al., 1996). The adaptive changes included reduced expression of CYPIIB, CYPIIF, CYP reductase, and secretory protein. The possible development of Clara cell resistance to the acute toxicity of 2-methylnaphthalene, however, has not been studied.

There are limited data to suggest that rats may be less sensitive than mice to the lung damage caused by acute exposure to 2-methylnaphthalene. Wistar rats given intraperitoneal doses of 140 mg/kg 2-methylnaphthalene did not lead to any pulmonary toxicity (Dinsdale and Verschoyle, 1987). In contrast, bronchiolar necrosis was induced in Swiss-Webster mice injected with the same dose (Rasmussen et al., 1986) and C57BL/6J and DBA/2J mice injected with 100 mg/kg 2-methylnaphthalene (Griffin et al., 1981, 1982, 1983). These data are consistent with findings that rats are more resistant than mice to the acute Clara cell toxicity of naphthalene (NTP, 2000; O'Brien et al., 1985). No data are available for interspecies comparisons of the chronic toxicity of 2-methylnaphthalene.

2-Methylnaphthalene does not appear to target the liver or kidneys. No histopathological damage in these organs was reported in mice following oral exposure to doses as high as 114 mg/kg-day for 81 weeks (Murata et al., 1997) or following acute intraperitoneal injections to doses associated with mortality (1000 mg/kg) (Griffin et al., 1981, 1983). Additionally, no changes in clinical chemistry markers of liver or kidney damage were seen in the 81-week study (Murata et al., 1997). Rasmussen et al. (1986) reported minimal changes in the livers of mice intraperitoneally injected with 2-methylnaphthalene, but did not further describe these changes or specify the dose levels at which they occurred. In addition, *in vitro* assays have demonstrated cytotoxicity caused by 2-methylnaphthalene in Sprague-Dawley rat cortical tubular epithelial cells and glomerular mesangial cells (Bowes and Ramos, 1994; Parrish et al., 1998; Zhao and Ramos, 1998), but the relevance of these changes is suspect given the absence of kidney changes in the acute and chronic *in vivo* exposure studies with 2-methylnaphthalene in mice.

4.5.2. Inhalation Exposure

No human studies regarding the inhalation toxicity of 2-methylnaphthalene are available.

In addition, no chronic or subchronic animal inhalation studies with 2-methylnaphthalene are available.

There are several acute inhalation toxicity studies available. Signs of nervous system depression were seen in rats exposed for 4 hours, and a transient decrease in respiratory rate occurred in mice exposed for 6 minutes (Korsak et al., 1998). No signs of hemotoxicity in dogs were found after exposure to mists of an unknown concentration of 2-methylnaphthalene for 50 minute periods over 4 consecutive days (Lorber et al., 1972). An acute inhalation study with a methylnaphthalene mixture (exposure concentration unknown) reported that no clinical signs, mortality, or gross pathology were found in rats (Union Carbide, 1982).

The pulmonary toxicity of 2-methylnaphthalene appears dependent on the route of exposure. Dermal exposure to a methylnaphthalene mixture induced pulmonary alveolar proteinosis in all exposed mice within 30 weeks, compared to oral exposure to 2-methyl-naphthalene, which induced pulmonary alveolar proteinosis in roughly half of exposed animals within 81 weeks at approximately equal administered dose levels respectively (Murata et al., 1992, 1997). Moreover, focal interstitial fibrosis in restricted areas and decreased survival were observed following dermal exposure to a methylnaphthalene mixture (Emi and Konishi, 1985), but not following oral exposure to 2-methylnaphthalene (Murata et al., 1997). These observations suggest that toxicity differences may exist across oral and dermal routes.

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION—SYNTHESIS OF HUMAN, ANIMAL, AND OTHER SUPPORTING EVIDENCE, CONCLUSIONS ABOUT HUMAN CARCINOGENICITY, AND LIKELY MODE-OF-ACTION

4.6.1. Summary of Overall Weight-of-Evidence

Under the Draft Revised Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999), the data are *inadequate for an assessment of human carcinogenic potential*, based on the absence of data concerning the carcinogenic potential of 2-methylnaphthalene in humans and limited equivocal evidence in animals as discussed below.

4.6.2. Synthesis of Human, Animal, and Other Supporting Evidence

No epidemiological studies or case reports regarding the carcinogenic potential of 2-methylnaphthalene in humans are available. Animal cancer bioassays are limited to an 81-week dietary study (Murata et al., 1997) and a 78-week dermal study with exposure to 2-methylnaphthalene and BaP (Schmeltz et al., 1978). Murata et al. (1997) observed statistically significantly increased incidences of lung adenomas and total lung tumors (adenomas and carcinomas combined) in male mice orally exposed to 54.3 mg/kg-day 2-methylnaphthalene, but not in males orally exposed to 113.8 mg/kg-day or in females exposed to either dose. The incidences of lung carcinomas alone were not significantly different from controls for any exposure group. No increased incidence was seen for other tumor types. The dermal cancer study did not find an increased incidence of skin tumors in mice dermally exposed biweekly to 2-methylnaphthalene (equivalent to 32 μg/kg-day) plus BaP for 78 weeks, compared to mice receiving BaP alone (Schmeltz et al., 1978). The incidences of non-skin tumors were not reported. This study is of limited toxicological value, since 2-methylnaphthalene was not tested alone. No mutagenicity was observed in tests using *Salmonella typhimurium* or in cultured human lymphocytes (Florin et al., 1980; Hermann, 1981; Kopper Co. Inc., 1982; Kulka et al., 1988).

There is no data indicating that metabolism of 2-methylnaphthalene and the structurally related 1-methylnaphthalene are similar, thus this lack of information precludes the use of evidence for 1-methylnaphthalene carcinogenicity as supporting evidence for 2-

methylnaphthalene carcinogenicity. Carcinogenicity of the structurally related naphthalene has been hypothesized to be due to, at least in part, to metabolism via CYP-mediated ring epoxidation to reactive metabolites such as the 1,2-epoxide or 1,2-quinone derivatives (Cho et al., 1995; Greene et al., 2000; Lakritz et al., 1996; NTP, 2000; Van Winkle et al., 1999). The metabolic formation of ring epoxides is a relatively minor pathway for 2-methylnaphthalene, whereas it is the principal pathway for naphthalene (NTP 2000; U.S. EPA, 2003) and thus precludes the use of naphthalene carcinogenicity data to as supporting evidence for 2-methylnaphthalene carcinogenicity.

In summary, there are no studies of the potential carcinogenicity of 2-methylnaphthalene in humans, and one chronic oral cancer bioassay in animals (Murata et al., 1997). The incidence of lung tumors (predominately adenomas) was increased in male mice, (not females) at only the lower of two dietary doses that were evaluated (54.3 and 113.8 mg/kg-day). As discussed in Section 4.4.4., results from short-term genotoxicity tests with 2-methylnaphthalene provide no consistent evidence of mutagenic activity in bacteria or of clastogenic activity in human lymphocytes *in vitro*. Overall, the weight of evidence for 2-methylnaphthalene carcinogenicity in animals is limited and insufficient to determine its carcinogenic potential in humans.

4.6.3. Mode-of-Action Information

Information regarding the possible mode of action for the potential carcinogenicity of 2-methylnaphthalene is limited to findings that no clear short-term genotoxic activity was observed in *Salmonella typhimurium* or in cultured human lymphocytes (Florin et al., 1980; Hermann, 1981; Kopper Co. Inc., 1982; Kulka et al., 1988) and suggestive evidence that the mode of action for lung tumor formation does not involve a progression from either bronchiolar necrosis or pulmonary alveolar proteinosis. No evidence of bronchiolar necrosis or Clara cell damage was seen in the mice exhibiting lung tumors after 81 weeks of dietary exposure to 2-methylnaphthalene (Murata et al., 1997). With respect to a possible relationship between the development of pulmonary alveolar proteinosis and lung tumors (in male mice), the available data do not support the hypothesis that pulmonary alveolar proteinosis might be a precursor effect to lung tumor formation (Murata et al., 1993, 1997). For example, compared with 1-methylnaphthalene, 2-methylnaphthalene induced equal or slightly higher incidences of pulmonary alveolar proteinosis, but lower incidences of lung tumors. In addition, Murata et al. (1993) reported that the numbers of mice developing pulmonary alveolar proteinosis and tumors following exposure to 1-methylnaphthalene were not statistically correlated, and that the sites of development of alveolar proteinosis and lung tumors were also not always clearly linked.

4.7. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.7.1. Possible Childhood Susceptibility

No studies are available regarding the adverse effects of 2-methylnaphthalene in children or prenatal animals.

Studies in children with pulmonary alveolar proteinosis have provided suggestive evidence that congenital deficiencies in expression of some proteins, such as surfactant protein B, may contribute to this disease (Mazzone et al., 2001; Mildenberger et al., 2001; Wang et al., 1997). Such individuals may be more sensitive than the general population to the toxic effects of repeated exposure to 2-methylnaphthalene.

The toxicokinetics of xenobiotics can vary widely between children and adults due to immaturity of the phase I and phase II enzyme systems and clearance mechanisms (Ginsberg et al., 2002). Metabolism studies suggest that alterations in CYP enzyme activity may be involved in the adverse noncancer pulmonary effects that are observed following exposure to 2-methylnaphthalene (Griffin et al., 1982, 1983). The importance and identity of the specific isozymes and/or metabolites responsible for these effects are not well understood. In addition, conjugation reactions catalyzed by enzymes such as glutathione transferase have been shown to decrease the pulmonary toxicity of exposure to 2-methylnaphthalene in mice (Griffin et al., 1982; Honda et al., 1990). Taken together, these studies suggest that children may be at increased risk for bronchiolar necrosis and pulmonary alveolar proteinosis after exposure to 2-methylnaphthalene. However, data are not available to demonstrate that this is the case, either in rodents or humans.

4.7.2. Possible Gender Differences

The extent to which men and women differ in susceptibility to 2-methylnaphthalene toxicity is not known. Clinical cases of pulmonary alveolar proteinosis are 3-fold more common in men than in women (Mazzone et al., 2001), but no data are available regarding gender sensitivity to 2-methylnaphthalene in humans.

The available animal data do not provide definitive information for gender differences in susceptibility to 2-methylnaphthalene toxicity. Acute animal testing data suggested that females were somewhat more sensitive to 2-methylnaphthalene toxicity than males (Union Carbide, 1982). For example, gavage studies in rats determined LD_{50} values of 4.29 mL/kg for males and 3.73 mL/kg for females (4310 and 3270 mg/kg) and dermal studies in rabbits calculated LD_{50} values of 6.1 mL/kg in males and 4.8 mL/kg in females (6130 and 4790 mg/kg). No significant differences in the incidences of pulmonary alveolar proteinosis were observed between male and female B6C3F1 mice given equivalent dietary doses of 2-methylnaphthalene for 81 weeks, but only exposed male mice, and not females, showed increased incidences of lung tumors in these studies (Murata et al., 1993;1997)

4.7.3. Other

No data are available regarding the effects of 2-methylnaphthalene on other potentially susceptible populations. Individuals with existing clinical pulmonary alveolar proteinosis may be more susceptible to the effects of 2-methylnaphthalene than healthy individuals. In addition, individuals with risk factors for pulmonary alveolar proteinosis which include persons who have myelongeous leukemia, pulmonary infection, a history of smoking, and inhalation exposure to silica or some heavy metals (Mazzone et al., 2001; Seymour and Presneill, 2002; Wang et al., 1997).

5. DOSE RESPONSE ASSESSMENT

5.1. ORAL REFERENCE DOSE (RfD)

There has been no RfD for 2-methylnaphthalene on IRIS before this assessment.

5.1.1. Choice of Principal Study and Critical Effect - with Rationale and Justification

No epidemiology studies or case reports are available which examined the potential effects of human exposure to 2-methylnaphthalene by oral exposure.

Only one chronic study is available in which animals were orally exposed to 2-methylnaphthalene (Murata et al., 1997). This study was chosen as the principal study. Male and female B6C3F1 mice (50/sex/group) were fed diets containing 0, 0.075%, or 0.15% of 2-methylnaphthalene for 81 weeks, and numerous endpoints were evaluated, including histology for more than 24 tissues, hematology, and serum chemistry. Incidences of pulmonary alveolar proteinosis were statistically significantly increased at both doses for males (54.3 and 113.8 mg/kg-day) and females (50.3 and 107.6 mg/kg-day). Incidences for control through high-dose groups were: 4/49, 21/49, and 23/49 for males; and 5/50, 27/49, and 22/49 for females. Mean growth curve data showed reduced weight gain in males at both doses and in females exposed to the high dose. Final body weight was statistically significantly reduced only for the high-dose male group, but because the magnitude of the decrease was less than 10% compared with controls, this was not considered a biologically significant effect. Murata et al. (1997) also reported several other statistically significant differences between control and exposure groups; however, the biological significance of these differences was unclear because no data were provided regarding the magnitude or exposure levels at which they occurred. Affected variables included relative and absolute brain and kidney weights, serum neutral fat levels, and differential counts of neutrophils and lymphocytes. No other increased incidences of non-neoplastic effects were identified by histology in exposed groups of male and female mice. For these reasons, pulmonary alveolar proteinosis is chosen as the critical effect.

As discussed in Sections 4.5.1., selection of pulmonary alveolar proteinosis as the critical effect following oral exposure to 2-methylnaphthalene is supported by dermal studies with a methylnaphthalene mixture containing 2-methylnaphthalene and 1-methylnaphthalene respectively, in an approximate 2:1 ratio. All female B6C3F1 mice dermally exposed to 119 mg/kg methylnaphthalene twice weekly for 30 weeks (34 mg/kg-day), or 238 mg/kg twice weekly for 20 weeks (68 mg/kg-day) exhibited pulmonary alveolar proteinosis (Murata et al., 1992). Similarly, a 61-week study reported lipid pneumonia in B6C3F1 mice dermally exposed to a methylnaphthalene mixture (Emi and Konishi, 1985).

Other available oral toxicity studies for 2-methylnaphthalene are of prechronic duration. Fitzhugh and Buschke (1949) did not observe cataract formation in rats fed 2-methylnaphthalene for at least 2 months, and did not investigate any other endpoints. Murata et al. (1997) conducted a range-finding study in which groups of B6C3F1 mice (10/sex/group) were fed diets containing 2-methylnaphthalene for 13 weeks delivering approximate average daily doses of 0, 31, 92, 276, 827, or 2500 mg/kg-day. No histopathological effects were observed in tissues and organs of male or female mice exposed to 827 or 2500 mg/kg-day. Decreased weight gain was seen at the three highest dose levels in both males and females, and was attributed to food refusal (Murata et al., 1997). The absence of pulmonary alveolar proteinosis in the prechronically exposed mice, which were exposed to much higher doses than those used in the chronic study, suggests that the development of pulmonary alveolar proteinosis may require chronic duration exposure.

No other adverse noncancer effects were found in tissues other than the lung in the Murata et al. (1997) dietary study with 2-methylnaphthalene. Similarly, no liver or kidney damage was found in mice administered single intraperitoneal injections of 2-methylnaphthalene. Although Rasmussen et al. (1986) reported minimal liver damage in male Swiss-Webster mice injected with 140 and 280 mg/kg of 2-methylnaphthalene, no histological evidence of liver or kidney damage was seen in male C57B1/6J (Griffin et al., 1981, 1982) or male DBA/2J mice (Griffin et al., 1983) at doses up to 1000 mg/kg. The acute dose of 1000 mg/kg of 2-methylnaphthalene was frankly toxic, as evidenced by mortality observed in 3/10 mice dosed with this concentration (Griffin et al., 1981, 1983).

A limitation of the principal study (Murata et al., 1997) is the occurrence of pulmonary alveolar proteinosis in control mice. The authors described this condition as being less pronounced but similar to the adverse lung effects seen in the 2-methylnaphthalene exposed mice. The authors also indicated that pulmonary alveolar proteinosis had not been observed in more than 5000 B6C3F1 control mice, and speculated that the background incidence may have been elevated by inhalation exposure to evaporated test chemicals and poor room ventilation. The 81-week study testing 0.075% and 0.15% 2-methylnaphthalene in the diet (Murata et al., 1997) was conducted simultaneously with a study testing 0.075% and 0.15% 1-methylnaphthalene in the diet (Murata et al., 1993). Both studies shared a common control group of mice, and all mice were housed in the same room. Murata et al. (1993, 1997) did not quantitate the concentration of either chemical in the air. It should be noted that 2-methylnaphthalene is slightly more volatile than 1-methylnaphthalene (vapor pressure of 0.068

mm of Hg compared to 0.087 mm Hg, respectively). Potential confounding from possible inhalation exposure to 2-methylnaphthalene and 1-methylnaphthalene adds some uncertainty to the dose-response relationship between oral exposure to 2-methylnaphthalene and pulmonary alveolar proteinosis.

5.1.2. Methods of Analysis - Including Models

While the principal study for 2-methylnaphthalene shows a dose-response relationship between oral exposure to 2-methylnaphthalene and pulmonary alveolar proteinosis (Murata et al., 1997), the data are somewhat uncertain for characterizing risk at lower exposures. First, the potential confounding (noted in Section 5.1.1) from possible inhalation exposure to 2-methylnaphthalene and 1-methylnaphthalene by all animals complicates the quantitative assessment of the dose-response relationship, at least in how to interpret the incidence of pulmonary alveolar proteinosis in control animals. This incidence was reported to be unusually high compared with historical controls, suggesting it may not be a relevant baseline. In addition, the similar degree of pulmonary alveolar proteinosis in the two exposed groups, both averaging about 45%, provides very little information concerning the shape of the dose-response relationship expected at lower exposures (see Table B1 and the BMDS graph in the model output in Appendix B). Nevertheless, some judgments about these issues can be made which allow estimating a reference dose from these data, as discussed below.

Note that the incidences of pulmonary alveolar proteinosis for male and females were not statistically significantly different from each other ($p \leq 0.05$, using Fisher's exact test), indicating neither sex was clearly more sensitive. Consequently, this analysis also considers the combined incidences for each exposure group where appropriate, in order to strengthen quantitative results.

Concerning the possible simultaneous inhalation exposure to 2-methylnaphthalene and 1-methylnaphthalene, consideration of the concurrent and historical control information may provide some bounds on the degree of effect that can be associated with oral exposure to 2-methylnaphthalene. First, the concurrent control group is generally the most relevant comparison group, unless there is documentation that the control group was treated differently than the exposed groups, that is, apart from direct exposure to the test material. In this study, there is no reason to believe the control group was treated any differently than the other groups. That is, if there were secondary exposure to volatilized test materials, and it can be assumed that all animals were similarly exposed, then the concurrent control group is especially important to consider in any assessment of adverse effects.

Alternatively, use of the historical control information may provide an upper bound on the magnitude of effect associated with oral 2-methylnaphthalene exposure. Two situations can be characterized, determined by the contribution of 1-methylnaphthalene to the observed effects. In the simplest case, if co-exposure to 1-methylnaphthalene has no adverse effects, and 2-methylnaphthalene exposure by inhalation is an unavoidable consequence of exposure to 2-methylnaphthalene in the diet, then the appropriate control group would be one which could

have been isolated from any possible inhalation exposure of 2-methylnaphthalene. The historical control group would then be considered the best available comparison group. This comparison would yield the largest difference in effect level between control and exposed groups.

It is not clear, however, that inhalation exposure to 1-methylnaphthalene would not be associated with pulmonary alveolar proteinosis. The animals exposed concurrently to 1-methylnaphthalene in their diet demonstrated similar incidences of pulmonary alveolar proteinosis to the 2-methylnaphthalene-exposed animals (see Footnote 3, Section 4.2.1.2.), indicating that oral 1-methylnaphthalene exposure is associated with pulmonary alveolar proteinosis. Consequently, an association of pulmonary alveolar proteinosis and 1-methylnaphthalene exposure by inhalation cannot clearly be ruled out. Therefore, use of the concurrent control is relevant to minimize the influence of any 1-methylnaphthalene exposure on effects associated with 2-methylnaphthalene exposure.

In summary, use of the historical control provides an upper bound on the degree of effect associated with oral exposure to 2-methylnaphthalene, while the concurrent control serves to control for any contribution of simultaneous exposure to 1-methylnaphthalene in this study. Both groups are considered below in characterizing the point of departure.

Both commonly used approaches for identifying a point of departure for low-dose extrapolation, LOAEL/NOAEL methodology and benchmark dose modeling, have some relevance for this data set. LOAEL/NOAEL methodology is not as dependent upon the level of response in the control group as benchmark dose modeling is, as long as the response level in the exposed group is significantly different from control (or any lower exposure groups without a significant response). The lower dose in the Murata et al. (1997) data set is easily identified as the LOAEL, regardless of whether it is compared with the concurrent or the historical control group. In addition, the similarity of the responses in the orally exposed groups has very little impact on identification of the LOAEL.

On the other hand, benchmark dose modeling can provide a point of departure which is consistent with more of the observed data than the LOAEL/NOAEL approach uses, taking into account the degree of response at the point of departure, and addressing the variability inherent in the data. In this case, the shape of the dose-response at lower exposures would still be somewhat uncertain, however. That is, the similar responses in the dose groups suggest that the observed plateau may continue somewhat into the lower exposure range, but not much more can be inferred about the low-dose behavior of the relationship. In order to explore what points of departure could be estimated when the full data set is considered, the incidence data for males, females, and males and females combined were fit to all dichotomous variable models available in the BMDS Version 1.3 software (U.S. EPA, 2002b). The results are shown in Appendix B.

Most of the benchmark modeling did not provide adequate fits, as indicated by Chi-square goodness-of-fit p-values less than 0.1 (see Appendix B). The application of the Log-

Logistic model to the male mouse data provided the best fit among the three adequate fits ($p>0.1$), as indicated by the lowest Akaike Information Criterion (AIC) among those three.

A benchmark response level of 10% extra risk of pulmonary alveolar proteinosis was selected for this assessment. As discussed earlier (Section 4.5.1), pulmonary alveolar proteinosis produces mild symptoms, and is a treatable condition in adult humans. It is not considered a frank effect following exposure to 2-methylnaphthalene, nor is there any indication that it is a precursor of more severe adverse effects. Thus, a 10% extra risk of pulmonary alveolar proteinosis was judged to be an acceptable level of extra risk for this critical effect. From this model, the ED_{10} was 14 mg/kg-day for pulmonary alveolar proteinosis in male mice exposed to 2-methylnaphthalene in the diet for 81 weeks (Murata et al, 1997). The lower 95% confidence limit on the ED_{10} (i.e., LED_{10}) was 9.1 mg/kg-day.

Note that while the overall log-logistic curve fit was technically adequate using the criteria in the draft BMDS guidance (U.S.EPA, 2000), the largest deviation in the fit occurs at the low dose response, where it is especially important to have an adequate prediction. Specifically, the fit suggests that this model may underestimate responses just below the experimental data. Given the lack of information suggesting a plausible underlying mode of action supporting a particular dose-response relationship, and the limited design of the study (only two exposed groups), the main recourse is to compare the ED_{10} with what would be predicted by assuming a linear dose-response between the control and low dose response. This line passes very close to the modeled ED_{10} (see Figure 6), providing additional support for the ED_{10} and the LED_{10} to characterize the point of departure.

Limited modeling was carried out using the male mice incidence data from the exposed groups and the reported historical incidence of 0 cases of pulmonary alveolar proteinosis in ~5000 control mice. The fits showed a similar pattern with the log-logistic, again providing the best fit. The ED_{10} was 11 mg/kg-day, and LED_{10} was 7.7 mg/kg-day. This LED_{10} is only marginally lower than the one based on the concurrent control group. Since the concurrent control can serve to adjust for any simultaneous 1-methylnaphthalene exposure, this LED_{10} appears to be the strongest point of departure for developing an RfD.

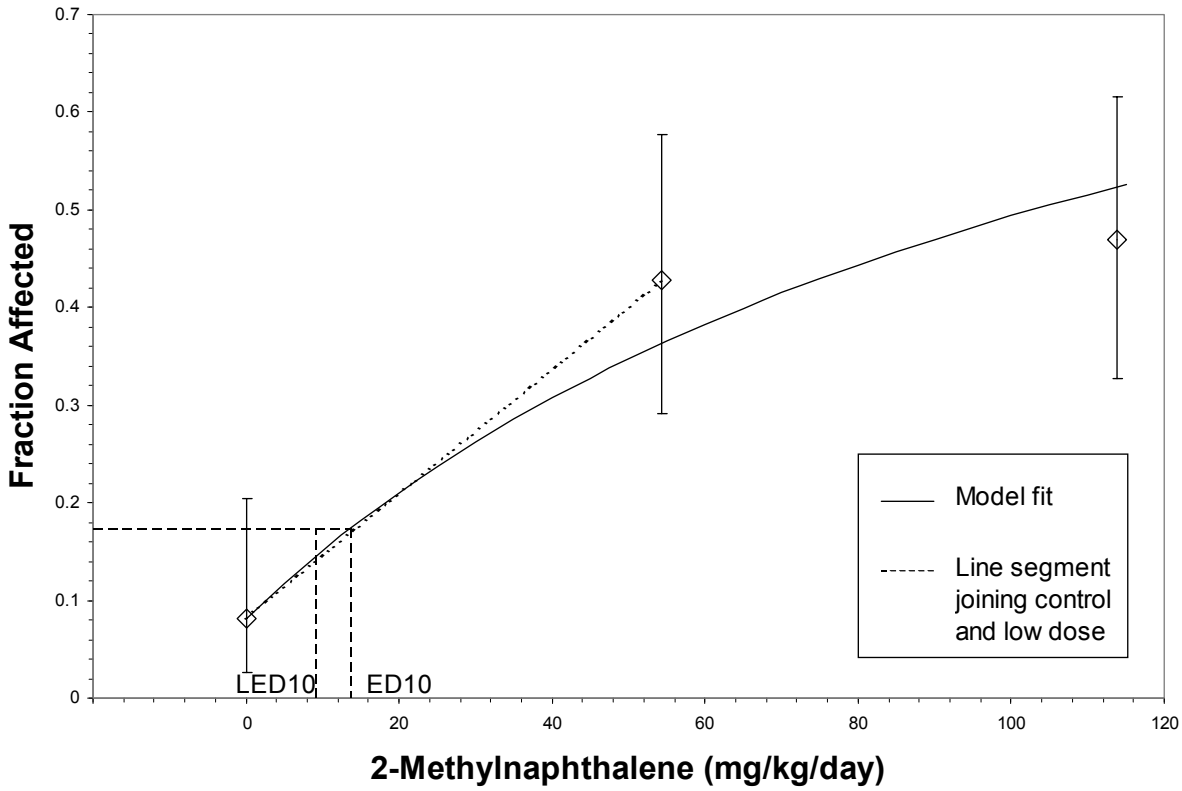

Figure 3: Log-logistic model fit to pulmonary alveolar incidence associated with oral exposure to 2-methylnaphthalene (Murata *et al.*, 1992), with the ED_{10} and LED_{10} indicated. A straight line joining the concurrent control and low dose responses is provided for comparison.

5.1.3. RfD Derivation - Including Application of Uncertainty Factors (UFs)

Using benchmark dose modeling, the LED_{10} of 9.1 mg/kg-day for 10% extra risk for pulmonary alveolar proteinosis in male mice exposed to 2-methylnaphthalene in the diet for 81 weeks (Murata et al., 1997) was selected as the point of departure for the RfD. To calculate the RfD using the LED_{10}, several uncertainty factors (UFs) were applied.

A total UF of 1000 was applied to this effect level: 10 for extrapolation for interspecies differences (UF_A: animal to human); 10 for consideration of intraspecies variation (UF_H: human variability); and 10 for deficiencies in the database (UF_D). Uncertainty factors for subchronic to chronic exposure extrapolation and for LOAEL to NOAEL extrapolation were not considered necessary. These decisions are described in greater detail below.

A 10-fold UF was used to account for uncertainty in extrapolating from laboratory animals to humans (i.e., interspecies variability). No information was available regarding the toxicity of 2-methylnaphthalene in humans exposed orally. No information was available to assess toxicokinetic differences between animals and humans. In the absence of data to the contrary, the pulmonary alveolar proteinosis observed in mice is assumed to be relevant to humans.

A 10-fold UF was used to account for variation in sensitivity among members of the human population (i.e., interindividual variability). This UF was not reduced, due to a lack of human oral exposure data.

A 10-fold UF was used to account for uncertainty associated with deficiencies in the data base. One chronic duration oral toxicity study in one animal species (mice) is available (Murata et al., 1997). The data base lacks adequate studies of oral developmental toxicity, reproductive toxicity, and neurotoxicity. The data base also lacks a 2-generation reproductive toxicity study.

An UF was not needed to account for subchronic to chronic extrapolation because a chronic study (81 weeks) was used to derive the RfD.

An UF for LOAEL-to-NOAEL extrapolation was not considered as such, since benchmark dose modeling was used to determine the point of departure[7]. The 10% increased response level used to derive the RfD is not a no-response level, however, so some consideration of what level of extra risk of pulmonary alveolar proteinosis constitutes a minimal health risk is

[7] The NOAEL/LOAEL approach would yield a LOAEL of 52.3 mg/kg-day, using the combined male and female data. This exposure level would be adjusted by a LOAEL-to-NOAEL extrapolation uncertainty factor of up to 10 in order to estimate an RfD. The observed response at the LOAEL, relative to the concurrent control, was approximately 44%, in terms of extra risk: ER = [P(d)-P(0)]/[1-P(0)] = [48/98 - 9/99]/[1 - 9/99] = 0.44, where P(d) is the proportion responding at dose d (here the low dose), and P(0) is the proportion responding at dose 0 (control). Use of the full LOAEL-to-NOAEL uncertainty factor of 10 would appear justified, although it would contribute to a total UF of 10,000 given the other uncertainties.

appropriate. EPA is developing guidance on the application of effect level extrapolation factors as uncertainty factors to extrapolate to risks below the effect level at the point of departure. Since pulmonary alveolar proteinosis is a treatable disorder, with mild symptoms, it is considered to be of limited severity. Further, it is not thought to be a precursor to a more severe adverse noncancer or cancer effect. Pending final guidance on this issue, it was determined that an effect level extrapolation factor was not necessary for the derivation of the RfD for 2-methylnaphthalene from the LED_{10}.

The RfD for 2-methylnaphthalene was calculated as follows:

$$RfD = LED_{10} \div UF$$
$$= 9.1 \text{ mg/kg-day} \div 1000$$
$$= 0.009 \text{ mg/kg-day}$$

In addition, note that there is model uncertainty owing to the lack of actual dose-response information or mode of action information in the region of the dose-response where the point of departure is estimated. The approach used here is unbiased in that it is plausible that a 10% extra risk of pulmonary alveolar proteinosis could occur at a higher or lower exposure level than was estimated. Given the lack of additional information, this is the most reasonable estimate that can be derived from these data.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

No epidemiology studies or case reports are available which examined the potential effects of human inhalation exposure to 2-methylnaphthalene.

No chronic or prechronic studies are available that exposed animals by inhalation to 2-methylnaphthalene.

Two reports are available for acute exposure of animals to 2-methylnaphthalene; neither are suitable for RfC derivation. Lorber et al. (1972) investigated hematoxicity endpoints in intact and splenectomized dogs exposed to mists of 2-methylnaphthalene (at unknown concentrations) for 41-50 minutes for 4 consecutive days. No clear evidence of hematoxicity was observed. Korsak et al. (1998) exposed rats by inhalation to 2-methylnaphthalene for 4 hours to evaluate neurotoxicity, and mice for 6 minutes to evaluate sensory/respiratory irritation (Korsak et al., 1998). In rats, none of the concentrations tested affected a neuromuscular test (rotarod performance) but two concentrations decreased pain sensitivity (measured by latency of paw-lick response to a heated surface). In mice, rapid but reversible decreases in respiratory rate were seen, with the response magnitude increasing with increasing exposure concentration.

In the absence of an inhalation study of sufficient duration that evaluated a comprehensive array of endpoints to establish a NOAEL or LOAEL, no RfC for 2-methylnaphthalene can be derived.

A route-to-route extrapolation is not currently possible. No toxicokinetic models are available for 2-methylnaphthalene, and there is evidence to suggest that its ability to induce pulmonary alveolar proteinosis in mice may vary across routes of exposure (as discussed in Section 4.5.2).

5.3. CANCER ASSESSMENT

As discussed in Section 4.6.1, the available data base for 2-methylnaphthalene *is inadequate to assess human carcinogenic potential*. Limited evidence of carcinogenicity in animals was provided by an 81-week dietary study in B6C3F1 mice (Murata et al., 1997). Incidences for lung adenomas and total lung tumors (adenomas and carcinomas combined) for the low-dose male group (54.3 mg/kg-day) were statistically significantly elevated compared to controls. However, no evidence of carcinogenicity was seen in male mice exposed to the high dose (113.8 mg/kg-day), or in female mice exposed to either dose. No evidence of a trend of increasing tumor incidence with increasing dose was seen for males or females. Lack of an apparent dose-response relationship makes these data unsuitable for quantitative assessment of carcinogenic potential. No statistically significant elevations in other tumor incidences were seen in any exposure group.

A dermal co-carcinogenicity study was an unsuitable test of 2-methylnaphthalene carcinogenicity because 2-methylnaphthalene was tested only in a mixture with benzo[a]pyrene (BaP) (Schmeltz et al., 1978).

In addition, no genotoxicity studies in humans or animals are available. No studies investigating potential germ-line mutations are available. Data from *in vitro* short-term assays provide limited evidence for genotoxic activity of 2-methylnaphthalene (Florin et al., 1980; Harvey and Halonen, 1968; Hermann, 1981; Kopper Co. Inc., 1982; Kulka et al., 1988; Weis et al., 1998).

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

2-Methylnaphthalene (CAS No. 91-57-6) is a natural component of crude oil and coal, and is found in pyrolysis and combustion products such as cigarette and wood smoke, emissions from combustion engines, asphalt, coal tar residues and used oils. It is also used as a chemical intermediate in the synthesis of vitamin K.

No data are available regarding the potential toxicity of 2-methylnaphthalene in exposed humans via the oral route. The available animal data indicate that the lung is a sensitive target organ. The critical effect observed in mice following chronic oral exposure to 2-methylnaphthalene (Murata et al., 1997) and chronic dermal exposure to methylnaphthalene mixtures containing 2-methylnaphthalene (Emi and Konishi, 1985; Murata et al., 1995) was pulmonary alveolar proteinosis. This is a condition characterized by accumulation of foamy cells, cholesterol crystals, and proteinaceous materials rich in lipids in the lumen of the pulmonary alveoli (Murata et al., 1997). In the absence of data to the contrary, the assumption is made that these observations are relevant to humans.

In humans, pulmonary alveolar proteinosis is characterized by symptoms such as dyspnea and cough with possible decreased pulmonary function, identified by decreased functional lung volume and reduced diffusing capacity. It has not been associated with airflow obstruction (Lee et al., 1997; Mazzone et al., 2001; Wang et al., 1997). Cases of pulmonary alveolar proteinosis in humans have not been directly associated with exposure to 2-methylnaphthalene.

The effects of prechronic or chronic inhalation exposure to 2-methylnaphthalene have not been studied in humans or animals. No suitable toxicokinetic models are available to extrapolate between routes of exposure. Since chronic exposure to 2-methylnaphthalene by oral and dermal routes target the lung to cause pulmonary alveolar proteinosis, it is plausible that similar adverse effects may be seen after chronic inhalation exposure to 2-methylnaphthalene. However, no conclusions can be drawn from the current data regarding potential exposure-response relationships for chronic inhalation exposure.

Under the Draft Revised Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999), the available data for 2-methylnaphthalene provided *are inadequate to assess human carcinogenic potential*. There are no studies of the potential carcinogenicity of 2-methylnaphthalene in humans, and only one adequate cancer animal bioassay for 2-methylnaphthalene is available (Murata et al., 1997). The study found increased incidence of total lung tumors and adenomas in male mice, but not female mice, exposed to 2-methylnaphthalene in the diet for 81 weeks. However, the incidence was only increased at the lower of two exposure levels. The relevance of these observations to humans is uncertain. Other animal species have not been tested and results from short-term genotoxicity tests provide no

supporting evidence for the carcinogenicity of 2-methylnaphthalene. As such, the available evidence of 2-methylnaphthalene carcinogenicity is limited and insufficient to determine that 2-methylnaphthalene is carcinogenic to humans.

6.2. DOSE RESPONSE

6.2.1. Noncancer/Oral

The RfD of 0.01 mg/kg-day was calculated from a $BMDL_{10}$ of 9.1 mg/kg-day for 10% extra risk for pulmonary alveolar proteinosis in male mice exposed to 2-methylnaphthalene in the diet for 81 weeks. A total UF of 1000 was used: 10 for interspecies variability, 10 for interindividual variability, and 10 for database deficiencies.

In the absence of human data regarding the potential health effects caused by oral exposure, the pulmonary alveolar proteinosis observed in mice are assumed to be relevant to humans. No suitable toxicokinetic or toxicodynamic models have been developed to reduce uncertainty in extrapolating from mice to humans.

The extent of variability in susceptibility to 2-methylnaphthalene among humans is unknown, representing another important area of uncertainty in the RfD. Chronic experiments relevant to 2-methylnaphthalene exposure have only been performed in one strain of one species, B6C3F1 mice. Subpopulations expected to be more susceptible to 2-methylnaphthalene toxicity include those with limited or altered capacity to metabolize and detoxify 2-methylnaphthalene, and people with existing pulmonary alveolar proteinosis or those with risk factors for the disease.

The principal study for the RfD (Murata et al., 1997) examined a comprehensive number of endpoints, including extensive histopathology, and tested two dose levels using sufficient numbers (50/group) of both sexes of B6C3F1 mice. Potential confounding from possible inhalation exposure of controls to 2-methylnaphthalene and 1-methylnaphthalene in this study adds some uncertainty to the dose-response relationship. Aside from this study, the oral data base is sparse. No information is available for the testing of 2-methylnaphthalene in assays of developmental toxicity, reproductive toxicity, and neurotoxicity.

Relative to a NOAEL/LOAEL approach for RfD derivation, the use of BMD modeling reduces the uncertainty associated with the RfD. BMD modeling reduces uncertainty by incorporating information available for the control and high-exposure groups in addition to the LOAEL. Additional uncertainties arise from extrapolation from the relatively high exposure levels used in the study (Murata et al., 1997) to lower exposure levels, and a lack of empirical data identifying a NOAEL. The Log-Logistic model was selected because it provided the best fit to the data (Appendix B).

6.2.2. Noncancer/Inhalation

The data base for inhalation exposure is limited to several acute studies and therefore, was unsuitable for calculation of an RfC value.

6.2.3. Cancer/Oral and Inhalation

Under the Draft Revised Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999), the data base for 2-methylnaphthalene is *inadequate to assess human carcinogenic potential*. As such, the data are unsuitable to calculate quantitative cancer risk estimates for humans.

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APPENDIX A. Summary of External Peer Review and Public Comments and Disposition

APPENDIX B. Benchmark Dose (BMD) Analysis

BMD modeling was performed to identify a point of departure (POD) for the RfD for 2-methylnaphthalene according to draft EPA guidelines (U.S. EPA, 2000) using the BMDS Version 1.3 software.

The modeled data were the incidences of pulmonary alveolar proteinosis observed in male and female B6C3F1 mice exposed to 2-methylnaphthalene in the diet (Murata et al., 1997) as shown in Table B1.

Table B1. Incidence of pulmonary alveolar proteinosis in B6C3F1 mice fed 2-methylnaphthalene for 81 weeks (from Murata et al., 1997).

	Combined (males + females)		Females		Males	
Dietary dose	Dose (mg/kg-day)	Incidence	Dose (mg/kg-day)	Incidence	Dose (mg/kg-day)	Incidence
0	0	9/99	0	5/50	0	4/49
0.075%	52.3	48/98	50.3	27/49	54.3	21/49
0.15%	110.7	45/97	107.6	22/48	113.8	23/49

The benchmark response (BMR) was defined as a 10% increase in extra risk for the critical effect, pulmonary alveolar proteinosis. Table B2 shows the statistical results used to evaluate the goodness of fit. Models which were clearly not relevant, that is, those with a concave shape or which completely missed the low dose response, are not included in the summary.

For each model, the software performed residual and overall Chi-Square Goodness of Fit tests, and determined the Akaike's Information Criterion (AIC). Lower AIC and higher Chi-Square value p-values are associated with better fit. The Chi-Square p-value is a measure of the closeness between the observed data and the predicted data (i.e., predicted using the modeled fit). Models with Chi-Square p-values ≥ 0.1 were considered to be adequate fits. The AIC is a measure of the model fit based on the log-likelihood at the maximum likelihood estimates for the parameters. Among models with adequate Chi-Square p-values, AIC values were compared. Based on these criteria, the fit of male data to the Log-Logistic model was chosen as the best fitting model. Output from the software for the Log-Logistic model run (of the male mouse incidence data) follows Table B2.

Since there is some validity to using the historical control data as reference group (assuming 0 responses from 5000 mice), additional runs were considered using the male mice incidence data from the exposed groups. These results are shown for comparison in Table B2. The best-fitting model was also the Log-Logistic model. These results follow the results which incorporated the concurrent control data.

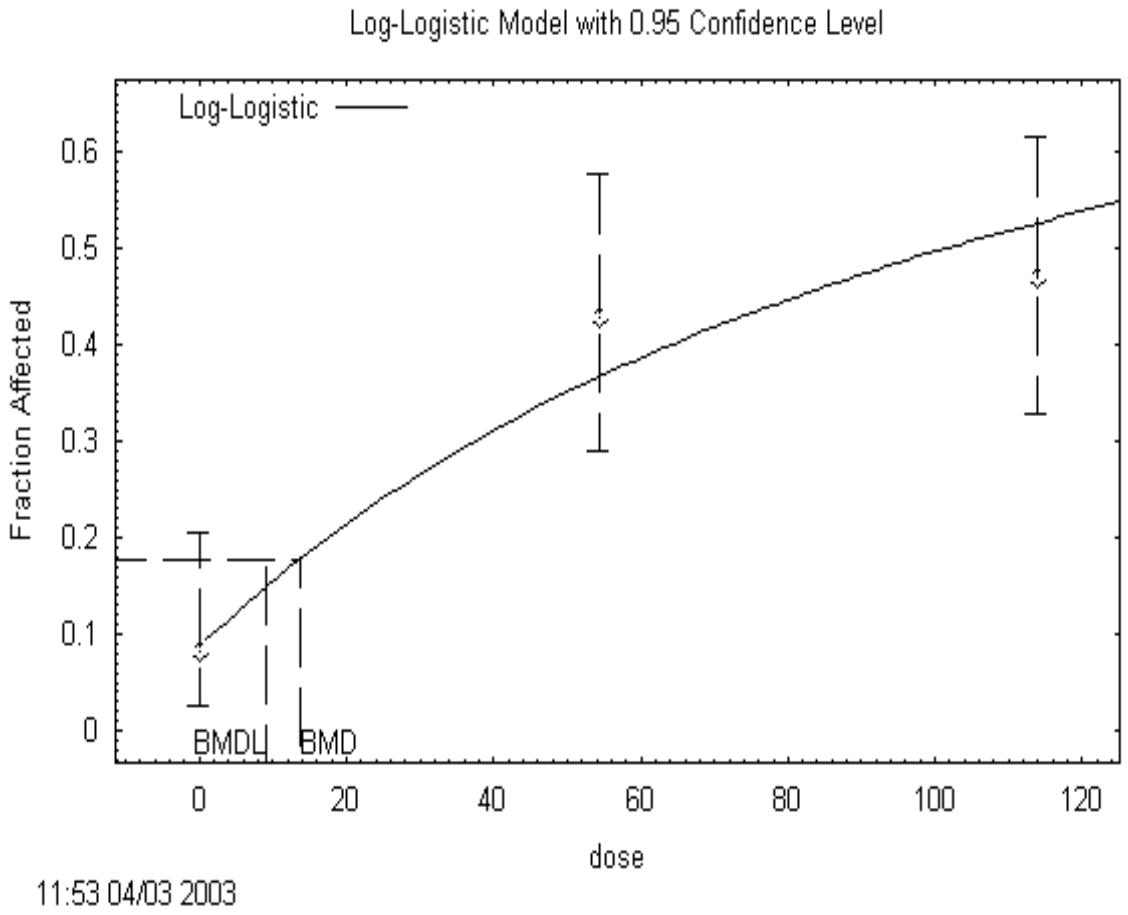
Table B2. Benchmark dose modeling for critical effect (settings of 10% Extra risk, Confidence Level 0.95).

Model	Model results	Combined (males + females)	Females	Males	Males[a]
Log-Logistic	AIC Chi Square P-value ED_{10} LED_{10}	341.177 0.0073 12.0874 8.95797	176.68 0.0099 10.6553 6.9703	167.813 0.2282 13.6562 9.07745	138.65 0.3654 10.7953 7.69436
Log-Probit	AIC Chi Square P-value ED_{10} LED_{10}	350.114 0.0000 27.7951 22.6773	182.437 0.0004 25.6622 19.2007	170.987 0.0293 30.0784 22.9322	142.923 0.0386 24.9254 20.1968
Probit	AIC Chi Square P-value ED_{10} LED_{10}	352.958 0.0000 30.037 25.5111	183.773 0.00002 28.3195 22.2763	172.401 0.0140 32.1091 25.9429	-[c]
Other models[b]	AIC Chi Square P-value ED_{10} LED_{10}	344.763 0.0009 16.6797 13.2069	179.224 0.0023 15.5088 11.1906	168.932 0.1063 17.9894 13.1441	140.617 0.1302 14.7148 11.543

[a] Using historical controls, rather than concurrent control.
[b]The output for the Gamma, Quantal-linear (one-stage multistage), and Weibull model fits were identical.
[c] Fit information not shown; dose-response shape (concave) not suitable for this data set.

BMDS MODEL RUN: Logistic Model, male mouse incidence data for pulmonary alveolar proteinosis, with concurrent control

Figure B1:



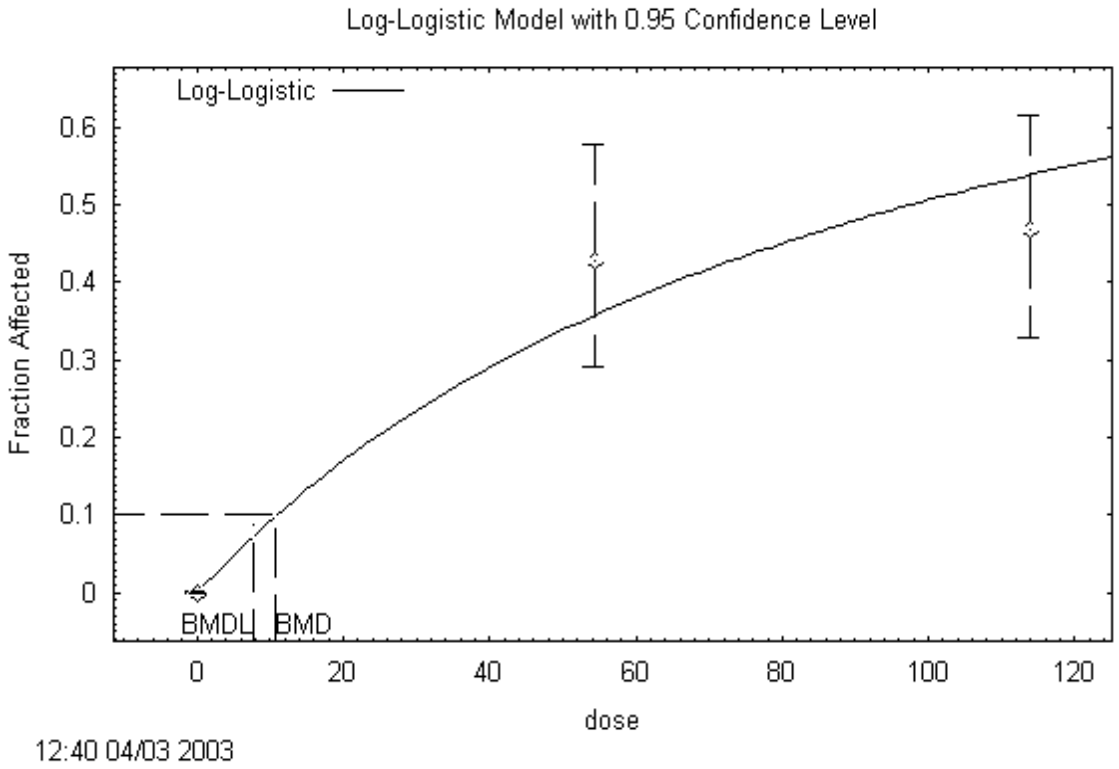

```
BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = PAP_males
   Independent variable = mg_kg_d
   Slope parameter is restricted as slope >= 1

   Total number of observations = 3
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
```

```
   User has chosen the log transformed model


                 Default Initial Parameter Values
                    background =    0.0816327
                     intercept =     -4.52857
                         slope =            1


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -slope
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

            background    intercept

background            1        -0.47

 intercept        -0.47            1


                          Parameter Estimates

       Variable           Estimate            Std. Err.
     background           0.0869488           0.0414032
      intercept            -4.81142            0.266769
          slope                   1                  NA

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.


                       Analysis of Deviance Table

       Model      Log(likelihood)  Deviance Test   DF     P-value
     Full model         -81.189
    Fitted model       -81.9066       1.43511      1          0.2309
   Reduced model       -92.8591       23.3401      2         <.0001

          AIC:         167.813


                     Goodness  of  Fit

                                                                  Scaled
    Dose     Est._Prob.    Expected    Observed     Size        Residual
  ---------------------------------------------------------------------
    0.0000      0.0869          4.260          4           49      -0.1321
   54.3000      0.3667         17.970         21           49       0.8983
  113.8000      0.5259         25.770         23           49      -0.7924

 Chi-square =       1.45     DF = 1        P-value = 0.2282


   Benchmark Dose Computation

Specified effect =           0.1
```

```
Risk Type        =      Extra risk
Confidence level =            0.95
             BMD =         13.6562
            BMDL =         9.07745
```

BMDS MODEL RUN: Logistic Model, male mouse incidence data for pulmonary alveolar proteinosis, with historical controls

Figure B



```
The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = PAP_males
   Independent variable = mg_kg_d
   Slope parameter is restricted as slope >= 1

   Total number of observations = 3
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008

Parameter Convergence has been set to: 1e-008




   User has chosen the log transformed model


                 Default Initial Parameter Values
                    background =            0
                     intercept =     -4.31854
                         slope =            1


          Asymptotic Correlation Matrix of Parameter Estimates


          ( *** The model parameter(s)  -background    -slope
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

             intercept

 intercept           1




                         Parameter Estimates

       Variable          Estimate            Std. Err.
     background                 0               NA
      intercept           -4.57634          0.206535
          slope                 1               NA

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.




                        Analysis of Deviance Table


       Model      Log(likelihood)  Deviance  Test DF     P-value
     Full model        -67.3348
   Fitted model        -68.3269       1.98424      2          0.3708
  Reduced model        -252.916       371.162      2         <.0001

          AIC:         138.654



                     Goodness  of  Fit
```

Dose	Est._Prob.	Scaled Expected	Observed	Size	Residual
0.0000	0.0000	0.000	0	5000	0
54.3000	0.3585	17.567	21	49	1.023
113.8000	0.5394	26.433	23	49	-0.9839

Chi-square = 2.01 DF = 2 P-value = 0.3654

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 10.7953

BMDL = 7.69436