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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)

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U.S. Environmental Protection Agency
Washington, DC

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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 202-566-1676.

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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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Summaries of the external peer reviewers' comments and the disposition of their recommendations are in Appendix A.

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 $µg/m^3$ 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 $-methylnaphthalene. Certain physical and chemical properties are shown below (ATSDR, 1995; CRC, 1990).

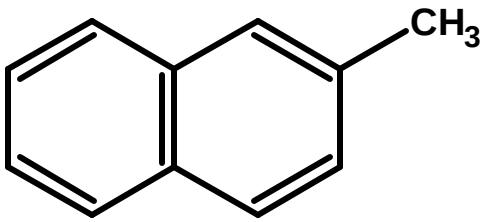

Figure 1: 2-Methylnaphthalene

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-methyl-naphthalene and one-third 1-methylnaphthalene (CASRN 90-12-0). Methylnaphthalene is manufactured from coal tar through the extraction of heteroaromatics and phenols. Distillation of methylnaphthalene removes 1-methylnaphthalene, leaving 2-methylnaphthalene. 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 (approximately 70-80% within 48 hours in guinea pigs and 55% in rats), primarily as urinary metabolites (Melancon et al., 1982; Teshima et al., 1983).

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, or 48 hours after exposure and radioactivity was measured in various organs and tissues. The amount of the radiolabel detected outside the gastrointestinal tract (i.e., various internal organs, blood, and urine) 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. 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. However, the percentages may underestimate the actual amounts absorbed since there may be significant enterohepatic cycling (see 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 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 1.2% mixture of 2-methylnaphthalene and 1-methylnaphthalene (approximate 2:1 ratio) in acetone twice weekly for 30 weeks induced pulmonary toxicity in virtually all exposed mice (Murata et al., 1992). By comparison, oral exposure of B6C3F1 mice to 52.3 mg/kg 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), when taken together the results indicate considerable dermal absorption or significant first pass metabolism may have occurred following oral exposure to 2-methyl-naphthalene.

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. While no human distribution data are available, two animal studies that measured the 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 at 3 hours in the blood and gallbladder, and at 6 hours in all other tissues (see 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 the calculation.

Table 1. Distribution of radioactivity in guinea pigs after oral administration of 2-[^{3}H]methylnaphthalene

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

Source: Adapted from Teshima et al., 1983.

Griffin et al. (1982) administered single intraperitoneal injections of 400 mg/kg [^{14}C]-2-methylnaphthalene to male C57BL/6J mice. Groups of 4 mice were sacrificed at 0.5, 1, 3, 6, 12, or 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/mg), and lung (0.7 nmol/mg). The results demonstrate 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 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, no lesions were evident until 8 hours after injection. The authors also evaluated distribution by measurement of irreversible binding of label from [^{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). Maximum irreversible binding of 2-methylnaphthalene metabolites was observed in lung, liver, and kidney tissues at 8 hours post administration. The 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 [^{14}C]-2-methylnaphthalene (50-500 mg/kg) for the measurement of irreversible binding of label from [^{14}C]-2-methylnaphthalene to organ macromolecules. The CYP enzyme inhibitor piperonyl butoxide significantly decreased irreversible binding in the liver, lung, and kidney by approximately 70, 40, and 50%, respectively. Administration of the CYP enzyme inducer phenobarbital 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 glutathione by treatment with diethylmaleate 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. The pathway has been elucidated through the identification of urinary metabolites eliminated by laboratory animals following acute exposure (Breger et al., 1983; Teshima et al., 1983; Melancon et al., 1985), by studies measuring the effects of enzyme modulators on the toxic and biochemical changes caused by 2-methylnaphthalene exposure in mice (Griffin et al., 1982, 1983), and by *in vitro* analyses of purified enzyme preparations (microsomal fractions and recombinant enzymes) from liver, lung, and kidney tissues (Melancon et al., 1985).

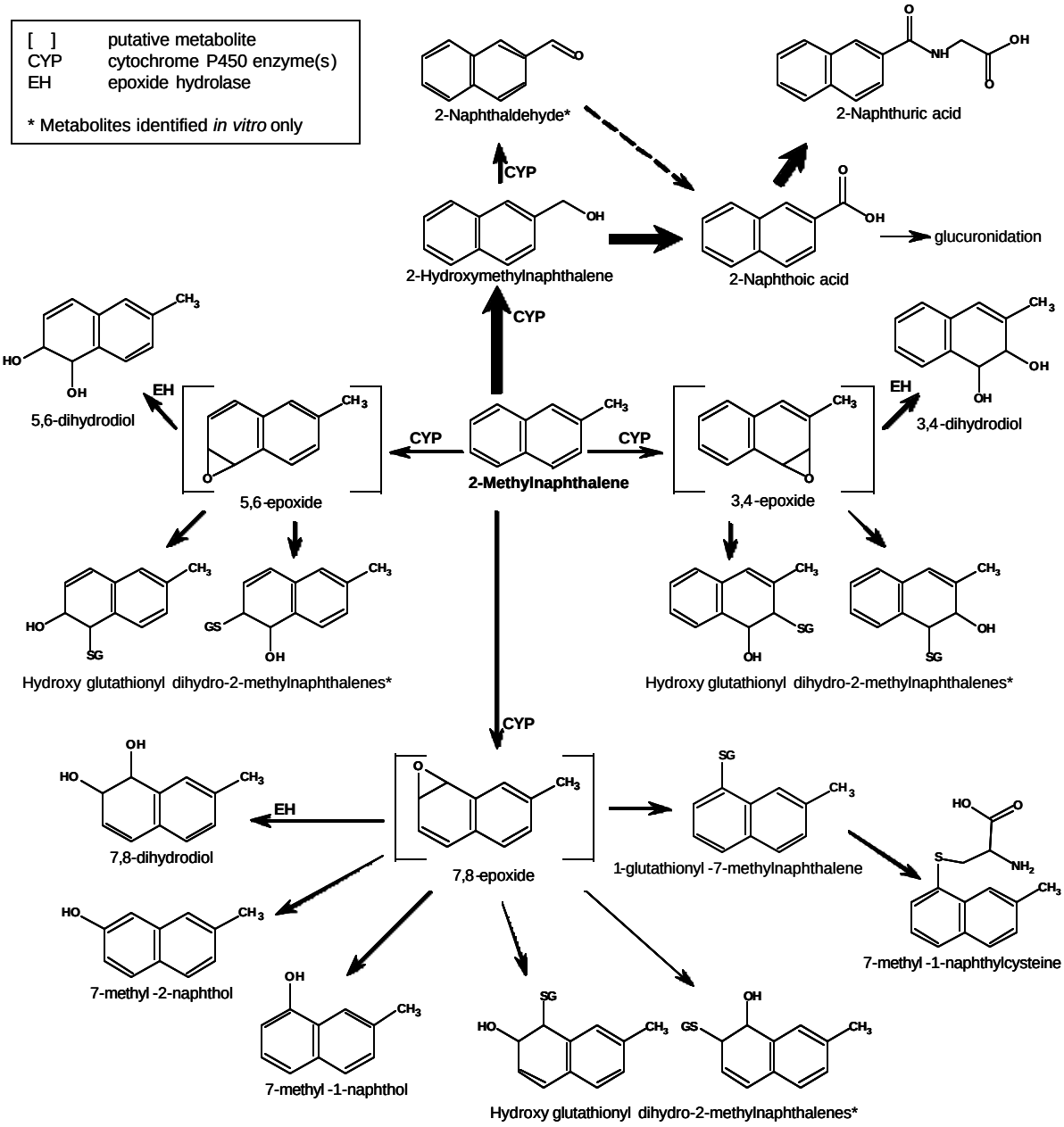

Figure 2: Metabolism of 2-methylnaphthalene (adapted from Buckpitt and Franklin, 1989; Shultz et al., 2001; Teshima et al., 1983).

CYP enzymes catalyze the first competing steps, involving 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 methyl group to produce 2-hydroxymethyl-naphthalene (Breger et al., 1983; Melancon et al., 1982; Teshima et al., 1983) which 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). CYPIIF2, or naphthalene dehydrogenase, has been shown to rapidly metabolize the structurally related chemical naphthalene (Schultz et al., 1999). Schultz et al.(2001) demonstrated that CYPIIF2 metabolizes 2-methylnaphthalene with a relatively high turnover rate (67.7 min^{-1}) and a low K_m (3.7 :m). 2-Naphthoic acid may be conjugated with glycine or glucuronic acid. Both 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. While the epoxides have not been isolated, they are proposed putative intermediates based on observed metabolites and are thought to be further oxidized by epoxide hydrolase to produce dihydrodiols (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 2-methylnaphthalene was incubated 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).

Three additional minor metabolites are 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 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 2-[^{3}H]methyl-naphthalene 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 the metabolism of 2-methylnaphthalene is rapid (approximately 55% in rats within 3 days and approximately 80% in guinea pigs within 1 day) and that 80-85% of the metabolism 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) 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). The experiments further demonstrate 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 are inhibited by heat denaturation or carbon monoxide. Other studies that measured covalent binding of label from 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 $-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) 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 inhibitor) nor diethylmaleate (depletes glutathione) had significant effects on metabolite formation (Griffin et al., 1982). Conversely, Griffin et al. (1983) observed no significant changes in the metabolism of 2-methylnaphthalene in lung and liver microsomes of DBA/2J mice by pretreatment with 3-methylcholanthrene, piperonyl butoxide, or diethylmaleate. However, 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, the data suggest that different isozymes are responsible for different steps in the metabolism of 2-methylnaphthalene and 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. The studies are further discussed in Section 4.4.3.

High pressure liquid chromatography (HPLC) was used to determine the metabolism of ^{14}C-2-methylnaphthalene in rat hepatic microsomes and purified CYP enzymes (Melancon et al., 1985). The study 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, or 24 hours post injection. Compared with controls, exposed mice showed a statistically significant decrease in levels of glutathione in the liver (32-37% reduction) 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. Results indicate that this dose of 2-methylnaphthalene led to a short-lived depletion of glutathione 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 tissue or intracellular levels of glutathione in response to exposure to high acute doses of 2-methylnaphthalene, demonstrative of glutathione conjugation (Griffin et al., 1982, 1983; Honda et al., 1990). Similarly, glutathione depletion (35% when 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 daily intraperitoneal injections of 100 mg/kg 2-methyl-naphthalene for 3 days. Endpoints measured included: O- or N-demethylation of p-nitroanisole and aminopyrene; metabolism of benzphetamine, piperonyl butoxide, pyridine, and n-octylamine; microsomal protein levels; and 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, 23.8% naphthalene, 13.3% 1-methylnaphthalene, and 0.22% indan. MROD is a measure of CYPIA2 while EROD measures CYPIA1 and IA2 enzyme activity. Doses of the mixture containing 300 mg/kg 2-methylnaphthalene did not induce lung microsomal EROD activity or hepatic MROD activity, and hepatic EROD activity was only minimally induced by doses containing 150 and 300 mg/kg 2-methylnaphthalene (2.4- and 6-fold induction, respectively).

Important differences exist in the metabolism of 2-methylnaphthalene and naphthalene (ATSDR, 1995; Buckpitt et al., 1986; Buckpitt and Franklin, 1989; NTP, 2000). CYP enzymes catalyze the initial metabolic step for both compounds, but ring epoxidation is the only initial reaction for naphthalene. 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 1-methylnaphthalene may follow a similar pathway as that described for 2-methylnaphthalene (i.e., side chain oxidation) since the chemicals are structurally related. 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-methyl-naphthalene. Melancon et al. (1982) and Teshima et al. (1983) indicate that absorbed 2-methylnaphthalene is rapidly eliminated (approximately 70-80% within 48 hours in guinea pigs and 55% in rats). Approximately 85% of the administered dose is eliminated, approximately 72% in urine, and 11-14% in feces (Melancon et al., 1982; Teshima et al., 1983). No studies are available describing the elimination of 2-methylnaphthalene through exhalation or other routes.

Table 1 shows the percent of urinary and fecal elimination of an oral dose of 10 mg/kg 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 reabsorption 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 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). The models were designed for oral, inhalation, intraperitoneal, and intravenous exposure and are 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) and naphthalene oxide is 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 examine 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. While no cataracts were found in a group of 5 weanling F344 rats fed a diet of 2% 2-methylnaphthalene (equivalent to 2,000 mg/kg-day[1]) for at least 2 months, 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, 2,000 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% 2-methylnaphthalene for 13 weeks. Estimated doses were: 0, 29.4, 88.4, 265, 794, or 2,400 mg/kg-day for males and 0, 31.8, 95.6, 287, 859, or 2,600 mg/kg-day for females, respectively. Approximate average doses (across sexes) were 0, 31, 92, 276, 827, or 2,500 mg/kg-day, respectively. The 0.147% 2-methylnaphthalene diet reduced weight gain in both sexes by 20-21%, while the 0.44 and 1.33% 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 the data is limited by inadequate reporting of study results. In this

[1]A daily dose of approximately 2,000 mg/kg, assumes 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 = 2,000 mg/kg-day of 2-methylnaphthalene.

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; 10 mice/cage) diets of 0, 0.075, or 0.15% 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, respectively. Mice were monitored daily for clinical signs of toxicity. For the first 16 weeks, food consumption and body weight were measured weekly, and 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 adrenal glands, 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. Pulmonary function was not evaluated in the control or treated groups. 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 # 0.05% was used as the threshold for statistical significance.

Both 2-methylnaphthalene and 1-methylnaphthalene were tested simultaneously under the same experimental conditions and protocols (Murata et al., 1993, 1997)[2]. A shared group of control mice (50 males and 50 females) was used in both of these studies. All dose and control groups 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 the Murata et al. (1993) study. They were also omitted

[2]Mice exposed to 0.075% or 0.15% 1-methylnaphthalene showed increased incidences of pulmonary alveolar proteinosis in males and females and 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 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.

from the later study (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). While 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 reported mean final body weights for the male and female high-dose groups were reduced by 7.5 and 4.5%, respectively, when compared with controls. The decrease in body weight was not considered to be biologically significant for the 2-methylnaphthalene assessment.

As shown in Table 2, dietary exposure to 2-methylnaphthalene was associated with a statistically significant ($p<0.05$; Cochran-Armitage trend tests performed by Syracuse Research Corporation) increased incidence of pulmonary alveolar proteinosis in male and female mice in both exposure groups, when compared with controls (Murata et al., 1997). Pulmonary alveolar proteinosis was characterized by the authors as being similar to those lesions described in an earlier study (Murata et al., 1993; 1997). According to the Murata et al. (1993) report, pulmonary alveolar proteinosis was characterized by an accumulation of phospholipids in the alveolar lumens and, upon gross inspection, white protuberant nodules approximately 1–5 mm in diameter were also observed. Histologically, there was visible filling of alveolar lumens with cholesterol crystals, foamy cells, and an amorphous acidophilic material. No prominent fibrosis, edema, alveolitis, or lipidosis were observed in alveolar walls or in epithelial cells. No evidence of bronchiolar Clara cell necrosis or sloughing was reported in the Murata et al., (1997) study nor was there histopathological evidence of non-neoplastic effects in any other tissue.

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).

Table 2. Incidence of pulmonary alveolar proteinosis in B6C3F1 mice fed 2-methylnaphthalene for 81 weeks

	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*	22/48*	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 ($p<0.05$)
Source: Adapted from Murata et al., 1997.

The authors indicated that the control non-zero incidence of pulmonary alveolar proteinosis (9/99) for males and females was unusual because pulmonary alveolar proteinosis has not appeared spontaneously in more than 5,000 B6C3F1 mice housed in the same room in the past. The appearance of pulmonary alveolar proteinosis in the control mice was similar, but 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 the inhalation of volatilized 1- or 2-methylnaphthalene due to insufficient room ventilation.

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 (immature) and segmented (mature) neutrophils were significantly decreased, and lymphocyte counts were increased when compared to controls (Murata et al., 1997). Although statistical significance was indicated for some of the effects, 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 (adenomas plus adenocarcinomas) in mice exposed to 2-methylnaphthalene. No significant differences were observed in the total tumor bearing mice between controls and 2-methylnaphthalene-exposed groups for either sex. While, the male low dose group (54.3 mg/kg-day) had a statistically

significant increased incidence of lung adenomas and total lung tumors when compared with controls, the incidence of lung tumor in the higher dose male group was not increased in a statistically significant manner. Analysis of the male total lung tumor data by the Cochran-Armitage trend test at the p # 0.05 level did not find a statistically significant trend with increasing dose (performed by Syracuse Research Corporation). The 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 not explicitly stated whether the total lung tumor incidences cited in the study refer to the number of lung-tumor bearing mice or to the number of lung tumors found in a group. The study authors also noted that the lung tumors were mostly single incidences.

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, 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 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#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. Mean latencies in the 2 highest exposure groups were higher than the control mean (statistically significant), indicating a decreased sensitivity to pain when compared with controls. 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. Respiratory rates were measured before, during, and 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 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 hematotoxicity in intact or splenectomized dogs following acute whole-body exposure to 2-methylnaphthalene. The Lorber (1972) study was conducted because an earlier unpublished study of exposure to a pyrethrin-based pesticide dissolved in a 3% mixture of methylnaphthalenes reportedly affected blood counts in intact and splenectomized dogs. Accordingly, Lorber (1972) tested the individual napthalenes to determine if they could account for the

hematotoxicity individually. 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 lasting 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[3]; therefore, no accurate exposure concentration could be estimated[4]. 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 # 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 evaluated the effects of 2-methylnaphthalene on lung histopathology, data supporting the fact that the lung is a target of 2-methylnaphthalene exposure has been provided by acute injection studies. In mice, histological changes and sloughing of Clara cells (a type of nonciliated cell that lines the bronchioles of the lungs) 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). In these studies, higher doses of 2-methylnaphthalene also produced bronchiolar and pulmonary necrosis.

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[3]Lorber (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 percent 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.

[4]Lorber (1972) reported that dogs were exposed in cages as far as possible from the fogger, in a 10 x 9 x 8 foot room. Given that 1 foot = 0.3048 meters, the volume was approximately 20 m^3. Homogenous dispersion of 30 or 100 g into 20 m^3 would have produced atmospheres of 1,000 or 5,000 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 amount 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 may have resulted in substantially reduced inhaled concentrations. The potential for dermal absorption due to deposits of the mixture on fur of the animals also exists.

Griffin et al. (1981) administered single intraperitoneal injections of 0, 0.1, 1, 10, 100, 200, 400, 600, 800, or 1,000 mg/kg 2-methylnaphthalene in corn oil to male C57BL/6J mice (5/group), with sacrifice 24 or 48 hours later. Endpoints measured included: 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. However, 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 observed in 2/5 mice and was limited to irregularities of cells lining the bronchioles, with cells present in the lumen. More severe pulmonary necrosis was seen in all mice exposed to doses $200 mg/kg, with minimal-to-prominent sloughing of nonciliated cells (Clara cells) lining the bronchioles. At 1,000 mg/kg, all mice 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 the 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, or 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. While no pulmonary necrosis was observed between 1 and 4 hours, all mice exhibited some evidence of necrosis beginning at 8 hours, 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 1,000 mg/kg 2-methylnaphthalene in corn oil and were sacrificed 24 hours later. Mortality was observed in 2/5 mice in the 1,000 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 100 mg/kg level, 2 mice showed irregularities of cells lining one or two bronchioles with sloughed 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. Prominent sloughing of bronchiolar lining cells into the lumen (scored as 3+) was observed 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 1,000 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. However, electron microscopic analysis detected bronchiolar damage at 400 mg/kg and exfoliated Clara cells in the bronchiolar lumen at 600 mg/kg. The number of animals per group was 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 in 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 (0, 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 tissues 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 or 3 mmol/kg (71 or 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 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 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 2-methylnaphthalene 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 LOAEL for bronchiolar necrosis in male Swiss Webster mice is 300 mg/kg 2-methylnaphthalene.

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 exposure.

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 (4,000 mg/kg)[5] 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 (4,200 mg/kg) for males and 3.25 mL/kg (3,180 mg/kg) for females. The same report also indicated that female New Zealand white rabbits (4/group) exposed dermally to 8.0 ml/kg (8,000 mg/kg) developed dark red lungs and blanched livers. The calculated dermal LD_{50} value for females was 5.38 mL/kg (4,660 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 119 mg/kg of a methylnaphthalene mixture 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. 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 to be filled with cholesterol crystals, an amorphous eosinophilic material, and many mononucleated giant cells with foamy cytoplasm. The alveolar spaces in areas were proteinosis was present were also filled with free myelinoid structures. The authors

[5]Based on a density of 0.978 g/ml for methylnaphthalene (NTP, 2002a). Example calculation: 4.0 ml/kg x 0.979 g/ml x 1,000 mg/g = 4,000 mg/kg.

reported that most myelinoid structures appeared to originate from hyperplastic and hypertrophic type II pneumocyte meocrine secretions. The enlarged mononucleated giant cells contained myelinoid structures similar to those observed in the alveolar space, along with lipid droplets. The myelinoid structures consisted of concentrically arranged and multilayered membranes interspersed with amorphous materials. Various numbers and sizes of needle-like crystals were also observed in the mononucleated giant cells cytoplasm. Alveolar walls were partially thickened but there was no prominent fibrosis. The thickening was due to hyperplasia and hypertrophy of type II pneumocytes, or focal hyperplasia of cells resembling type I pneumocytes in appearance. 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, twice weekly) induced a 100% incidence of pulmonary alveolar proteinosis in a shorter time period (20 weeks), but noted that this was unpublished data (Murata et al., 1992). Murata et al. (1992) stated that the incidence of pulmonary alveolar proteinosis observed in mice exposed to a methylnaphthalene mixture via the dermal route had been demonstrated in an earlier study conducted by their laboratory (Emi and Konishi, 1985).

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. 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 at 38 weeks was attributed to lipid pneumonia. Lipid pneumonia was observed (in animals that died) as early as 10 weeks in which Emi and Konishi (1985) described the condition as severe. 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 was also observed but was considered a compensatory reaction by the authors.

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 observed in the lungs of all exposed animals, but not in controls. Exposure to the 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 containing 2-ring PAHs (consisting of 38.3% 2-methylnaphthalene, 39.3% naphthalene, 22.0% 1-methylnaphthalene, and 0.36% indan). Two days later, the mice were challenged with injections of T-cell-independent or T-cell-dependent antigens (trinitrophenyl-lipopolysaccharide [TNP] or TNP-haptenated sheep red blood cells, respectively). Mice were sacrificed 2 days after the challenge. Levels of serum anti-TNP IgM and the ability of spleen cells to form plaque in the presence of the administered antigen and complement were measured as a determinant of immune function. Decreased plaque formation following the 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, 3-ring, and $4-ring PAHs. The 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. Other Cancer Studies

No evidence of cocarcinogenic activity was found in female ICR/Ha Sprague-Dawley mice (30/group) dermally exposed to 0 or 25 :g (32 :g/kg-day) 2-methylnaphthalene plus 300 ng of

benzo[a]pyrene (BaP) in acetone 3 times per week for 78 weeks (Schmeltz et al., 1978). While negative (acetone only) and positive (BaP plus 12-o-tetradeconoyl phorbol-13-acetate) controls were included, 2-methylnaphthalene was not tested alone. Compared to positive controls, the exposure 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) regarding the number of tumor-bearing animals were found with mixtures of BaP with naphthalene, 1-methylnaphthalene, 1,2-dimethylnaphthalene, 2-ethylnaphthalene, or the naphthalene-fraction of cigarette smoke.

4.4.4. Genotoxicity Studies

No genotoxicity studies in humans or animals are available. No studies investigating potential germline mutations are available. Data from *in vitro* short-term tests 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).

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 fractions produced statistically significant increases in the incidence of sister chromatid exchanges (#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 observed 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. They also considered the chromatid breaks to be minor because no damage was observed at concentrations #2 mM.

In vitro assays in WB-F344 rat liver epithelial cells indicated that 2-methylnaphthalene, as well as naphthalene and 1-methylnaphthalene, inhibits gap junctional intercellular communication (Weis et al.,

1998). The inhibition of intracellular communication has been postulated by the authors to be an epigenetic mechanism of tumor promotion by preventing intercellular transport of regulatory molecules.

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). While the experiment provides suggestive evidence that 2-methylnaphthalene may interact with DNA (even in the absence of metabolic activation), 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. A number of studies in laboratory animals indicate pulmonary toxicity following exposure to 2-methylnaphthalene. One 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 when 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 22/48 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). Pulmonary function in the control and exposed mice was not measured in this study. The findings indicate that the alveolar region of the lung is the critical target of chronic oral exposure to 2-methylnaphthalene.

In addition to pulmonary alveolar proteinosis, other effects observed in the study included changes in brain and kidney weights, blood variables (decreased differential counts of neutrophils and increased lymphocytes), and serum levels of neutral fat, total lipids, and phospholipids (Murata et al., 1997). The biological significance of these 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. The authors proposed that additional research is warranted to determine whether the elevated serum levels of fat is related to the induction of pulmonary alveolar proteinosis or is a subsequent effect of this condition. The authors were contacted and the data on these variables was requested. The authors provided the data concerning the number of neutrophils and lymphocytes along with the serum levels of fats and lipids. The authors did not provide brain and kidney weight data. Currently, no U.S. EPA guidance exists detailing the biological significance of changes in immunological parameters and their use as critical effects. Thus, the altered number of neutrophils and lymphocytes was deemed inappropriate for use as the critical effect. However, it should be recognized that decreased neutrophils, also referred to as leukopenia, may alter immune function and promote infection. In addition, altered activity of the hematopoietic growth factor, granulocyte-macrophage colony stimulating factor (GM-CSF), a growth factor responsible for the proliferation and differentiation of neutrophils and macrophage lineage hemopoietic cells, has been suggested to be involved in the pathogenesis of pulmonary alveolar proteinosis in humans (Mazzone et al., 2001). Finally, there was no definitive evidence indicating whether changes in the serum levels of neutral fats, lipids, and phospholipids are related to the induction of pulmonary alveolar proteinosis or are a result of this condition following exposure to 2-methylnaphthalene.

The oral toxicity data base for 2-methylnaphthalene is sparse (Table 3). A poorly reported study in rats that found no evidence for cataracts after $2 months exposure to 2,000 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 when 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 0.15% 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-methyl-naphthalene 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; 2,000 mg/kg-day	2,000 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 2,500 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 2,500 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 examining 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). Murata et al. (1992) stated that their results were consistent with those previously observed in their laboratory (Emi and Konishi, 1985). Emi and Konishi (1985) identified lipid pneumonia in 0/4 control mice and in 3/11 mice treated dermally with 29.7 mg/kg doses of a methyl-naphthalene mixture for 61 weeks. Emi and Konishi (1985) also observed lipid pneumonia in 31/32 female B6C3F1 mice exposed to 118.8 mg/kg doses of a methylnaphthalene mixture applied dermally twice per week for 61 weeks. The authors of the Murata et al. (1992) study were contacted concerning the discrepancy in the classification of these similar lesions following dermal exposure to methylnaphthalene mixtures (i.e., lipid pneumonia versus pulmonary alveolar proteinosis). The authors stated that the lesions seen in both dermal studies with a mixture of methylnaphthalenes were basically the same and that during the earlier study they were

unfamiliar with such lesions and wavered on their classification. The authors also indicated that in studies subsequent to Emi and Konishi (1985), where consistent and similar pulmonary effects were observed (Murata et al., 1992, 1993, 1997) following either dermal exposure to a mixture of

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		29.7 or 118.8 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.

methylnaphthalenes or dietary exposure to 2-methylnaphthalene, they determined that pulmonary alveolar proteinosis was a more appropriate description of pulmonary toxicity. A subsequent study

reported that 119 or 238 mg/kg of methylnaphthalene, applied twice weekly (dermal) to female B6C3F1 mice for 50 weeks, produced changes in lung lipid contents that were indicative of lipid pneumonia (Taki et al., 1986).

Lipid pneumonia is characterized by inflammation and fibrotic changes in the lungs resulting from the inhalation of oils or fatty substances (exogenous lipid pneumonia) or the accumulation of endogenous lipid material, typically cholesterol and/or lipids (endogenous lipid pneumonia). Lipid pneumonia often develops from obstructive pneumonitis and typically is observed in the vicinity of lung tumors. The disorder is characterized by the alveolar accumulation (without conclusive evidence of the involvement of epithelial cells) of foamy macrophages that contain lipid droplets in their cytoplasm. While lipid pneumonia and pulmonary alveolar proteinosis usually occur independently, they are often observed simultaneously. The disorders correspond to two separate and morphologically distinct presentations of lipid accumulation in the alveoli of the lung. Pulmonary alveolar proteinosis is characterized by the accumulation of lamellar bodies in the alveoli (described later in Section 4.5.1.). The lamellar bodies are composed of apoproteins and lipids that appear to be surfactant related. Few foamy macrophages are present in the alveoli. Altered function of the type II pneumocytes (epithelial cells of the pulmonary alveoli) is believed to be involved in the development of pulmonary alveolar proteinosis (Mazzone et al., 2001; Seymour and Presneill, 2002). In contrast, lipid pneumonia is characterized by the accumulation of foamy macrophages that are filled with lipid droplets. There is suggestive, but not definitive evidence that type II pneumocytes may be involved in the development of lipid pneumonia (Sulkowska et al., 1997; Sulkowski and Sulkowska, 1999). Specifically, Sulkowska et al. (1997) and Sulkowski and Sulkowska (1999) found evidence of type II pneumocyte proliferation in late stage or fully advanced forms of lipid pneumonia, but not in the early stages of this disorder upon histological examination of lung fragments from patients with non-small cell lung carcinomas and in rodents administered cyclophosphamide intraperitoneally to induce lung damage. Thus, it is unclear whether lipid pneumonia and pulmonary alveolar proteinosis share a common pathogenesis or etiology.

The suggested mode of action in animals 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 needle-like crystals. Murata et al. (1992) hypothesized that, in response to a mixture containing 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 methyl-naphthalene 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 (also referred to as alveolar lipoproteinosis, alveolar phospholipidosis, alveolar proteinosis, and pulmonary alveolar lipoproteinosis) is a disorder in humans that is characterized by the accumulation of surfactant lipids and proteins in the alveoli. The condition develops most commonly between the ages of 20-50 and more often in males then females (3:1, respectively) and in smokers when compared to nonsmokers. The primary symptom associated with this condition is dyspnea that may be accompanied with cough. Altered serum lactate dehydrogenase (LDH) levels have been observed in few patients. Patients examined physically may appear normal, but may have nonspecific pulmonary symptoms such as sporadic reduction in diffusing capacity to modest reduction in vital capacity. In the majority of cases, pulmonary alveolar proteinosis is diagnosed by the presence of a milky bronchiolar lavage fluid containing large amounts of granular acellular eosinophilic proteinaceous material with abnormal foamy macrophages filled with periodic acid-Schiff base (PAS) positive intracellular material. Upon examination of the bronchiolar lavage fluid by electron microscopy, concentrically laminated phospholipid structures, known as lamellar bodies, may be present and are used to confirm pulmonary alveolar proteinosis. Histopathologically, pulmonary

alveolar proteinosis is diagnosed by the almost complete filling of the alveolar space with PAS positive surfactant material while the architecture of the alveoli is well preserved. 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). During the whole lung lavage procedure, the patient is anesthetized and intubated. While one lung is ventilated the other is lavaged with saline. The lung is infused with 3-5 mL increments of saline until the drained effluent is clear. The second lung is either lavaged the same day or 3-7 days later. Persons with pulmonary alveolar proteinosis may require several whole lung lavage treatments for recovery, 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 few incidence of reported death (Mazzone et al., 2001). In addition, cases of this condition (approximately 8% of patients as reported in the available literature) 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*, *Nocardia*, or *Mycobacterium* is the major complication associated with this condition.

It was initially suggested that the occurrence of pulmonary alveolar proteinosis in humans was the result of inhaled irritant particles. However, inhalation toxicity studies in animals failed to produce the clinical features associated with pulmonary alveolar proteinosis and human lung biopsy samples did not contain actual particulate matter. Advances in the understanding of the pathogenesis of pulmonary alveolar proteinosis in humans have led to the realization that there are three distinct forms of this condition (primary acquired, secondary, and congenital pulmonary alveolar proteinosis) each of which have similar histologic presentations. Approximately 80% of pulmonary alveolar proteinosis cases occur as the primary acquired disorder of unknown etiology, and are not associated with a familial predisposition. 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 GM-CSF which is responsible for the transformation of monocytes into mature macrophages in the lungs (Shah et al., 2000; Mazzone et al., 2001; Seymour and Presneill, 2002). These mature macrophages

degrade surfactant. Altered activity of the growth factor (GM-CSF) may be due to the production of a neutralizing antibody to GM-CSF. The inhibition of GM-CSF activity leads to immature macrophages, undegraded surfactant, and surfactant buildup in the lung (Shah et al., 2000; Mazzone et al., 2001; Seymour and Presneill, 2002).

In rare instances, several underlying conditions such as lysinuric protein intolerance, acute exposure to silica dust or other inhaled environmental or industrial chemicals, immunodeficiency disorders, malignancies and hematopoietic disorders rarely lead to the development of secondary acquired proteinosis in humans. The low occurrence of pulmonary alveolar proteinosis following exposure to silica and other inhaled environmental and industrial chemicals, reported in a few selected case studies is most likely due to improved occupational health and safety standards (Seymour and Presneill, 2002).

Congenital pulmonary alveolar proteinosis 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). Since whole lung lavage is difficult to perform on neonates, the most promising treatment for infants is lung transplantation (Seymour and Presneill, 2002; Vaughan and Zimmerman, 2002). Difficulties associated with whole lung lavage are magnified by the difficulty in passing the intubation tube and instruments used to perform the lavage through the glottis of infants. Children that develop pulmonary alveolar proteinosis later in life generally require repeated lavage treatments, but have greater chance of survival (Seymour and Presneill, 2002; 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 children that are homozygous recessive for this disorder (Seymour and Presneill, 2002).

It is unknown whether 2-methylnaphthalene by 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-methyl-naphthalene alone at comparable doses (Murata et al., 1997), suggests that first pass hepatic metabolism associated with oral exposure may limit parent compound 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 Section 3.3.).

It is evident that the primary effect of 2-methylnaphthalene exposure is pulmonary toxicity. However, it is unknown whether 2-methylnaphthalene by itself or one or more of its metabolites are responsible for the development of pulmonary alveolar proteinosis. Several metabolism studies have evaluated the effect of CYP enzyme inducers and inhibitors or glutathione depletion on 2-methylnaphthalene-induced toxicity. The studies provide equivocal evidence indicating whether 2-methylnaphthalene or potentially reactive metabolites are responsible for lung toxicity. For example, as described in Section 3.2., Griffin et al. (1982) pretreated male C57BL/6J mice with 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. None of the pretreatments alone nor any of the pretreatments plus 200 mg/kg-day 2-methylnaphthalene resulted in pulmonary toxicity or lethality when compared to controls. Exposure to 400 mg/kg 2-methylnaphthalene alone resulted in the induction of bronchiolar necrosis in all exposed mice compared to controls (Griffin et al., 1982). On the other hand, pretreatment with the CYP enzyme inducers phenobarbital and 3-methylcholanthrene appeared to provide some protection from 2-methylnaphthalene-induced pulmonary toxicity, indicating that 2-methylnaphthalene, rather than its metabolites, were responsible for the toxicity.

In contrast to the effects observed in C57BL/6J mice (Griffin et al., 1982), pretreatment of male DBA/2J mice with the same CYP enzyme inducers or inhibitors prior to intraperitoneal exposure to 2-methylnaphthalene (as described in Sections 3.2. and 4.4.1.) did not decrease the severity of 2-methylnaphthalene-induced bronchiolar lesions (Griffin et al., 1983).

Griffin et al. (1982) suggested that glutathione conjugation of reactive metabolites may play a detoxifying role in response to the acute toxicity of 2-methylnaphthalene. Pretreatment of male C57BL/6J mice (5/group) with 625 mg/kg diethylmaleate (to deplete glutathione) 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 2-methylnaphthalene. Pretreatment with diethylmaleate (600 :l/kg) 1 hour prior to injection caused extensive sloughing and exfoliation of bronchiolar epithelial cells in all animals (5/5) (Honda et al., 1990).

These observations suggest that metabolism of 2-methylnaphthalene may play a role in the pathogenesis of pulmonary alveolar proteinosis in type II pneumocytes.

Additional evidence on whether 2-methylnaphthalene or its metabolites are responsible for lung toxicity comes from intraperitoneal injection studies with 2-methylnaphthalene (Table 5). Castranova et al. (1988) noted that two types of cells in the lung (type II pneumocytes in the alveoli and the nonciliated Clara cells lining the bronchioles) exhibit substantial CYP enzyme activity and are expected to metabolize foreign chemicals. Both cell types are secretory and 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 appeared to target the type II pneumocytes, inducing pulmonary alveolar proteinosis (Murata et al., 1992, 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 (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 the possible involvement of reactive metabolites produced via CYP enzymes, but the mode of action at the molecular level has not been elucidated and 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 1,2-epoxides 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).

Findings from mode of action studies regarding the acute response in mice to intraperitoneal injection with 2-methylnaphthalene support the understanding 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). The 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 nontoxic initial dose of 200 mg/kg naphthalene for 7 days made the Clara cell lining of the bronchioles more resistant to a subsequent dose of 300 mg/kg naphthalene compared with mice given only 300 mg/kg naphthalene without pretreatment (Lakritz et al., 1996). The authors suggested that reduced expression of CYPIIB, CYPIIF, CYP reductase, and secretory protein led to this increased resistance in the Clara cells. However, the possible development of Clara cell resistance to the acute toxicity of 2-methylnaphthalene has not been studied.

There are limited data to suggest that rats may be less sensitive than mice to lung damage caused by acute exposure to 2-methylnaphthalene. Wistar rats given intraperitoneal doses of 140 mg/kg 2-methylnaphthalene did not lead to pulmonary toxicity (Dinsdale and Verschoyle, 1987). In contrast, bronchiolar necrosis was induced in Swiss-Webster mice injected with approximately 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). The 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 (1,000 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 exposure 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.

Several acute inhalation toxicity studies are available. Signs of nervous system depression were observed in rats exposed for 4 hours, and a transient decrease in respiratory rate was observed in mice exposed for 6 minutes (Korsak et al., 1998). No signs of hematotoxicity in dogs were found after exposure to mists of an unknown concentration of 2-methylnaphthalene for 50 minute periods over 4 consecutive days (Lorber, 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).

4.5.3. Dermal Exposure

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-methylnaphthalene, which induced pulmonary alveolar proteinosis in roughly half of exposed animals within 81 weeks at approximately equal administered dose levels (Murata et al., 1992, 1997). The findings suggest that first pass hepatic metabolism associated with oral exposure may limit the amount of parent material reaching the lung. 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. It should be noted that the higher incidence of disease following dermal exposure to methylnaphthalene mixtures may be partly attributed to increased absorption of methylnaphthalene in an acetone solution rather than methylnaphthalene in an aqueous solution.

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 EPA's 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-methyl-naphthalene in humans are available. Animal cancer bioassays are limited to an 81-week dietary study (Murata et al., 1997). Murata et al. (1997) observed a statistically significant increase in the incidence 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 50.3 or 107.6 mg/kg-day 2-methylnaphthalene. 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 study (Murata et al., 1997) was conducted in conjunction with a study testing 0.075 and 0.15% 1-methyl-naphthalene 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. While Murata et al. (1993, 1997) did not quantitate the concentration of 1- or 2-methylnaphthalene in the air, it should be noted that 2-methylnaphthalene is slightly more volatile than 1-methylnaphthalene (vapor pressure of 0.068 and 0.087 mm Hg, respectively). Potential confounding from possible inhalation exposure to 1- and 2-methylnaphthalene adds some uncertainty to the relationship between oral exposure to 2-methylnaphthalene and the increased incidence of lung tumors. Historical controls for B6C3F1 male and female control mice typically develop lung adenomas and carcinomas spontaneously at an incidence of 19-24.8% and 7-8.5%, respectively (NTP, 2002b, c).

In addition, in a skin painting study where female mice were exposed to 2-methylnaphthalene (equivalent to 32 :g/kg-day) plus BaP for 78 weeks, 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%) when compared to mice treated only with BaP (Schmeltz et al., 1978). In this study 2-methylnaphthalene was not tested alone. The statistical significance of these findings could not be determined from the data presented. The incidences of non-skin tumors were also not reported. No mutagenicity was observed in tests using *Salmonella typhimurium* or cultured human lymphocytes (Florin et al., 1980; Hermann, 1981; Kopper Co. Inc., 1982; Kulka et al., 1988).

There are no data indicating that the metabolism of 2-methylnaphthalene and the structurally-related 1-methylnaphthalene are similar. This lack of information precludes the use of evidence for 1-methylnaphthalene carcinogenicity (see Footnote 3, Section 4.2.1.2) as supporting evidence for 2-methylnaphthalene carcinogenicity. It should be noted that evidence of carcinogenicity of the structurally-related PAH 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, 1998c) and, thus, the use of naphthalene carcinogenicity data as supporting evidence for 2-methylnaphthalene carcinogenicity is of limited value.

4.6.3. Mode of Action Information

The mode of action for tumor formation in mid-dose male mice in the Murata et al. (1997) study is not known. 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). In addition, the available data do not support the hypothesis that pulmonary alveolar proteinosis might be a precursor 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 lung tumors following exposure to 1-methylnaphthalene were not statistically correlated, and the sites of development of alveolar proteinosis and lung tumors were 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, neonatal, or postnatal animals.

When compared to adults, infants and children who develop pulmonary alveolar proteinosis have more severe symptoms and a poor prognosis for survival. Whole lung lavage, the standard treatment for this disorder, is unavailable to infants because of the difficulty in performing intubation and lavage techniques on their small airways. In addition, studies in children with pulmonary alveolar proteinosis provide suggestive evidence that congenital deficiencies in the 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 in children (Ginsberg et al., 2002). Studies in animals indicate an equivocal effect of metabolism on the toxicity following 2-methylnaphthalene exposure (Griffin et al., 1982, 1983; Honda et al., 1990). The importance and identity of the specific isozymes and/or metabolites responsible for these adverse effects are not well understood.

4.7.2. Possible Gender Differences

Gender-specific susceptibility to 2-methylnaphthalene toxicity is not known. While clinical cases of pulmonary alveolar proteinosis are 3-fold more common in men than in women (Mazzone et al., 2001), 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 (4,310 and 3,270 mg/kg, respectively) and dermal studies in rabbits calculated LD_{50} values of 6.1 mL/kg in males and 4.8 mL/kg in females (6,130 and 4,790 mg/kg, respectively). Although no significant differences in the incidence of pulmonary alveolar proteinosis were observed between male and female B6C3F1 mice given equivalent dietary doses of 2-methylnaphthalene for 81 weeks, only exposed male (rather than female) mice showed an increased incidence of lung tumors (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 include persons with myeloid leukemias, pulmonary infection, a history of smoking, or inhalation of silica or certain heavy metals may be more susceptible (Mazzone et al., 2001; Seymour and Presneill, 2002; Wang et al., 1997).

5. DOSE RESPONSE ASSESSMENT

5.1. ORAL REFERENCE DOSE (RfD)

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

No epidemiology studies or case reports are available which examine 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-methyl-naphthalene (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. Numerous endpoints were evaluated, including histology for more than 24 tissues, hematology, and serum chemistry. Pulmonary function was not evaluated in either control or exposed mice. Mean growth curve data showed reduced weight gain in males at both doses and in females exposed to the high dose. While a statistically significant reduction in final body weight was observed only in the high-dose male group, the decrease was not considered a biologically significant effect in this assessment. Several other statistically significant differences in blood parameters and organ weights between control and exposure groups were reported. However, the biological significance of these differences is unclear because no data were provided regarding the magnitude of the response 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. A statistically significant increase in the incidence of pulmonary alveolar proteinosis was observed 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, 27/48, and 22/48 for females, respectively. No other incidence of non-neoplastic effects were identified in exposed groups of male and female mice. For these reasons, pulmonary alveolar proteinosis is chosen as the critical effect.

The 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 both 2- and 1-methylnaphthalene, in an approximate 2:1 ratio, respectively. All female B6C3F1 mice

dermally exposed to 119 mg/kg methylnaphthalene twice weekly for 30 weeks, or 238 mg/kg twice weekly for 20 weeks exhibited pulmonary alveolar proteinosis (Murata et al., 1992). Similarly, a 61-week study reported lipid pneumonia in B6C3F1 mice dermally exposed to 118.8 mg/kg-day methylnaphthalene mixture (Emi and Konishi, 1985).

Other available oral toxicity studies of 2-methylnaphthalene are of prechronic duration. Fitzhugh and Buschke (1949) conducted a study on cataract formation in rats fed 2-methyl-naphthalene for at least 2 months. No cataracts were observed and no other endpoints were studied. Murata et al. (1997) conducted a range-finding study in which groups of B6C3F1 mice (10/sex/group) were fed diets containing approximate average daily doses of 0, 31, 92, 276, 827, or 2,500 mg/kg-day 2-methylnaphthalene for 13 weeks. No histopathological effects were observed in tissues and organs of male or female mice exposed to 827 or 2,500 mg/kg-day. Decreased weight gain was observed 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 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.

Although Rasmussen et al. (1986) reported minimal liver damage in male Swiss-Webster mice injected with 142 or 284 mg/kg 2-methylnaphthalene, no histological evidence of liver or kidney damage was seen in male C57BL/6J (Griffin et al., 1981, 1982) or male DBA/2J mice (Griffin et al., 1983) at doses up to 1,000 mg/kg. An acute dose of 1,000 mg/kg 2-methylnaphthalene was frankly toxic, as evidenced by mortality observed in 3/10 mice dosed at this concentration (Griffin et al., 1981, 1983).

A limitation of the principal study by Murata et al. (1997) is the occurrence of pulmonary alveolar proteinosis in control mice. The authors described the condition as being less pronounced but similar to the adverse lung effects observed in the 2-methylnaphthalene exposed mice. The authors also indicated that pulmonary alveolar proteinosis had not been observed in more than 5,000 B6C3F1 control mice, and speculated that the background incidence may have been elevated by the inhalation of volatilized test chemicals and poor room ventilation. The study was conducted in conjunction 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). While Murata et al. (1993, 1997) did not quantitate the concentration of either 1- or 2-methylnaphthalene in the air, it should be noted that 2-methylnaphthalene is slightly more volatile than 1-methylnaphthalene (vapor pressure of 0.068 mm Hg compared to 0.087 mm Hg, respectively). Potential confounding from possible inhalation exposure to 1- and 2-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

The data were analyzed using benchmark dose (BMD) modeling for the derivation of the point of departure. Based on the Murata et al. (1997) study, the critical effect is pulmonary alveolar proteinosis. 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 from possible inhalation exposure to 1- and 2-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. No data were found which characterize the response of mice to either chemical alone by inhalation. Since the incidence of pulmonary alveolar proteinosis was reported to be unusually high compared with historical controls, it may not be a relevant baseline. Moreover, the similar degree of pulmonary alveolar proteinosis in the two exposed groups, both averaging about 45%, provides 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 an RfD from these data, as discussed below.

Given the possible simultaneous inhalation exposure to 1- and 2-methylnaphthalene during the oral exposure study, consideration of concurrent and historical control information may provide some bounds on the degree of effects that can be associated with oral exposure to 2-methylnaphthalene. 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. There is no reason to believe that the control group was treated any differently than the exposure groups in the principal study. Even if there were secondary exposure to volatilized test materials and it can be assumed that all animals

were similarly exposed, the concurrent control group provides the most relevant baseline for assessing adverse effects in the exposed groups.

Alternatively, use of the historical control information may provide an upper bound on the magnitude of effects associated with oral exposure to 2-methylnaphthalene. Several hypothetical situations can be used to characterize the contribution of 1-methylnaphthalene to the observed effects. In the simplest case, if coexposure 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 has no association with pulmonary alveolar proteinosis. The animals exposed simultaneously to 1-methylnaphthalene in their diet demonstrated incidences of pulmonary alveolar proteinosis similar to that of the 2-methylnaphthalene-exposed animals (see Footnote 2, Section 4.2.1.2.), indicating that oral 1-methylnaphthalene exposure is associated with pulmonary alveolar proteinosis. Therefore, use of the concurrent control is important to adjust for any effect of simultaneous 1-methylnaphthalene exposure. If the effect of 1-methylnaphthalene is additive and constant across the experimental groups, the concurrent control incidence effectively accounts for this. If, however, 1-methylnaphthalene and 2-methylnaphthalene interact, then the concurrent control incidence may be a low estimate of the contribution of 1-methylnaphthalene in the groups purposely exposed to 2-methylnaphthalene. In that case, use of the concurrent control is more relevant than the historical control, but would still lead to an overestimate of the effect attributable to 2-methylnaphthalene at a given dose level, due to underestimating the contribution of 1-methylnaphthalene in the 2-methylnaphthalene-exposed groups. In other words, the benchmark dose (BMD) estimate would be lower than it should be. Without data to clarify whether there is an interaction (or how large it may be), there is no way to estimate the impact on the BMD.

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 accounts for any

additive effects of simultaneous inhalation exposure to 1-methylnaphthalene in this study. If simultaneous inhalation exposure to 1-methylnaphthalene is associated with an interactive effect with 2-methylnaphthalene, use of the concurrent control would also provide a high-end estimate of the risk of pulmonary alveolar proteinosis at a given exposure level. Both control groups are considered below in characterizing the point of departure.

Both commonly used approaches for identifying a point of departure for low-dose extrapolation, the LOAEL/NOAEL methodology and BMD modeling, have some relevance for this data set. The LOAEL/NOAEL methodology is not as dependent on the level of response in the control group as BMD modeling, as long as the response level in the exposed group is significantly different from the control. 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 groups. In addition, the similarity of responses in the orally exposed groups has very little impact on identifying the LOAEL. The NOAEL/LOAEL approach would yield a LOAEL of 52.3 mg/kg-day, using the combined male and female data.

On the other hand, BMD modeling can provide a point of departure which is consistent with more of the observed data than the LOAEL/NOAEL approach uses, by 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. 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. The incidence data for males and females were fit using all dichotomous variable models available in the BMDS Version 1.3.2. software (U.S. EPA, 2002); the results are shown in Appendix B. Note that the incidence of pulmonary alveolar proteinosis for male and females were not different or statistically significant from each other (p #0.05, using Fisher's exact test), indicating neither sex was clearly more sensitive. Consequently, the BMD modeling analysis also considers the combined incidences for each exposure group to strengthen the quantitative results.

Much of the BMD modeling did not provide adequate fits, as indicated by chi-square goodness-of-fit p-values less than 0.1 (see Appendix B). None of the models fit the female mouse data well, nor the combined female and male data, due to the non-monotonic response pattern in female

mice. For the male mouse data, the application of the log-logistic model provided the best fit, as indicated by the lowest Akaike Information Criterion (AIC) among the models with adequate fits ($p>0.1$), according to the criteria in the draft BMDS guidance (U.S. EPA, 2000c). The BMD_{10} and BMD_{05} from the log-logistic model were 13.7 and 6.9 mg/kg-day, respectively, 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 BMD_{10} and BMD_{05} (i.e., $BMDL_{10}$ and $BMDL_{05}$) were 9.1 and 4.5 mg/kg-day, respectively. However, this model does not fit the data well since the largest deviation in the fit occurs at the low dose response where it is especially important to have an adequate prediction. Also, even though the male and female responses were not different or statistically significant from each other, the female response (55%) was somewhat higher than the male response (43%) at the low dose (see Table B1). The biological significance of this difference is not clear, especially because of the uncertain nature of the background incidence of pulmonary alveolar proteinosis in this study, as noted earlier (see Sections 5.1.1. and 5.1.2.). Therefore, reliance on the combined male and female incidence data appears to be the most appropriate approach.

Another approach to fit the low dose responses observed in male and female mice is to exclude the high dose groups from the combined data set. This practice is justified by the following considerations (U.S. EPA, 2000c). Without a mechanistic understanding of how pulmonary alveolar proteinosis results from exposure to 2-methylnaphthalene, data from exposures much higher than that associated with the benchmark response do not provide very much information about the shape of the response in the region of the benchmark response. The lack of fit for the full data set appears to be due to the characteristics of the high dose groups, where the response plateaus. Although dropping the high dose groups ignores some of the data and decreases the degrees of freedom for modeling, it is a reasonable approach because the focus of BMD analysis is on the low dose and response region (U.S. EPA, 2000c). The BMDS quantal-linear model provides a model that is closest to a straight line, which is all that can be justified for modeling essentially two data points (the combined control groups and the similar male and female low dose groups). The resulting model parameters are provided in Appendix B. Note that goodness-of-fit measures are irrelevant in this case, since a straight line is defined by two points.

A benchmark response level of 5% extra risk of the critical effect, pulmonary alveolar proteinosis, was selected for this assessment. This effect is similar to a disorder of unknown etiology

that has been identified in humans. If this disorder were to occur in humans following exposure to 2-methylnaphthalene, it is anticipated that children may be more susceptible especially since children affected with the disorder often experience more severe symptoms than adults. Thus, a 5% extra risk of pulmonary alveolar proteinosis was judged to be an appropriate level of extra risk for this critical effect. The BMD_{10} and BMD_{05} from the quantal-linear model was 9.6 and 4.7 mg/kg-day for pulmonary alveolar proteinosis in male and female mice exposed to 2-methylnaphthalene in the diet for 81 weeks respectively (Murata et al., 1997). The lower 95% confidence limit on the BMD_{10} and BMD_{05} (i.e., $BMDL_{10}$ and $BMDL_{05}$) was 7.3 and 3.5 mg/kg-day respectively.

Limited modeling was carried out using the reported historical incidence of 0 cases of pulmonary alveolar proteinosis in ~5,000 control mice and the combined male and female incidence data from the exposed groups. The fits paralleled those using the concurrent control, with none providing an adequate fit. The BMD_{10} was 8.2 mg/kg-day, and the $BMDL_{10}$ was 6.5 mg/kg-day. This $BMDL_{10}$ is marginally lower than the value of 7.3 mg/kg-day derived using the concurrent control group. Given the lack of information confirming any confounding exposures and whether or not an interaction would be expected, the concurrent control appears to provide the most suitable baseline for estimating the extra risk of developing pulmonary alveolar proteinosis. Therefore, the high dose groups were dropped and a quantal-linear model was fit to the low-dose male and female data, as described below.

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

Using benchmark dose modeling, the $BMDL_{05}$ of 3.5 mg/kg-day for 5% extra risk of pulmonary alveolar proteinosis in 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 $BMDL_{05}$, 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.

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. While the 5% extra response level used to derive the RfD is not a no-response level, some consideration of what level of extra risk of pulmonary alveolar proteinosis constitutes a minimal health risk is appropriate.

The RfD for 2-methylnaphthalene was calculated as follows:

$$\begin{aligned} RfD &= BMDL_{05} \div UF \\ &= 3.5 \text{ mg/kg-day} \div 1000 \\ &= 0.004 \text{ mg/kg-day} \end{aligned}$$

In addition to the uncertainties noted above, 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. As noted earlier, the responses in 2-methylnaphthalene-exposed animals suggest a continuation of the plateau into the lower exposure region, so using a linear

model may provide a higher benchmark dose than is appropriate. In addition, while BMDS was used to generate a lower bound on the estimated benchmark dose, the lower bound probably describes too narrow a confidence limit on the benchmark dose. This is because the uncertainty in the data set cannot be adequately described without the high dose responses.

In comparison, the NOAEL/LOAEL approach would yield a LOAEL of 52.3 mg/kg-day, using the combined male and female data. This dose 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 appears justified, and it might be argued that a factor of 10 is not enough. However, the default of 10 would contribute to a total UF of 10,000 given the other uncertainty factors already considered. The RfD/RfC Technical Panel Report (U.S. EPA, 2002) recommended "limiting the total uncertainty factor applied for any particular chemical to no more than 3000 and avoiding the derivation of a reference value that involves application of the full 10-fold uncertainty factor in four or more areas of extrapolation."

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 on acute exposure of animals to 2-methylnaphthalene; neither are suitable for RfC derivation. Lorber (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
observed with the response magnitude increasing with increasing exposure concentration.

An RfC for 2-methylnaphthalene cannot be derived in the absence of an inhalation study of sufficient duration that evaluates a comprehensive array of endpoints to establish a NOAEL or LOAEL. A route-to-route extrapolation is not currently possible. No toxicokinetic models are available for 2-methylnaphthalene, but 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). A statistically significant increase in the incidence of lung adenomas and total lung tumors (adenomas and carcinomas combined) for the low-dose male group (54.3 mg/kg-day) was observed when compared to controls. However, no evidence of carcinogenicity was observed in male mice exposed to the high dose (113.8 mg/kg-day) or in female mice (50.3 or 107.6 mg/kg-day). 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 the data unsuitable for quantitative assessment of carcinogenic potential. No statistically significant elevations in other tumor incidences were seen in any exposure group.

A dermal cocarcinogenicity 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 and 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 as a pyrolytic byproduct from the combustion of tabacco, wood, petroleum-based fuels and coal. 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. However, 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 (Emi and Konishi, 1985; Murata et al., 1992) was pulmonary alveolar proteinosis. This effect was 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). Since the effect is similar to a disorder of unknown etiology that has been observed in humans, it is anticipated that humans exposed to 2-methylnaphthalene may develop pulmonary alveolar proteinosis.

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 targets the lung causing 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 *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 is available (Murata et al., 1997). While the study found an 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, 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 0.004 mg/kg-day was calculated from a $BMDL_{05}$ of 3.5 mg/kg-day for 5% extra risk for pulmonary alveolar proteinosis in 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 data base deficiencies.

No data are available regarding the potential toxicity of 2-methylnaphthalene in exposed humans via the oral route and 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 developing 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 1- and 2-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 by incorporating information available for the control and high-exposure groups in addition to the LOAEL. Additional uncertainties arise when extrapolating 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 BMDS quantal-linear 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 calculating 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 A: SUMMARY OF EXTERNAL PEER REVIEW AND PUBLIC COMMENTS AND DISPOSITION

The support document and IRIS summary for 2-methylnaphthalene have undergone both internal peer review performed by scientists within EPA and a more formal external peer review performed by scientists in accordance with EPA guidance on peer review (U.S. EPA, 1998b, 2000a). Comments made by the internal reviewers were addressed prior to submitting the documents for external peer review and are not part of this appendix. The four external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. A summary of significant comments made by the external reviewers and EPA's response to these comments follows:

The reviewers made several editorial suggestions to clarify specific portions of the text. These changes were incorporated in the document as appropriate and are not discussed further.

COMMENTS FROM EXTERNAL PEER REVIEW

Overall Document Quality

Questions 1 and 2: How well were the data from individual studies characterized and are the conclusions that are drawn from each study valid? How well are the data integrated into an overall conclusion and characterization of hazard as presented in Sections 4.5, 4.6, 5, and 6?

Comment: The reviewers agreed that the data from individual studies was well characterized and properly incorporated into an overall conclusion and characterization of hazard. One reviewer noted that despite the availability of previous studies evaluating the toxicity of 2-methylnaphthalene, the data base for this chemical has deficiencies such as the lack of human exposure and carcinogenicity studies. The reviewer felt that as a result of the sparse data base, data integration in the dose-response was lacking.

Response: The absence of additional long-term carcinogenicity and inhalation studies, in addition to

deficiencies in the data base is discussed in Sections 5.1.3., 5.2., and 5.3. In addition, all available data on 2-methylnaphthalene (oral, limited inhalation, intraperitoneal studies, and studies on methylnaphthalene mixtures containing 2-methylnaphthalene) has been described in depth and integrated in the dose-response assessment.

Question 3: What is the overall quality of the document?

Comment: All four reviewers commented on the high overall quality of the document.

General Questions and Issues

Question 1: Are any other data/studies available that are relevant (i.e., useful for the hazard identification or dose-response assessment) to the assessment of the adverse health effects, both cancer and noncancer, of this chemical?

Comments: All reviewers agreed that there were no other available studies that were relevant to the hazard identification or dose-response assessment for 2-methylnaphthalene (both cancer and noncancer)

RfD Derivation

Question 1: Under Section 5.1.1., *Choice of Principal Study and Critical Effect*, the RfD is based on an 81-week study in mice fed 2-methylnaphthalene (Murata et al., 1997). The critical effect observed was pulmonary alveolar proteinosis. This study was conducted concurrently with an 81-week study in mice fed 1-methylnaphthalene (Murata et al., 1993) with a shared control group between the 1- and 2-methylnaphthalene exposure groups. All animals were housed in the same room. While the incidence of pulmonary alveolar proteinosis in controls was increased (for the particular strain of mouse utilized in both studies), the authors suggested it may have been due to volatilized 1- and 2-methylnaphthalene. Is use of the Murata et al. (1997) study justified and is the rationale for this study adequately explained in the Toxicological Review (Section 5.1.1.) in light of incidence of the critical effect in the control group?

Comments: All reviewers agreed that the use of the Murata et al. (1997) study was justified and that the rationale for this study was adequately explained in the document.

One reviewer expressed interest in why the principal study was published after a study evaluating the toxicity of 1-methylnaphthalene, even though these studies were conducted concurrently and by the same laboratory. The reviewer also questioned why the principal study was published as a short communication. The reviewer suggested that the study authors could be contacted, but that the information was not necessary for inclusion in the document. This reviewer felt that the information would be helpful in determining the confidence in the principal study.

Another reviewer noted that the possibility of interactive effects was not throughly explained in relation to the hypothesized simultaneous exposures to airborne 1- and 2-methylnaphthalene in the Murata et al. (1997) study and the subsequent incidence of pulmonary alveolar proteinosis in the control group.

Response: The principal study authors were contacted and questioned about their choices concerning the type of publication and the delay in publication of the 2-methylnaphthalene data. The authors indicated that the delay in publishing the 2-methylnaphthalene data (Murata et al., 1997) following the publication of 1-methylnaphthalene data (Murata et al., 1992) was simply a matter of time constraints. The study authors also indicated that the short communication was used as a result of the existence of an extensive and detailed publication on 1-methylnaphthalene that was conducted concurrently under the same protocol and conditions.

Although the possibility of interactive effects was not thoroughly explained in the document, an expanded discussion of this issue has been added to the document.

Question 2: Under Section 5.1.1., *Choice of the Principal Study and the Critical Effect,* the critical effect is identified as pulmonary alveolar proteinosis. Is this the correct critical effect and is it adequately described? Is this critical effect biologically significant? Finally, does the information presented from animal studies mirror what is know about the disease in humans and is this information adequately described?

Comment: All reviewers agree that pulmonary alveolar proteinosis is the correct critical effect and it

has been throughly described. The reviewers also agreed that this effect is biologically significant. One reviewer indicated that the disease state in humans had not been adequately described in terms of cell types involved in reducing pulmonary function and histopathology of pulmonary alveolar proteinosis, and indicated that it was unclear as to whether the pathology of this disorder in humans is similar to that in rodents. The reviewer also commented that it has been shown that pulmonary alveolar proteinosis may lead to decreased pulmonary function in humans. The reviewer also noted that changes in type II pneumocytes could provided a logical explanation for reduced pulmonary function in mice treated with 2-methylnaphthalene. Another reviewer requested that the relationship of the critical effect to drug-induced phospholipidosis be addressed in this section of the assessment.

One reviewer noted that the dose-response assessment focused only on the incidence of the critical effect in males and this decision should either be explained more clearly and transparently or that both the male and female incidence data should be utilized in the dose-response assessment.

Response: Section 5.5.1. has been augmented and revised for clarity. The description of the cell types involved in the pathogenesis of primary acquired and the congenital forms of pulmonary alveolar proteinosis in humans (less is known concerning the pathogenesis of the secondary acquired form of the disorder) has been more extensively discussed. This section also provides evidence that the pathogenesis of pulmonary alveolar proteinosis may be similar for rodents and humans.

The concept that, changes in type II pneumocytes offers a logical explanation for reduced pulmonary function in mice treated with 2-methylnaphthalene is somewhat equivocal. Human pulmonary alveolar proteinosis has been associated with reduced pulmonary function. This association has not been shown in rodents. In addition, pulmonary function was not an endpoint measured in the Murata et al. (1997) study.

Drug-induced phospholipidosis is a condition that develops as the result of exposure to drugs that have a cationic lipophilic structure and is characterized by the: accumulation of phospholipids in cells, appearance of lamellar inclusion bodies, accumulation of the inducing drug in association with the increased phospholipids, and reversibility of alterations after removal of exposure to the drug (Reasor and Kacew, 2001). The disorder shares some common characteristics with pulmonary alveolar proteinosis (also known as pulmonary phospholipidosis), but is not identical. While both involve the

accumulation of lipid in the alveoli, pulmonary alveolar proteinosis is not characterized by the accumulation of an inducing agent in the alveoli of the lung and there is no evidence that removal of exposure to the inducing agent is associated with reversibility of the disease. Inhalation toxicity studies in animals have failed to produce the clinical features associated with pulmonary alveolar proteinosis and human lung biopsy samples from patients with pulmonary alveolar proteinosis typically do not contain actual particulate matter (Seymour and Presneill, 2002). In addition, acute exposure to silica dust or other inhaled environmental or industrial chemicals rarely leads to the development of secondary acquired proteinosis and has only been documented in a few case studies (Seymour and Presneill, 2002).

Since it is recognized that the female mouse incidence data have utility in the assessment, incidence data for both the males and females have been considered and used in the dose-response assessment. Additional modeling and explanatory text has been added to the document.

Question 3: Under Section 5.1.2, *Methods of Analysis, Including Models,* is the point of departure determined appropriately (i.e., benchmark dose approach)? Is the 10% response level appropriate and is the use of this response level supported adequately?

Comments: All reviewers felt that the point of departure had been determined appropriately and 10% was an adequate level of response.

One reviewer commented that the discussion of choice of a benchmark dose model in relation to the shape of the dose-response curve (Appendix B) was inaccurate. The reviewer disagreed with part of the description of the dose-response characterization, that models with a concave shape were clearly not relevant for these data.

Response: The dose-response characterization in the document was intended to describe fits of particular models to the data, not that a concave shape could not be consistent with the available data. Additional language has been added to the document for clarity.

Question 4: Under Section 5.1.3, *RfD Derivation-Including the Application of Uncertainty Factors,* are the appropriate uncertainty factors applied? Is the explanation for each transparent?

Specifically, is the recommendation for not applying an effect level extrapolation factor justified adequately?

Comments: All reviewers felt that the proper uncertainty factors had been applied and were adequately justified in the assessment.

Cancer Weight-of-Evidence Designation

Question 1: The weight-of-evidence and cancer characterization are discussed in Section 4.6. Have appropriate criteria been applied from EPA's Draft Revised *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1999)?

Comment: All reviewers agreed that the data were inadequate to assess human carcinogenic potential of 2-methylnaphthalene and felt that the appropriate criteria from EPA's Draft Revised *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1999) had been applied.

One reviewer noted that while agreeing with the overall conclusion that the Murata et al. (1997) study did not provide adequate evidence for determining the carcinogenic potential of 2-methylnaphthalene, additional carcinogenicity studies should be performed in order to evaluate the possibility that 2-methylnaphthalene toxicity masks any potential carcinogenic response. The reviewer suggested that since lung tumors in mice are typically derived from type II pneumocytes and Murata et al. (1997) speculated that the type II pneumocytes may be the specific cellular target of 2-methylnaphthalene toxicity, the potential exists that 2-methylnaphthalene-induced toxicity masks the carcinogenic potential of this chemical in the mouse lung. This reviewer suggested that 2-methylnaphthalene may be carcinogenic in the mouse lung at lower doses, administered for shorter durations and recommended that further experiments be conducted to determine whether this may or may not be the case.

One reviewer noted the need for further long-term carcinogenicity and inhalation studies to determine the potential carcinogenicity of 2-methylnaphthalene. The absence of additional long-term carcinogenicity and inhalation studies is recognized in Sections 5.1.3., 5.2., and 5.3.

Response: It is recognized that further studies should be conducted to better evaluate the pathogenesis and carcinogenicity of 2-methylnaphthalene. However, since there is currently no definitive evidence to suggest that 2-methylnaphthalene may be carcinogenic in the mouse lung at lower doses and administered for shorter durations, a discussion of this reviewer's hypothesis is not included in the document. The specific molecular mode of action of 2-methylnaphthalene-induced pulmonary alveolar proteinosis is not completely understood. The suggestion by the principal study authors that 2-methylnaphthalene may target type II pneumocytes is consistent with the pathogenesis of the disease in humans and is substantiated by the available evidence in mice exposed to a mixture of methylnaphthalenes. Specifically, the lungs of mice chronically exposed to a mixture of methylnaphthalenes (containing both 1- and 2-methylnaphthalene) show hypertrophy and hyperplasia of

type II pneumocytes (Murata et al., 1992).

PUBLIC COMMENTS (RECEIVED SEPTEMBER 2003)

Comment: A commenter did not consider the Murata et al. (1997) study adequate for the derivation of an RfD based on the possible simultaneous exposure of the animals to 1- and 2-methylnaphthalene. In addition, the commenter indicated that the BMD modeling did not adequately use all of the data (i.e., the female data was omitted from the analysis). Also, the commenter suggested that BMD modeling of the study's data set may be technically inappropriate.

Response: An expanded discussion of the possible simultaneous exposure to 1- and 2-methylnaphthalene was added to the document following external peer review. In addition, both male and female data were used in the BMD modeling for derivation of the RfD. An added discussion of the BMD modeling has also been added to the document.

APPENDIX B. BENCHMARK DOSE (BMD) ANALYSIS

APPENDIX B: BENCHMARK DOSE (BMD) ANALYSIS

Using BMDS version 1.3.2., the modeled data included the incidence 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

	Females		Males	
Dietary dose (%)	Dose (mg/kg-day)	Incidence	Dose (mg/kg-day)	Incidence
0	0	5/50 (10%)	0	4/49 (8%)
0.075	50.3	27/49 (55%)	54.3	21/49 (43%)
0.15	107.6	22/48 (46%)	113.8	23/49 (47%)

Source: Adapted from Murata et al., 1997.

The benchmark response (BMR) was defined as a 5% increase in extra risk for the critical effect, pulmonary alveolar proteinosis. Tables B2 and B3 show the statistical results used to evaluate the goodness-of-fit. Models which were clearly not relevant, that is, those which completely missed any of these dose-response points and their confidence intervals, such as the quantal-quadratic model, are not included in the summary. The BMD_{05} and BMD_{10} and a $BMDL_{05}$ and $BMDL_{10}$ were estimated as a consistent point of comparison across chemicals as recommended by the Benchmark Dose Technical Guidance Document (U.S. EPA, 2000c).

For each model, the software performed residual and overall chi-square goodness-of-fit tests, and determined the Akaike's Information Criterion (AIC). The chi-square p-value is a measure of the closeness between the observed data and the predicted data (predicted using the modeled fit). Models with chi-square p-values $0.1 were considered adequate fits. The AIC is a measure of the model fit based on the log-likelihood at the maximum likelihood estimates for the parameters. Models with lower AIC values among those with adequate chi-square p-values were identified. Based on these criteria, the fit of male data to the log-logistic model is the best fitting model. Output from the software for the log-logistic model run (of the male mouse incidence data) follows Tables B2 and B3.

Since female mice demonstrated a somewhat higher response at the low-dose than male mice, modeling

of both low-dose groups was also carried out (Table B3).

Since there is some validity to using the historical control data as a reference group (assuming 0 responses from 5,000 mice), additional runs were considered using the male and female incidence data from the low dose group. The results are shown for comparison in Table B3.

Table B2. Benchmark dose modeling (both high and low dose groups compared to concurrent controls) for critical effect, settings of 10% extra risk, confidence level 0.95

Model	Model results			
		Females	Males	Combined (males + females)
Log-Logistic	AIC	176.7	167.8	341.6
	Chi Square p-value	0.0099	0.228	0.027
	BMD_{10} (mg/kg-day)	10.7	13.7	12.1
	$BMDL_{10}$ (mg/kg-day)	7.0	9.1	9.0
Log-Probit	AIC	182.4	171.0	350.1
	Chi Square p-value	<0.001	0.029	<0.001
	BMD_{10} (mg/kg-day)	25.7	30.1	27.8
	$BMDL_{10}$ (mg/kg-day)	19.3	22.9	22.7
Probit	AIC	183.8	172.4	350.8
	Chi Square p-value	<0.001	0.014	<0.001
	BMD_{10} (mg/kg-day)	28.3	32.1	27.9
	$BMDL_{10}$ (mg/kg-day)	22.3	25.9	22.7
Quantal-linear [a]	AIC	179.2	168.9	345.2
	Chi Square p-value	0.002	0.106	0.005
	BMD_{10} (mg/kg-day)	15.5	18.0	16.7
	$BMDL_{10}$ (mg/kg-day)	11.2	13.1	13.2

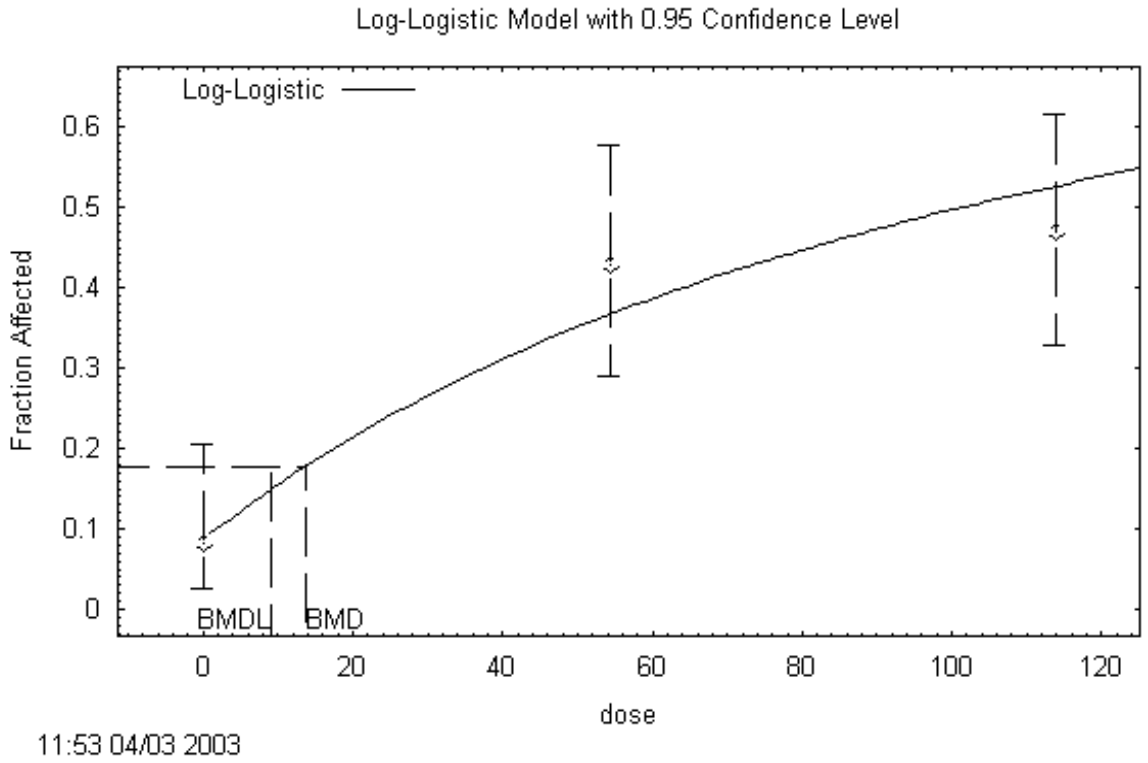
[a] The output for the gamma, quantal-linear (one-stage multistage), and weibull model fits were identical.

Table B3. Comparison of benchmark dose modeling results considering 5% and 10% extra risk levels (with confidence level 0.95) and comparison against historical and concurrent controls

Data Set	Model	Model results	
Male mice, low and high dose groups (concurrent controls)	Log-Logistic	AIC Chi Square p-value BMD_{10} (mg/kg-day) $BMDL_{10}$ (mg/kg-day) BMD_{05} (mg/kg-day) $BMDL_{05}$ (mg/kg-day)	167.8 0.228 13.7 9.1 6.9 4.5
Male and female mice, low-dose only (concurrent controls)	Quantal-linear[a]	AIC Chi Square p-value BMD_{10} (mg/kg-day) $BMDL_{10}$ (mg/kg-day) BMD_{05} (mg/kg-day) $BMDL_{05}$ (mg/kg-day)	200.7 0.15 9.6 7.3 4.7 3.5
Male and female mice, low-dose only (historical controls)	Quantal-linear[a]	AIC Chi Square p-value BMD_{10} (mg/kg-day) $BMDL_{10}$ (mg/kg-day)	138.5 0.34 8.2 6.5

[a] The quantal-linear model provides a fit closest to a straight line.

Output B1: Log-logistic model, male mouse incidence data for pulmonary alveolar proteinosis, with concurrent control (10% BMR)




```
BMDS MODEL RUN
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   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
```
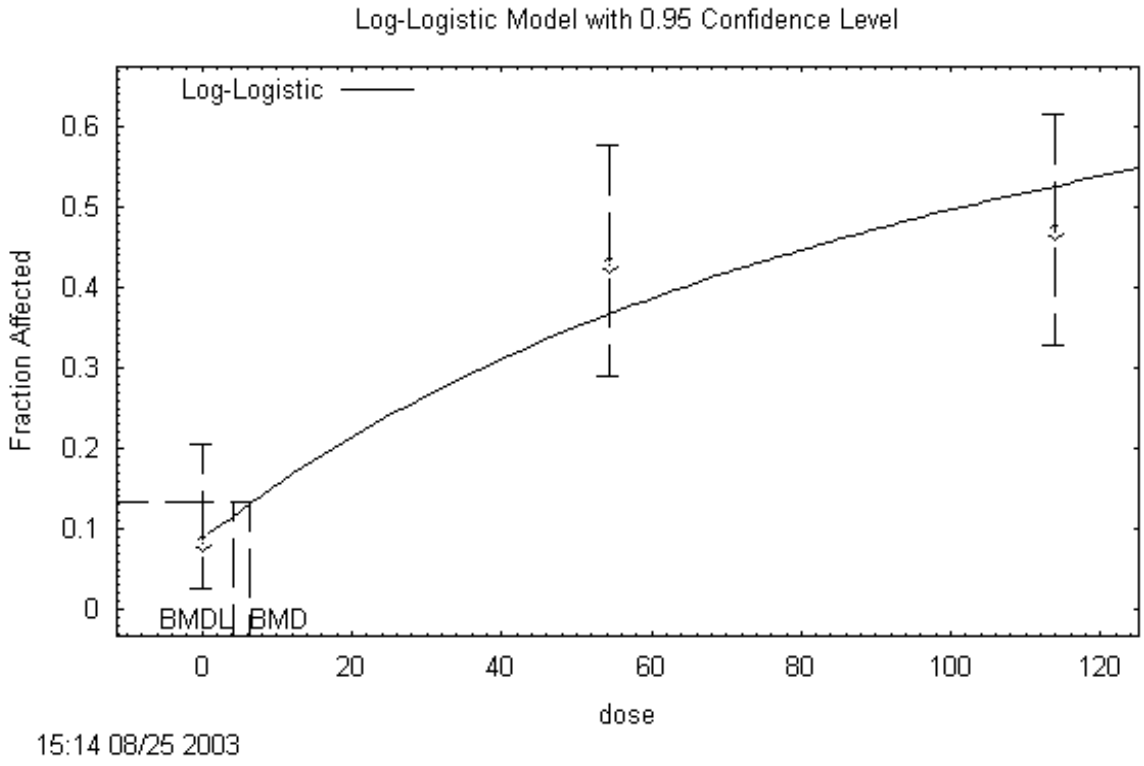

```
                    intercept =     -4.52857
                        slope =            1

          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
```

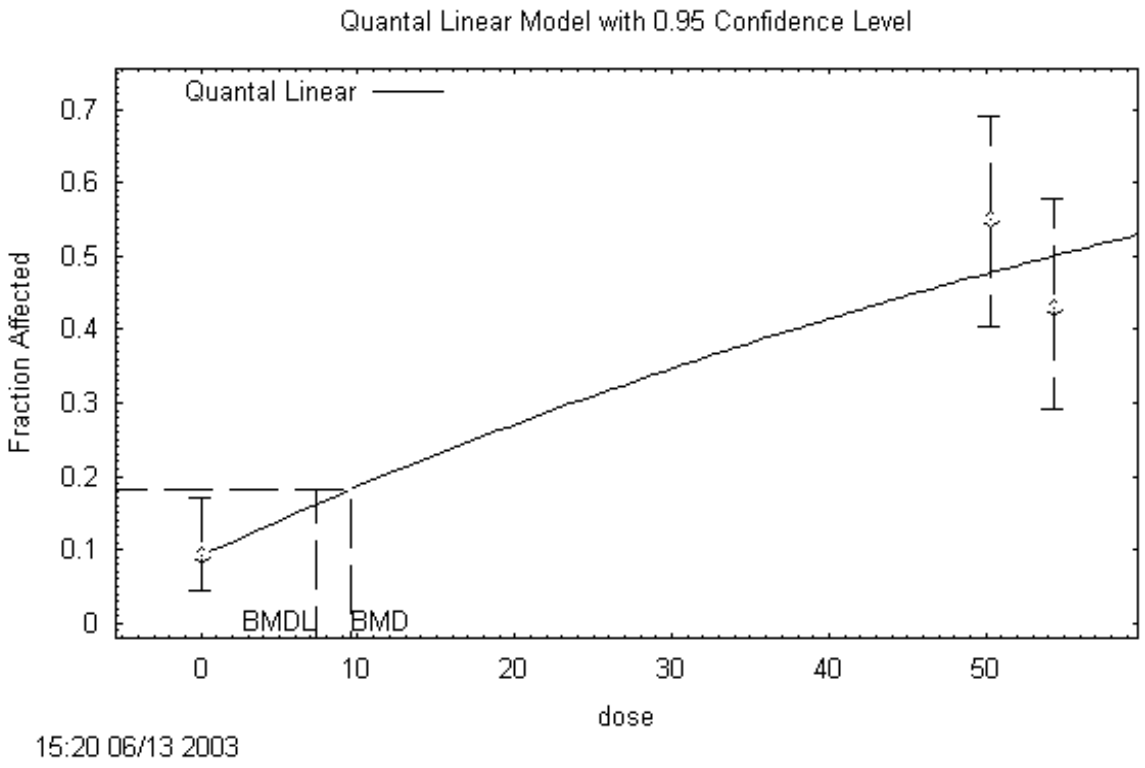
Output B1 (con'd): Log-logistic model, male mouse incidence data for pulmonary alveolar proteinosis, with concurrent control (5% BMR)



See Output B1 for model fit details.

```
   Benchmark Dose Computation

Specified effect =           0.05

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        6.46871

            BMDL =       4.29985
```

Output B2: Quantal-linear fit of low-dose male and female mice incidence data for pulmonary alveolar proteinosis, with concurrent control (10% BMR)




```
BMDS MODEL RUN
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   The form of the probability function is:

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

   Dependent variable = PAP
   Independent variable = mg_kg_d

   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

                  Default Initial (and Specified) Parameter Values
                     Background =        0.095
                          Slope =   0.00851379
                          Power =            1   Specified
```

Asymptotic Correlation Matrix of Parameter Estimates

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

	Background	Slope
Background	1	-0.31
Slope	-0.31	1

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0.0914229	0.0290457
Slope	0.0109971	0.00198579

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-97.3301			
Fitted model	-98.3622	2.06418	1	0.1508
Reduced model	-118.507	42.3543	2	<.0001

AIC: 200.724

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0914	9.051	9	99	-0.01774
54.3000	0.4999	24.497	21	49	-0.9991
50.3000	0.4774	23.395	27	49	1.031

Chi-square = 2.06 DF = 1 P-value = 0.1510

Benchmark Dose Computation

Specified effect = 0.1

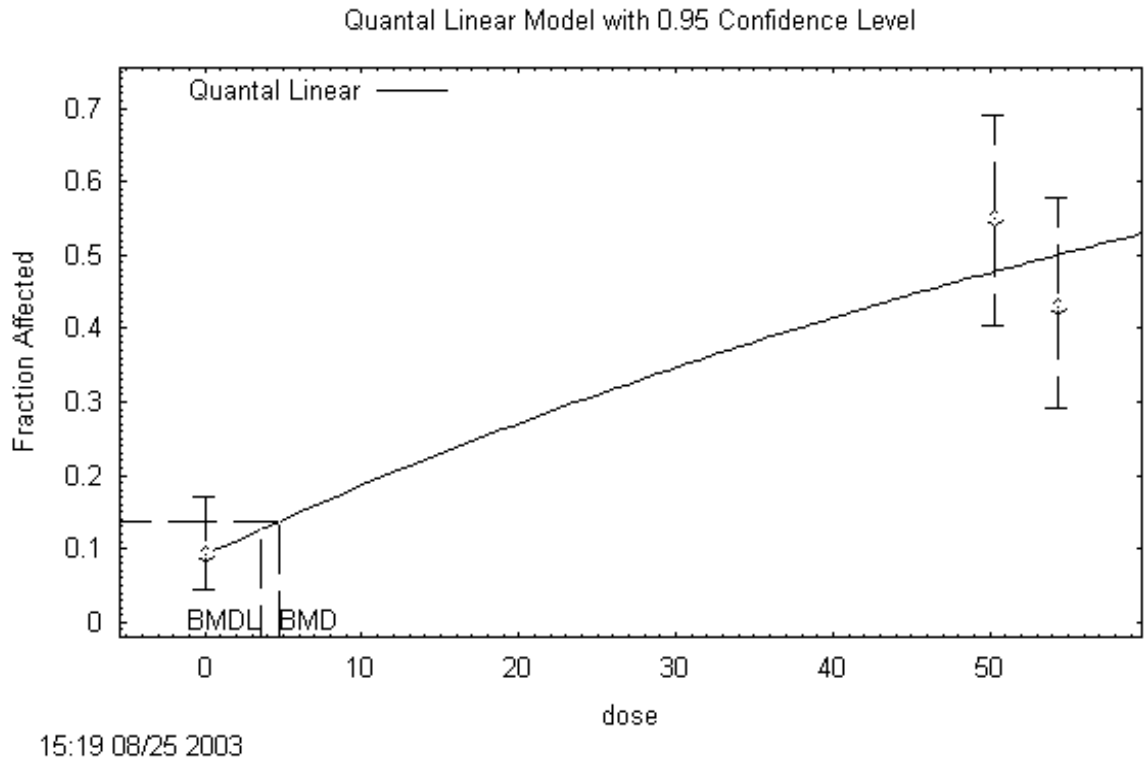
Risk Type = Extra risk

Confidence level = 0.95

BMD = 9.58077

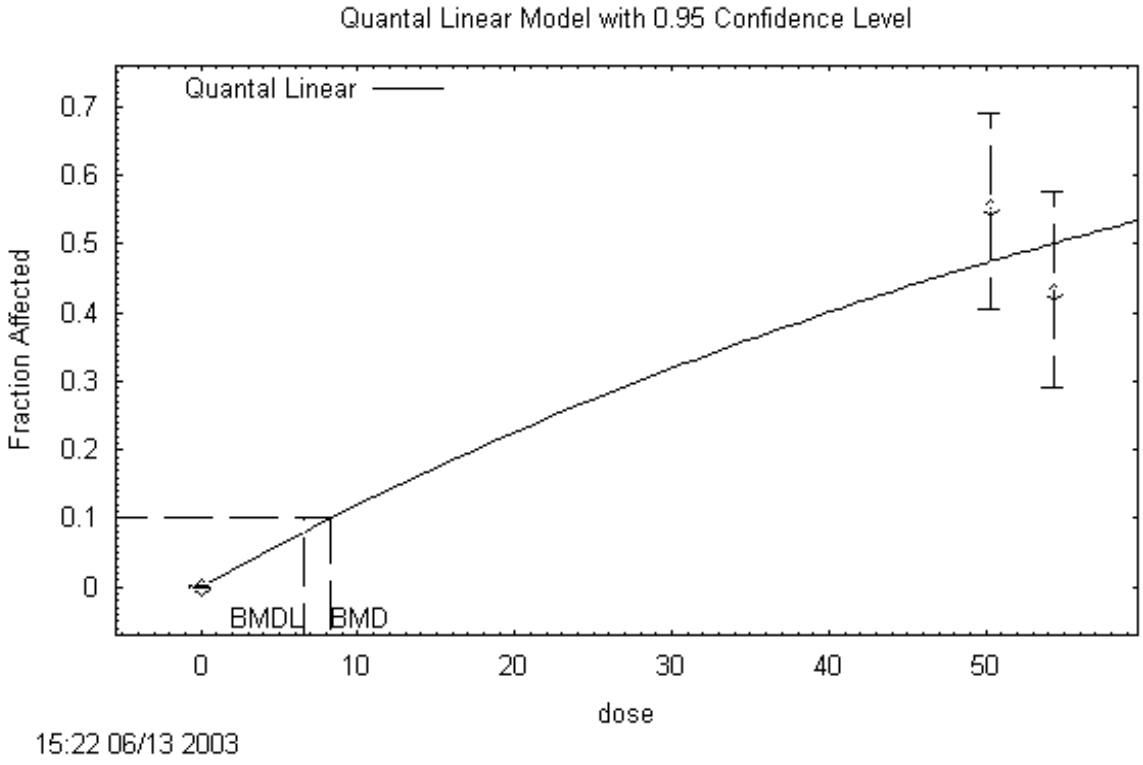
BMDL = 7.26842

Output B2 (con'd): Quantal-linear fit of low-dose male and female mice incidence data for pulmonary alveolar proteinosis, with concurrent control (5% BMR)




```
  Benchmark Dose Computation

Specified effect =           0.05

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        4.66426

            BMDL =       3.53853
```

Output B3: Quantal-linear fit of low-dose male and female mice incidence data for pulmonary alveolar proteinosis, with historical control (10% BMR)




```
BMDS MODEL RUN
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   The form of the probability function is:

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

   Dependent variable = PAP_hcont
   Independent variable = mg_kg_d

   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

                  Default Initial (and Specified) Parameter Values
                     Background =   9.998e-005
                          Slope =    0.0103503
                          Power =            1   Specified
```

Asymptotic Correlation Matrix of Parameter Estimates

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

	Slope
Slope	1

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0	NA
Slope	0.0128415	0.00188826

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	-67.1712			
Fitted model	-68.2571	2.17188	2	0.3376
Reduced model	-271.713	409.083	2	<.0001

AIC: 138.514

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0	5000	0
54.3000	0.5021	24.601	21	49	-1.029
50.3000	0.4758	23.315	27	49	1.054

Chi-square = 2.17 DF = 2 P-value = 0.3380

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 8.20467

BMDL = 6.49803