TOXICOLOGICAL REVIEW

of

NAPHTHALENE

(CAS No. 91-20-3)

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

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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 naphthalene. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of naphthalene.

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 Risk Information Hotline at 202-566-1676.

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

LIST OF ABBREVIATIONS

BMC	benchmark concentration
BMD	benchmark dose
BMR	benchmark response
CNS	central nervous system
G6PDH	glucose 6-phosphate dehydrogenase
Hct	hemotocrit
HEC	human equivalent concentration
Hgb	hemoglobin
LEC_{10}	95% confidence internal lower boundary on concentration at an extra risk level of 0.1
LED_{10}, LED_{05}, LED_{01}	95% confidence interval lower boundary on dose at extra risk levels of 0.10, 0.05, or 0.01
LOAEL	lowest-observed-adverse-effect level
MLE	maximum likelihood estimate
NADPH	nicotinamide adenine dinucleotide phosphate, reduced form
NOAEL	no-observed-adverse-effect level
PBPK	physiologically based pharmacokinetic
q1*	95% confidence interval upper boundary on the slope of a polynomial dose-response function in the low-dose region
RBC	red blood cells
RfC	chronic inhalation reference concentration
RfD	chronic oral reference dose
SAR	structure-activity relationship

1. INTRODUCTION

This document presents background and justification for the hazard and dose-response assessment summaries in EPA's Integrated Risk Information System (IRIS).

The reference dose (RfD) and reference concentration (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 effects during a lifetime. The inhalation RfC is analogous to the oral RfD. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). It is generally expressed in units of mg/m^3.

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

Development of these hazard identification and dose-response assessments for naphthalene has followed the general guidelines for risk assessments as set forth by the National Research Council (1983). EPA guidelines that were used in the development of this assessment may include the following: the *Guidelines for Carcinogenic Risk Assessment* (U.S. EPA, 1986a), *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986b), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986c), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991b), *Proposed Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1995a), *Proposed Guidelines for Carcinogenic Risk Assessment* (U.S. EPA, 1996a), and *Reproductive Toxicity Risk Assessment Guidelines* (U.S. EPA, 1996b); *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); *Peer Review and Peer Involvement at the U.S. Environmental Protection Agency* (U.S. EPA, 1994c); *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995b); *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998); and memorandum from EPA Administrator, Carol Browner, dated March 21, 1995, Subject: Guidance on Risk Characterization.

Literature search strategy employed for this compound was based on the CASRN and at least one common name. At a minimum, the following databases were searched: RTECS, HSDB, TSCATS, CCRIS, GENETOX, EMIC, EMICBACK, DART, ETICBACK, TOXLINE, CANCERLINE, MEDLINE, and MEDLINE backfiles. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Naphthalene (CAS no. 91-20-3) is a bicyclic aromatic hydrocarbon with the chemical formula $C_{10}H_8$ and a molecular weight of 128.16. Pure naphthalene is a white, water-insoluble solid at room temperature with a vapor pressure of 0.087 mmHg (U.S. EPA, 1987; ATSDR, 1993).

Naphthalene is produced by distillation and fractionation of either petroleum or coal tar. Naphthalene's principal use is as an intermediate in the production of phthalic anhydride. Phthalic anhydride is important in the manufacture of phthalate plasticizers, resins, dyes, and insect repellents. Naphthalene is also used in the manufacture of synthetic leather tanning agents and the insecticide carbaryl. Naphthalene has been used as a moth repellent and as a deodorizer for diaper pails and toilets (ATSDR, 1993; U.S. EPA, 1980, 1987).

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

3.1. ABSORPTION

On the basis of the occurrence of adverse effects following exposure, naphthalene is expected to be absorbed via the gastrointestinal tract, the respiratory tract, and the skin (U.S. EPA, 1987; ATSDR, 1993). Bock et al. (1979) reported that naphthalene was rapidly absorbed, predominantly unchanged, from isolated rat intestinal loops into the portal blood; major metabolites identified were naphthalene-1,2-dihydrodiol and 1-naphthol. No absorption studies were located for measuring the rate or extent of dermal absorption in humans. However, naphthalene toxicity was reported in neonates (Dawson et al., 1958; Schafer, 1951), presumably (through the dermal route, although exposure concentration measurements were not taken and inhalation exposure cannot be excluded) from contact of the diapers with mothballs or naphthalene flakes. With respect to animals, quantitative data were reported in rats (half-time of 2.1 hours) for dermal absorption (Turkall et al., 1994) using neat applications.

3.2. DISTRIBUTION

Absorbed naphthalene and/or its metabolites are expected to be distributed by the blood throughout the body. Limited information from a study of pigs, chickens, and a cow indicate that following 31-day oral exposures, the highest concentrations of naphthalene or its metabolites occurred in the lung, liver, kidney, heart, and spleen (Eisele, 1985). In pigs given doses of radiolabeled naphthalene for 31 days, radiolabel was detected at 31 days in the following tissues, listed in order of decreasing concentrations (specified in parentheses in units of percent dose/g tissue 10^{-3}): lung (0.15), liver (0.11), heart (0.11), kidney (0.09), spleen (0.09), ham (0.06), loin (0.05), and fat (0.03). The amount of naphthalene in adipose tissue in pigs actually changes with time. At 24 hours after a single dose of 0.123 mg/kg, adipose is the tissue with the highest concentration (in contrast to the lowest concentration after repeated oral doses) of 3.5%. The following results were found for chickens: kidney (2.40), lung (1.24), liver (0.74), spleen (0.71), heart (0.44), fat (0.37), dark meat (0.33), and white meat (0.16). In the cow, radiolabel was detected at 31 days in the following tissues: liver (0.006), heart (0.004), spleen (0.004), lung (0.003), loin (0.003), kidney (0.002), flank (0.002), and fat (0.001). Comparable distribution studies of rodent species used in the available toxicity studies with naphthalene were not located. In the case of 2-methylnaphthalene, the highest concentration in fat was reported in mice given a single dose via intraperitoneal administration (Griffin et al., 1982). The liver, kidney, and lung followed the fat in order of decreasing concentration.

3.3. METABOLISM

The in vivo and in vitro metabolism of naphthalene in mammalian systems has been studied extensively (for more complete reviews see U.S. EPA, 1987; ATSDR, 1993; Buckpitt and Franklin, 1989; Wells et al., 1989; Plopper et al., 1992a,b; Xu et al., 1992a,b) and is depicted in Figure 1. Due to its lack of a functional group for conjugation, the first step in the naphthalene metabolism is oxidative in nature and catalyzed by cytochrome P-450 oxygenases in the microsome that produces an electrophilic arene epoxide intermediate, 1,2-naphthalene oxide, that was first isolated by Jerina et al. (1970). Whereas the highest cytochrome P-450-specific activities in mammalian tissues are normally found in the liver, naphthalene metabolism also has been demonstrated to occur in other tissues (notably eye and lung tissues; see Wells et al., 1989; Xu et al., 1992a; Buckpitt and Franklin, 1989).

Once formed, the epoxide can spontaneously rearrange to form naphthols (predominantly 1-naphthol) and subsequently conjugate with glucuronic acid or sulfuric acid via enzymatic reactions to form conjugates. In addition to being converted to alcohols, the epoxides can be enzymatically (glutathione-S-transferase) conjugated with glutathione, ultimately forming a variety of cysteine conjugate derivatives known as thioethers through the loss of glutamyl or glycine moieties. Specifically, the 1,2 bonds in all the glutathione conjugates should be saturated. It is only in the presence of acid (and therefore an artifact of sample workup) that the thioether is

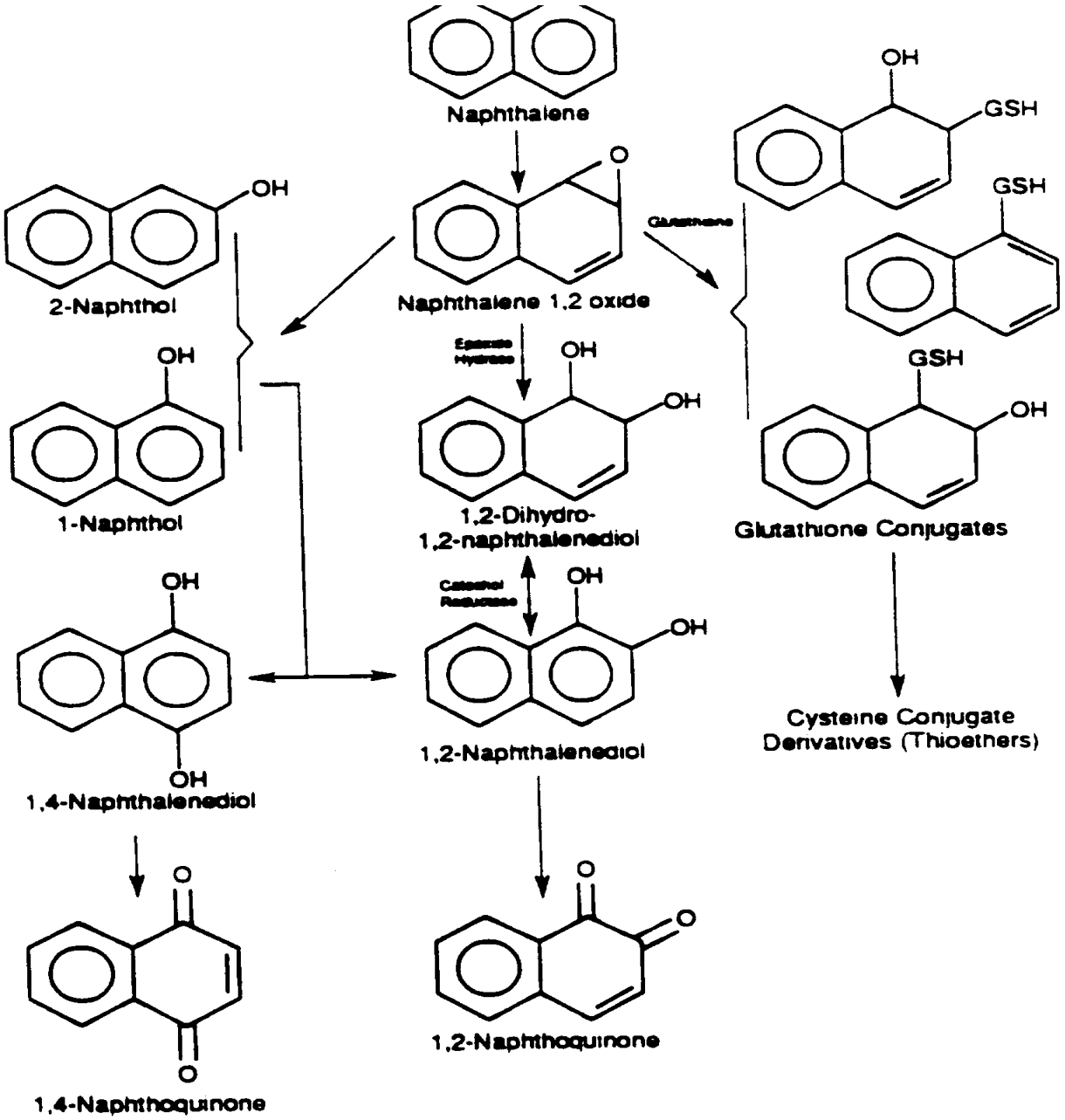

Figure 1. Proposed pathways for naphthalene metabolism.

Source: Modified from ATSDR, 1995.

dehydrated. Thioethers may be further catabolized to premercapturic and mercapturic acids before excretion in the urine or bile. Naphthols can also be enzymatically hydrated by epoxide hydrolase to form trans-1,2-dihydro-dihydroxynaphthalene (also known as naphthalene-1,2-dihydrodiol). The naphthalene-1,2-dihydrodiol can be converted to 1,2-napthalenediol by catechol reductase. The 1,2-naphthalenediol is subsequently oxidized to 1,2-naphthaquinone and hydrogen peroxide and subsequently rearranged to the 1,4-naphthaquinone and vice versa. The latter naphthaquinone can also be produced from 1-naphthol. The metabolites of 1-naphthol, 1,2-naphthoquinone and 1,4-naphthoquinone, were directly toxic to mononuclear leucocytes and depleted glutathione to 1% of the control levels. Both quinones were also genotoxic to human lymphocytes. In contrast, the primary metabolite of naphthalene, 1,2-epoxide, was neither cytotoxic or genotoxic and did not deplete glutathione. Overall, these data suggest that the cytotoxicity of naphthalene is associated with the formation of quinones from 1-naphthol rather than naphthalene-1,2-epoxide (Wilson et al., 1996).

In addition to understanding the importance of species and tissue differences in susceptibility to the toxicity of naphthalene, concentration also appears to be critical for the formation of certain metabolites, since naphthoquinones (assumed to cause cataracts) are produced in rats and rabbits only at levels of 700 mg/kg or greater. Xu et al. (1992a) proposed that naphthalene dihydrodiol is formed in the liver, transported to the aqueous humor, and penetrates into the lens where it is metabolized to form 1,2-naphthoquinone, the putative toxic species that oxidatively damages the lens. Such concentration dependency appears to be confirmed by Chichester et al. (1994) with respect to cell injury of intact Clara cells exposed in vitro to either naphthalene or naphthalene metabolites. It appears that the Clara cells are more susceptible to specific naphthalene metabolites (naphthaquinone and naphthalene oxide) than to the parent compound naphthalene.

In humans, little information is available pertaining to the metabolism of naphthalene (ATSDR, 1995); however, it has previously been shown that the two principal stable metabolites formed by human hepatic microsomes are 1-naphthol and naphthalene 1,2-dihydrodiol (Tingle et al., 1993).

The methylation of naphthalene to form 1-methylnaphthalene and 2-methylnaphthalene presents the opportunity for side chain oxidation reactions in addition to ring oxidation by the cytochrome P-450 monooxygenases. Such side chain oxidation is indeed a significant metabolic pathway in dissected airways from mouse lung, according to unpublished data by Buckpitt (memo from A.R. Buckpitt, to R. Bruce, Cincinnati, OH, June 23, 1997). The possible significance of this is related to the finding that aldehyde dehydrogenase activities are low or nondetectable in the lung (Patel et al., 1979). The potential toxicity of the aldehyde raises the possibility that there are distinct differences between naphthalene and the methylnaphthalenes that are related to differences in metabolism. At comparable molar doses, the cytotoxicity of 1-methylnaphthalene in mice appears to be much less (~ 75%) compared with naphthalene and 2-methylnaphthalene (Rasmussen et al., 1986; ATSDR, 1995). The three diol derivatives of 2-methylnaphthalene and naphthalene are similar, suggesting that ring oxidation appears to be very similar; however, no information was located that documented the metabolism of 1-methylnaphthalene.

3.4. ELIMINATION AND EXCRETION

The majority of the absorbed naphthalene is eliminated, in various forms of metabolites, in urine, with a small quantity found in feces. 1-Naphthol is subject to conjugation with glucuronic acid via glucuronyl transferase; the glucuronide conjugate represents a significant urinary metabolite of naphthalene. For example, Chen and Dorough (1979) reported that the glucuronide conjugate represented 12% of urinary radioactivity in rodents after administration of radiolabeled naphthalene. 1-Naphthol also can be metabolized to naphthoquinones (1,2- and 1,4-naphthoquinones); several enzyme systems have been proposed to perform this conversion (see Buckpitt and Franklin, 1989, for discussion). Naphthoquinones are thought to covalently bind to cellular macromolecules and to produce cellular oxidative stress (e.g., free radical generation), both leading potentially to cytotoxicity (Buckpitt and Franklin, 1989; Wells et al., 1989; Xu et al., 1992a,b). 1-Naphthol formation from naphthalene also may occur via enterohepatic circulation of premercapturic acids and intestinal microflora metabolism. Results from experiments with normal bile-duct-cannulated and germ-free rats treated with naphthalene suggest that after biliary excretion of premercapturic acids into the intestine, intestinal microflora convert them to 1-naphthol, which is subject to absorption and recirculation (Bakke et al., 1985).

Naphthalene-glutathione conjugates are catabolized to premercapturic and mercapturic acids before excretion in the urine or bile (U.S. EPA, 1987). Urinary excretion of premercapturic acids and mercapturic acids represents a major excretory path in mice and rats (Stillwell et al., 1978; Chen and Dorough, 1979), but one study with chimpanzees (Summer et al., 1979) and another with rhesus monkeys (Rozman et al., 1982) indicated that urinary excretion of mercapturic acids may not be as important in primates. In rodents, the majority (approximately 80% of which is composed of thioethers) of the label recovered from ingested or dermal absorbed radioactive labeled naphthalene is in urine with a small amount (about 7%) in feces (Summer et al., 1979; Turkall et al., 1994). Boyland and Sims (1958) also reported that only “traces” of mercapturic acids were detected in the urine of a man after oral administration of 500 mg naphthalene. Rozman et al. (1982) proposed that the apparent difference in formation of glutathione conjugates may be due to decreased rates of naphthalene epoxide formation (i.e., decreased cytochrome P-450 oxygenase activity) in primates relative to rodents or increased rates of naphthalene dihydrodiol formation (i.e., increased epoxide hydrolase activity).

Naphthalene-1,2-dihydrodiol has been proposed to be oxidized via dihydrodiol dehydrogenase, catechol dehydrogenase, and/or other enzymes to the cytotoxic 1,2-naphthoquinone (Van Heyningen, 1979; Wells et al., 1989; Xu et al., 1992a,b).

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

4.1.1. Hemolytic Anemia

Humans exposed to naphthalene via inhalation, combined inhalation and dermal exposure, and combined inhalation and oral exposure have developed hemolytic anemia. Hemolytic anemia is characterized by findings of lowered hemoglobin, hematocrit, and erythrocyte values; elevated reticulocyte counts; Heinz bodies; elevated serum bilirubin; and fragmentation of erythrocytes. In severe cases, the hemolytic anemia was accompanied by jaundice, high serum levels of bilirubin, cyanosis, and kernicterus with pronounced neurological signs. Case reports have described acute hemolytic anemia resulting from inhalation of naphthalene vapor from a naphthalene-containing medication by neonates (Hanssler, 1964; Irle, 1964), transplacental naphthalene exposure and neonatal hemolysis (Anziulewicz et al., 1959), inhalation of naphthalene vapor from excessive numbers of mothballs in the home by a child and adults exposed for several years (Linick, 1983), and inhalation of naphthalene vapor or combined inhalation and dermal absorption by neonates (Cock, 1957; Dawson et al., 1958; Grigor et al., 1966; Naiman and Kosoy, 1964; Schafer, 1951; Valaes et al., 1963) and adults (Younis et al., 1957) from clothing and bedding that had been stored in mothballs. In some of the neonatal cases, clinical signs of neurological effects (lethargy, decreased crying) were mentioned; these signs may have been secondary to the decreased oxygen-carrying capacity of the blood. In addition, acute hemolytic anemia was diagnosed in an infant whose only exposure was during gestation; his mother had inhaled and ingested naphthalene from moth balls during pregnancy, particularly during the last trimester (Zinkham and Childs, 1958).

The only one of the above case reports involving inhalation exposure that attempted to quantitate exposure was that by Linick (1983). Linick reported that a 26-year-old woman and her 4-year-old daughter, as well as seven relatives living in two other households, had anemia, jaundice, headache, confusion, nausea, and vomiting. In addition, visitors to the woman's apartment also became ill with headache, nausea, and vomiting. Excessive numbers of mothballs (used for years to curb odors and control insects) were found in all three households. Analysis of air samples collected on charcoal in the woman's apartment revealed exposure levels of 20 ppb naphthalene, but the author pointed out that levels may have been much higher when fresh supplies of mothballs were introduced. When the use of mothballs was discontinued, the above signs and symptoms disappeared.

Gidron and Leurer (1956) described two cases of unsuccessful suicide attempts in which individuals had ingested approximately 6 g (a 16-year-old girl) or 10 g (age and sex of this subject were not specified) of naphthalene and subsequently developed hemolytic anemia and other signs of naphthalene poisoning (estimated doses of 85.7 or 142.8 mg/kg can be calculated, assuming a 70 kg body weight).

Oxidized glutathione is reduced by glutathione reductase, an NADPH-requiring enzyme. The primary source of erythrocyte NADPH is glucose-6-phosphate oxidation by the enzyme G6PDH. Neonates and persons deficient in G6PDH or readily depleted of glutathione are believed to be particularly susceptible to naphthalene-induced hemolytic anemia (Valaes et al., 1963; U.S. EPA, 1987). Incomplete development of livers in neonates is thought to restrict the ability to conjugate naphthalene metabolites responsible for hemolysis. Persons with deficits in G6PDH have reduced capabilities to maintain adequate levels of reduced glutathione for the conjugation of naphthalene metabolites. G6PDH deficiency is particularly common among African and Mediterranean races. A study of 83 G6PDH-deficient neonates and 151 normal neonates admitted to the hospital found that exposure to naphthalene was correlated with the development of neonatal jaundice only in the G6PDH-deficient infants (Owa, 1989).

Owa et al. (1993) monitored 1-naphthol in the urine of 50 babies (age 1-19 days) admitted to a Nigerian hospital; 64% of the babies had neonatal jaundice. 1-Naphthol was detected in the urine of 5 babies who were among the 25 babies with a positive history of exposure to naphthalene (mothballs). Four of the five babies had jaundice, and three were G6PDH deficient. Information on possible exposure levels experienced by the babies was not reported.

4.1.2. Lethality

The majority of human deaths following naphthalene intoxication have resulted from intentional ingestion of moth balls (Gupta et al., 1979; Kurz, 1987). The reported case studies are descriptive in nature and do not provide sufficient information for an accurate determination of lethal doses and do not describe any lesions histopathologically. The single exception is a report in the Japanese literature of a child poisoned with approximately 5 g moth balls (Ijiri et al., 1987). At autopsy, there was congestion, edema, and hemorrhage of the lungs. In addition, histopathology of the liver showed infiltration of polymorphonuclear leucocytes and lymphocytes as well as fatty changes. Edema and hemorrhage of the lungs are not prominent findings in animals and with an N = 1, it is difficult to know whether these findings are significant. Blood levels of naphthalene were reported at 0.55 ppm (mg/L). Nevertheless, the potential of a lung lesion as well as changes in the liver, albeit at high doses (estimated to be 1g/kg), suggests that lesions in these two organs are possible in the human. The time to death was short (1 hour), and tissue injury (in mice, lung lesions are observed as early as 1 hour after treatment but are not maximal until 4-8 hours) may have been considerably greater had the child lived a few more hours. Death of an infant who wore diapers that had been stored with moth balls has also been reported (Schafer, 1951), and no estimates of exposure concentration were available. One study reported that ingestion of 2 g naphthalene over a 2-day period caused the death of a 6-year-old child (Gerarde, 1960). From this, Gerarde (1960) speculated that the probable oral lethal dose for adults may lie between 5 and 15 g (71.4-214.2 mg/kg, assuming a 70 kg body weight), but provided no evidence for this claim other than the cases cited in this paragraph. Reports of hemolytic anemia (or other effects on the blood) in humans following subchronic or chronic exposure are not available.

4.1.3. Cataracts

In humans, cataract formation also has been associated with exposure to naphthalene. A pharmacist ingesting 5 g naphthalene developed blindness and bilateral cataracts (Lezenius, 1902). (A dose of approximately 71 mg/kg is estimated, assuming a body weight of 70 kg; however, this is a bolus dose resulting in acute symptoms and cannot be compared to a no-observed-adverse-effect level [NOAEL] of 71 mg/kg-day resulting from oral chronic animal studies.) Corroborative human data regarding cataract-forming exposure levels are not available. Cataracts occurred in 8 of 21 workers employed for 5 years in a dye-producing plant where naphthalene was used (Ghetti and Mariani, 1956). Of the affected workers, seven were younger than 50 years old and, therefore, would not have been likely to have developed cataracts spontaneously. It is likely that exposure in these workers occurred predominantly via inhalation and dermal contact, but exposure levels were not estimated.

4.1.4. Cancer

Available data are inadequate to establish a causal association between exposure to naphthalene and cancer in humans. Adequately scaled epidemiologic studies designed to examine a possible association between naphthalene exposure and cancer were not located.

Naphthalene is a component of several complex mixtures associated with human cancer, including tobacco smoke and emissions from coal and wood combustion (IARC, 1984; U.S. EPA, 1987); however, exposure of humans to naphthalene alone has not been studied for an association with the occurrence of cancer.

Wolf (1976) reported that six cases of carcinomas occurred among 15 workers exposed to vapors of naphthalene and coal tar for 7-32 years at a coal-tar naphthalene production facility. Of the four workers who developed carcinomas of the larynx, all were tobacco smokers; the other two developed carcinomas of the stomach (pylorus) and cecum. This study is inadequate for evaluation of naphthalene's carcinogenicity for several reasons: there were no controls; limited numbers of subjects were included; exposure levels were not determined; and the subjects were exposed to complex mixtures (i.e., coal tar and tobacco smoke) containing other demonstrated carcinogens (e.g., other polycyclic aromatic hydrocarbons such as benzo[a]pyrene).

In an abstract report of an East German study, it was reported that 15 patients with cancer of the ear, nose, and/or throat were examined for an association with work-related causes (Kup, 1978). Among this group, there were 12 cases of laryngeal carcinomas, 2 cases of epipharyngeal cancer, and 1 case of nasal carcinoma. Only three of these patients were nonsmokers. Among the 12 laryngeal cases, 4 patients had occupational exposure to naphthalene. The author suggested that most of the cancers resulted from non-work-related causes.

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

4.2.1. Oral Exposure

4.2.1.1. *Hemolytic Anemia*

The only animal species in which naphthalene-induced hemolytic anemia has been demonstrated is the dog. Hemolytic anemia (decreased hemoglobin and hematocrit, Heinz body in red blood cells, reticulocytosis) occurred in three dogs following oral administration of naphthalene incorporated into a meat diet; one dog (weighing 7.3 kg body weight) received a single dose of 3 g (410 mg/kg), the second dog (5.9 kg) received a single 9 g dose (1.53 g/kg), and the third dog (6.8 kg) received seven consecutive daily doses ranging from 0.5 to 3.0 g (74-441 mg/kg; a total of 12.5 g naphthalene was administered = 262 mg/kg-day average) (Zuelzer and Apt, 1949). Examination of hematological parameters revealed no distinctive signs of naphthalene-induced hemolytic anemia in F344 rats given gavage doses of up to 400 mg/kg, 5 days/week for 13 weeks (BCL, 1980a); in B6C3F1 mice given gavage doses up to 200 mg/kg, 5 days/week for 13 weeks (BCL, 1980b); or in albino CD-1 mice given gavage doses up to 133 mg/kg for 90 consecutive days (Shopp et al., 1984). (These studies are reviewed in more detail later in this section.)

4.2.1.2. *Cataracts*

After acute or subchronic oral exposure to naphthalene, cataracts have been found in rabbits, rats, and mice. Individual studies that adequately describe dose-response relationships for naphthalene-induced cataracts were not found, but oral dose levels that produced significant increases in cataract incidence are higher than dose levels examined in other subchronic oral studies that did not find cataracts in rats (BCL, 1980a) or mice (BCL, 1980b; Shopp et al., 1984).

Cataract formation occurred in 10/16 pigmented and 11/12 albino rabbits given 3-28 consecutive daily oral doses of 1 g/kg; cataracts were noted as early as 2 days after administration of the first dose (Van Heyningen and Pirie, 1967, 1976; Van Heyningen, 1979). Mild cataracts were reported to occur in five weanling rats (sex and strain were not specified) given 2% naphthalene in the diet for 2 months (Fitzhugh and Buschke, 1949). A daily dose of approximately 2 g/kg is estimated using an assumed body weight of 0.18 kg and an equation described by U.S. EPA (1988) that relates the rate of food consumption to body weight. Koch et al. (1976) reported that when administered 1,000 mg/kg naphthalene on alternate days, all rats of pigmented strains developed cataracts within 16 to 28 days, whereas only some of the rats of the albino strains (Sprague-Dawley and Wistar) developed minor lens changes after a greater period of time. More recent experiments, using better diagnostic methods, found naphthalene-induced cataract formation in both pigmented and nonpigmented strains of rats treated for 28 days with 1,000 mg/kg naphthalene (see review of Xu et al., 1992a,b, below). Shichi et al. (1980) found incidence for cataracts of 1/15 and 1/15 in groups of C57BL/6N mice fed naphthalene in the diet at doses of approximately 60 or 120 mg/kg for 60 days and treated with twice-weekly injections

of an inducer of cytochrome P-450. No cataracts were found in DBA/2N mice given the same treatment regime.

Xu et al. (1992a) reported that administration of gavage doses of 1g/kg naphthalene in mineral oil for up to 28 days produced lens opacification and cataracts in pigmented strains of rats (Long-Evans and Brown-Norway) and in albino rats (Sprague-Dawley, Wistar, and Lewis). Groups of 6-10 male rats/strain were treated with mineral oil (control), naphthalene in mineral oil, naphthalene plus ALϕ1576 (an aldose reductase inhibitor), or ALϕ1576 alone. Eyes were examined by slit-lamp with focal- and retro-illumination techniques, twice a week during the first 2 weeks and once a week thereafter. The presence of brown opacities was confirmed in all lenses under a dissecting microscope after excision on day 28. Cataracts developed within the 4-week period in all naphthalene-treated rats, regardless of strain. The following order was noted in the rate of cataract development among the strains: Brown-Norway > Long-Evans = Lewis = Sprague-Dawley > Wistar. Naphthalene-treated rats displayed diarrhea, stunted growth, loss of hair, and occasional death, but incidence for these effects was not reported. The lenses from control normal, ALϕ1576-treated, and naphthalene-plus-ALϕ1576 rats remained clear (no opacification) for at least 6 weeks and indistinguishable from the baseline lens. From these in vivo studies, it was hypothesized that naphthalene dihydrodiol is the metabolite transported to the aqueous humor via the circulation and penetrates into the lens where it is metabolized to form 1,2-naphthoquinone, the putative toxic species that oxidatively damages the lens. In a companion in vitro experiment with cultured rat lenses, Xu et al. (1992b) found that morphological and biochemical changes induced by naphthalene and naphthalene dihydrodiol were similar and were similarly inhibited by ALϕ1537. Xu et al. (1992b) proposed that AL1537 prevents cataract formation, possibly by inhibiting key enzymes involved in the metabolism of naphthalene dihydrodiol in the lens.

Murano et al. (1993) reported that gavage doses of 1g/kg naphthalene in liquid paraffin, administered every other day for 6 weeks to groups of six male Brown-Norway rats or six male Sprague-Dawley rats, produced cataracts in all rats. The quality of the ocular opacities was identical, but the onset and time progression of development differed in the two rat strains. Brown-Norway rats showed a faster progression of cataract development than Sprague-Dawley rats. These results are in general agreement with the rat strain comparisons conducted by Xu et al. (1992a).

In a study comparing the ability of structurally distinct aldose reductase inhibitors to inhibit naphthalene cataractogenicity, Tao et al. (1991a,b) found cataracts in groups of female Brown-Norway rats administered gavage doses of naphthalene (700 mg/kg) for up to 102 days. The available reports of this study did not specify the incidence of affected animals. ALϕ1537 inhibited the development of naphthalene-induced cataracts, whereas another aldose reductase inhibitor with different structural characteristics, FK366, did not. Tao et al. (1991a,b) speculated that ALϕ1537 inhibits naphthalene metabolism at some step (not involving aldose reductase) following the formation of naphthalene epoxide. After 90 days, average body weights in the exposed groups (regardless of presence or absence of aldose reductase inhibitors) were 13%-15% lower than body weights in the control group.

4.2.1.3. *General Toxic Effects From Subchronic Exposure*

Naphthalene (> 99% pure) in corn oil was administered by gavage to groups of 10 male and 10 female Fischer 344 rats at dose levels of 0, 25, 50, 100, 200, or 400 mg/kg (duration adjusted 0, 17.9, 35.7, 71.4, 142.9, and 285.7 mg/kg-day), 5 days/week for 13 weeks (BCL, 1980a). Endpoints included weekly measurement of food consumption and body weight, twice daily observation for clinical signs of toxicity, measurement of hematological parameters for blood collected at termination (hemoglobin, hematocrit, total and differential white blood cell count, red blood cell count, mean cell volume, mean cell hemoglobin concentration), necropsy of all rats in the study, and complete histopathological examination of 27 organs and tissues (including the eyes, lungs, stomach, liver, reproductive organs, thymus, and kidneys) from all control and 400 mg/kg rats. Male kidneys and female thymuses from the 200 mg/kg group were also examined histopathologically (according to the histopathology tables; however, the report text states that the 100 mg/kg group was examined). Organ weight data were not reported.

At the highest dose level, two males died during the last week of treatment, and rats of both sexes displayed diarrhea, lethargy, hunched posture, and rough coats at intermittent intervals throughout the study (BCL, 1980a). Food consumption was not affected by exposure, but mean decreases in body weight (over the 13-week period) in several groups of exposed rats were decreased by more than 10% relative to the controls (29.2% and 12.1% decrease in the 400 and 200 mg/kg males, respectively, compared with controls and a 23% decrease in the 400 mg/kg females compared with controls). The terminal body weights at 13 weeks' exposure were 250.6, 306.7, 333.4, 351.2, 353.4, and 348.9 g for females and 156.7, 190.5, 197.2, 203.5, 197.8, and 203.4 g for males for the 400, 200, 100, 50, 25, and 0 dose groups, respectively. Differences between mean values of hematological parameters in exposed groups and those in control groups were < 10% of control values, except for a 94% increase in numbers of mature neutrophils and a 25.1% decrease in numbers of lymphocytes in male 400 mg/kg rats and a 37.2% increase in mature neutrophils in 400 mg/kg females. Histological examinations revealed low incidences of lesions in exposed male kidneys and exposed female thymuses; no lesions were observed in respective control kidneys or thymuses. Focal cortical lymphocytic infiltration or focal tubular regeneration were observed in kidneys in 2/10 male rats exposed to 200 mg/kg naphthalene, and diffuse renal tubular degeneration occurred in 1/10 male rats exposed to 400 mg/kg naphthalene. Lymphoid depletion of the thymus occurred in 2/10 females exposed to 400 mg/kg naphthalene, but not in any other females. No other tissue lesions were detected. The NOAEL/LOAEL (lowest-observed-adverse-effect level) based on mean terminal body weight decreases were 100 and 200 mg/kg, respectively.

Ten male and ten female B6C3F1 mice were administered gavage doses of naphthalene in corn oil at levels of 0, 12.5, 25, 50, 100, or 200 mg/kg, 5 days/week for 13 weeks (BCL, 1980b). Seven mice (three males and two females of the 200 mg/kg group, one female of the 25 mg/kg group, and one control male) died during the second, third, and fourth weeks from gavage trauma or accident. Transient signs of toxicity (lethargy, rough hair coats, and decreased food consumption) occurred between weeks 3 and 5 in the 200 mg/kg groups. All exposed male mice gained more weight during the study than did control males (weight gains expressed as a percentage of control weight gain were 154.3%, 116.0%, 125.9%, 122.2%, and 107.4% for the

12.5-200 mg/kg groups, respectively). In contrast, exposed female mice displayed decreased weight gain compared with controls (weight gains expressed as a percentage of control weight gain were 97.5%, 81.5%, 81.5%, 77.8%, and 76.5% for the 12.5-200 mg/kg groups, respectively). The average change in body weight between day 0 and the 13th week was 6.2 g/mouse for the 200 mg/kg female mice compared with 8.1 g/mouse for the control females. The investigators believed that a difference in weight gain of 1.9 g over a 13-week period "was not large enough to conclusively indicate a toxic effect." All mice were necropsied, and 27 organs (including the eyes, thymus, and lungs) from the mice in the control and high-dose groups noted were examined histologically. No exposure-related lesions were observed in any organs. The highest incidence of lesions observed was from minimal to mild, focal or multifocal, subacute pneumonia in both controls (4/10 males and 2/10 females) and high-dose mice (4/10 males and 5/10 females). Organ weight data were not reported. Hematological analyses were performed on all groups. Exposed groups displayed mean values that were within 10% of the control means for the following parameters: hemoglobin, hematocrit, total white blood cells, and total red blood cells. An increase in lymphocytes (18% increase) and a decrease in segmented neutrophils (38.8% decrease) in high-dose males were not considered biologically significant by the authors. The authors considered the highest dose level to be a NOAEL. The authors expressed the belief that the differences in weight gain did not conclusively indicate a toxic effect, because other consistent signs of toxicity were not observed and "such a marked indication of sex difference in (body weight) response" was observed. Adopting the authors' interpretation that the effects on female body weight gain in this study were not conclusive, but not their interpretation of the absence of other signs of toxicity, the highest dose level (200 mg/kg; 142.9 mg/kg-day) in the study is judged to be a LOAEL for transient clinical signs of toxicity

Groups of male and female albino CD-1 mice (approximately 6 weeks old at the start) were administered gavage doses of 0, 5.3, 53, or 133 mg/kg naphthalene (99.3% pure) in corn oil for 90 consecutive days (Shopp et al., 1984). A naive control group and the 5.3 and 53 mg/kg dose groups each contained 76 male mice and 40 female mice. The vehicle control group contained 112 male mice and 76 female mice. The high-dose group contained 96 male mice and 60 female mice. Significant chemical-related decreases in terminal body weights or survival were not observed in either sex. No significant alterations in absolute or relative organ weights occurred in exposed male mice. Significant decreases in absolute weights of brain, liver, and spleen and relative weight of spleen occurred in high-dose females. Histopathological examination of organs was not conducted, but the authors noted that cataracts were not formed in exposed mice (methods used to assess the presence of cataracts were not specified). Examination of hematological parameters (including numbers of leucocytes, erythrocytes, and platelets and determination of hematocrit and hemoglobin) at termination revealed only slight, but statistically significant, increases in hemoglobin in high-dose females only; however, the hematological data were not shown in the available report. Chemical analysis of serum showed statistically significant decreased blood urea nitrogen in all exposed female groups and increased serum globulin and protein in the two highest female dose groups. No exposure-related responses were found in a battery of immunological assays (humoral immune response, lymphocyte responsiveness, delayed-type hypersensitivity response, popliteal lymph node response, and bone marrow function); immunotoxic responses were observed in positive controls given intraperitoneal injections of 50 mg/kg cyclophosphamide on days 87, 88, 89, and 90. The study identified a LOAEL of 133

mg/kg-day and a NOAEL of 53 mg/kg-day with significant decreases in absolute weight of brain, liver, and spleen and relative weight of spleen in high-dose females; however, the organ-to-body weight ratios were significantly different only for the spleen. The toxicological significance of the statistically significant alterations in hematological and serum chemical parameters are not clear.

4.2.1.4. *Cancer*

Schmähl (1955) reported that naphthalene administered in food did not cause cancer in a group of 28 rats (in-house strains BDI and BDII). Naphthalene (purchased from Merck Co. and described as "Naphthalene puriss. cryst. alcoh. depur. [54935]") was dissolved in oil and given six times/week in food. The absorption spectrum of the test material displayed no atypical peaks compared with published data for naphthalene, suggesting "high" purity. The daily dose was reported to vary between 10 and 20 mg/rat, but further details regarding dose variation were not provided. After reaching a total dose of 10 g/rat (food intake and body weights were not reported), treatment was stopped on the 700th experimental day, and animals were observed until spontaneous death, between 700 and 800 days of age. Assuming an average daily dose of 15 mg/rat and a body weight of 0.36 kg (U.S. EPA, 1988, reference body weight for male Fischer 344 rats), an estimated average daily dose of 42 mg/kg is calculated. Autopsies were performed on dead animals, and organs that appeared unusual were examined histologically (the report did not specify which organs were histologically examined). The number of rats in the control group was not reported; survival for control and exposed rats was reported to be similar. Reported results from the autopsy and histological examinations were restricted to the statement that no toxic effects were seen, including eye damage and tumors. Inadequacies in experimental protocol design and implementation (e.g., only one dose level was administered, the histopathological examination was not complete, hematological endpoints were not evaluated, and some rats lived as long as 300 days beyond exposure before being examined) and inadequacies in reporting limit the conclusions that can be drawn from this study regarding either the carcinogenicity or chronic toxicity of naphthalene. Furthermore, the dose tested is not considered an adequately high dose to detect carcinogenic effects (i.e., absence of any toxicity).

4.2.2. Inhalation Exposure

In a National Toxicology Program (NTP, 1992a) cancer bioassay, groups of male and female B6C3F1 mice were exposed (whole body) to naphthalene (> 99% pure) vapors at concentrations of 0 (75 mice/sex), 10 (75 mice/sex), or 30 ppm (150 mice/sex) for 6 hours/day, 5 days/week for 2 years. Mice were housed five to a cage. There were 150 mice housed in each of four inhalation chambers; two chambers were used for the high-exposure level. A comprehensive histological examination was performed on all control and high-dose mice and on low-dose mice that died or were sacrificed before 21 months of exposure. After 21 months of exposure, only the nasal cavity and lung were examined in the low-dose group. In each chamber, 50 animals per sex were designated for the 2-year studies; 5 animals per sex were designated for hematological evaluations at 14 days, and 3, 6, 12, and 18 months. However, because of high mortality in the

male control group (see next paragraph), only the 14-day hematological evaluation was conducted. The other surviving interim mice were incorporated into the 2-year study.

Statistically significant decreases in survival were observed in the control male mice due to fighting compared with the exposed groups. Exposed male mice were observed to huddle in corners of the cages during exposure and were less inclined to fight. Survival percentages at the end of the study were 37% (26/70), 75% (52/69), and 89% (118/133) for the 0, 10, and 30 ppm male groups, respectively. Survival percentages did not include mice sacrificed at 14 days, mice that died before the study began, mice that were accidentally killed, or mice that were lost during the study. Survival at 2 years in the control female mice (86%; 59/69) was comparable to survival in the exposed groups; survival percentages were 88% (57/65) and 76% (102/135) for low- and high-dose females. Body weights were not affected by exposure in either sex.

Statistically significant increases in incidences of nonneoplastic lesions were found in the lung and nose of males and females at both exposure levels. Observed nonneoplastic effects included the following (with respective incidences listed in the order of control, low- and high-exposure groups): chronic inflammation of the lung (0/70, 21/69, and 56/135 for males; 3/69, 13/65, and 52/135 for females), chronic nasal inflammation, hyperplasia of the respiratory epithelium in the nose (0/70, 66/69, and 134/135 for males; 0/69, 65/65, and 135/135 for females), and metaplasia of the olfactory epithelium (0/70, 67/69, and 133/135 for males; 1/69, 65/65, and 135/135 for females).

The lung inflammation in the exposed mice was described as a chronic inflammatory response with the formation of granuloma. This consisted of “focal intra-alveolar mixed inflammatory cell exudates and interstitial fibrosis” that in more advanced lesions consisted “primarily of large foamy macrophages, sometimes accompanied by multinucleated giant cells” (NTP, 1992a). Foci of alveolar epithelial hyperplasia were noted to occur generally in regions distant to inflammation.

A statistically significant increase in the incidence of alveolar/bronchiolar adenomas was observed in the 30 ppm group of females (28/135), but not in the 10 ppm group (2/65), relative to the control female group (5/69). Among females, an additional mouse in the 30 ppm group displayed an alveolar/bronchiolar carcinoma. The historical combined incidence of alveolar/bronchiolar adenomas and carcinomas in control B6C3F1 female mice from NTP inhalation studies was cited as 39/466 (8.4%, range 0-12%). In the author’s opinion, alveolar/bronchiolar adenomas and carcinomas constitute a morphological continuum. The incidences of male mice with alveolar/bronchiolar adenomas were 7/70, 15/69, and 27/135 for the control, 10 ppm, and 30 ppm groups, respectively; for combined adenomas and carcinomas of the alveolar/bronchiolar region, the respective incidences were 7/70, 17/69, and 31/135. A statistical analysis that adjusted for intercurrent mortality (logistics regression analysis) determined that the tumor incidences for control and exposed groups of male mice were not significantly different (NTP, 1992a). Historical incidence for combined alveolar/bronchiolar adenomas and carcinomas in control male B6C3F1 mice from NTP inhalation studies was cited as 94/478 (20%, range 10%-30%). The adenomas were described as “locally compressive nodular masses consisting of cords of well-differentiated epithelial cells,” whereas the carcinoma was “composed of ribbons

and/or coalescing sheets of smaller, more anaplastic, cells which sometimes extended into adjacent parenchyma" (NTP, 1992a).

Hemangiosarcomas occurred at various sites within the vascular endothelium in five high-dose female mice (5/135), but not within the other groups of female mice (0/69 and 0/65 for control and 10 ppm females, respectively). The high-dose female incidence (3.7%) was not significantly different from the concurrent control incidence and was within the range of historical control incidences from NTP inhalation studies (range: 0%-8%; overall incidence: 17/467 or 3.6%). No significantly elevated incidences of tumors were found at other tissue sites in exposed male or female mice (NTP, 1992a).

Adkins et al. (1986) exposed groups of 30 female A/J strain mice (6-8 weeks old) to 0, 10, or 30 ppm naphthalene (98%-99% pure) vapors for 6 hours/day, 5 days/week for 6 months. After the 6-month exposure period, excised lungs were examined for tumors. Tumors were examined histologically. The authors did not describe any noncancer histopathological effects that their examinations may have revealed. Survival was not different between the exposed and control groups. Lung tumors were found in all 20 positive control mice given single intraperitoneal injections of 1 g urethane/kg; the mean number of tumors per mouse in the positive control was 28.9. Increased numbers of lung tumors were found in the naphthalene-exposed groups compared with the control group, but the differences were not statistically significant (6, 10, and 11 for the 0, 10, and 30 ppm groups, respectively). Tumors were described as alveolar adenomas consisting of "large cuboidal or columnar epithelial cells supported by a sparse fibroblastic stroma and arranged in poorly defined acinar structures with papillary formations." No carcinomas were found. Naphthalene exposure did not significantly increase the percentage of animals with tumors (21%, 29%, and 30% for 0, 10, and 30 ppm mice, respectively). Statistically significant increases in the number of adenomas per tumor-bearing lung were observed in the exposed mice, but there was no increase in response with increasing dose. Mean numbers of tumors per tumor-bearing lung (standard deviation noted in parentheses) were 1.00 (0.00), 1.25 (0.07), and 1.25 (0.07) for 0, 10, and 30 ppm mice, respectively. Applicability of this study to the assessment of risk for lifetime exposure is limited due to the less-than-lifetime exposure and observation periods and the restricted histopathology.

4.2.3. Other Routes of Exposure

Exposure to naphthalene or methylnaphthalenes by noninhalation routes appears to produce lung damage in rodents, especially mice. Several laboratories have found that single intraperitoneal injections of naphthalene or certain other chemicals that are metabolically activated, including methylnaphthalenes, bromobenzene, and carbon tetrachloride, produce bronchiolar epithelial cell injury in rodent species, with mice being the most sensitive species (e.g., Reid et al., 1973; Rasmussen et al., 1986; Buckpitt and Franklin, 1989). Mahvi et al. (1977) reported that single intraperitoneal doses of 0.05 mmol/kg naphthalene (6 mg/kg) produced only minor ultrastructural or histopathological changes in the bronchiolar epithelium of mice, but 1 and 2 mmol/kg doses (128 and 256 mg/kg) caused nonciliated bronchiolar epithelial (Clara) cells to expand and exfoliate within 12-24 hours, followed by morphological changes on the surface of the

ciliated cells and rapid division of the remaining cells. Five to seven days after dose administration, bronchioles appeared normal. Rasmussen et al. (1986) compared the potency of single 1 or 2 mmol/kg intraperitoneal injections of naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene in mice using a cytotoxicity scoring system for bronchiolar epithelial damage. 2-Methylnaphthalene and naphthalene were about equally cytotoxic, whereas 1-methylnaphthalene produced less severe damage.

Boyland et al. (1964) implanted naphthalene into the bladder of stock Chester Beatty mice and examined them after 30 weeks in an effort to determine the suitability of naphthalene as a potential vehicle for carcinogenicity testing. The original number of mice implanted with naphthalene was not reported, but 23 mice were reported to have survived 30 weeks. One mouse developed a bladder carcinoma (1/23; 4%); no adenomas or papillomas were found. Tumor incidence was as low as when paraffin wax was used (2%-4%) and lower than with the implantation of cholesterol (12%). There are limitations of this study that make it an inadequate lifetime cancer bioassay, including the short exposure and observation periods and the lack of untreated controls.

Coal-tar-derived naphthalene that contained approximately 10% unidentified impurities was tested for carcinogenicity by Knake (1956). White rats (40, sex unspecified) were given seven subcutaneous injections of 0 or 500 mg/kg naphthalene in sesame oil at 2-week intervals over an approximate 3.5-month period. Thirty-four of 38 treated rats and 32/38 control rats survived the injection period. Survival was somewhat reduced in the naphthalene-exposed rats compared with the vehicle-control rats during the following 18-month period. Survival incidences at 6, 11, and 17 months after the injection period were 21/34, 6/34, and 0/34 for the naphthalene-exposed rats and 17/32, 12/32, and 4/32 for the control rats. Lymphosarcomas were found in 5/34 (14.7%) exposed rats during the 18-month observation period; one exposed rat showed a mammary fibrosarcoma. Vehicle controls showed a 6% (2/32) incidence of tumors (one with lymphosarcoma and one with mammary fibrosarcoma). Mice (25, inbred black) were painted with 0.5% naphthalene in benzene 5 days/week for life; 21 control mice were painted with benzene alone. Four treated mice developed lymphomatic leukemia, three had lung adenomas, one had lymphosarcoma, and one had a nonspecified tumor (9/25 with tumors). In the benzene controls, one had lymphosarcoma, one had lung adenoma, and one had a nonspecified tumor (3/21 with tumors). These studies are limited for the assessment of carcinogenicity due to the presence of unknown impurities that may have carcinogenic properties. Moreover, the vehicle (benzene) in the mouse study has been shown to cause leukemia in humans and rodents, and the site of injection in the rat study was painted, prior to injection, with carbolfuchsin, a known carcinogen.

La Voie et al. (1988) administered naphthalene dissolved in dimethylsulfoxide via the intraperitoneal route in doses of 0.25, 0.50, and 1.0 μmol to male and female newborn CD-1 mice on days 1, 8, and 15 of life (total dose = 1.75 μmol naphthalene). The report did not specify the purity of the naphthalene tested. Forty-nine pups were treated with naphthalene, and 46 control pups were treated with dimethylsulfoxide alone. Mice were maintained (10 mice/cage) until moribund or until 52 weeks when survivors were killed. All gross lesions as well as liver sections from all mice were examined histologically. No statistically significant increased incidence of liver tumors (adenomas or hepatomas) was found in the exposed mice. Reported incidences for the

number of mice with liver tumors were (denominators are for the number of mice that lived at least 6 months): 0/16 and 2/31 for exposed females and males, and 0/21 and 4/21 for vehicle-control females and males. This assay is inadequate to assess the carcinogenicity of lifetime exposure to naphthalene because the exposure period (2 weeks) and observation period (52 weeks) were significantly less than the lifetime for mice (approximately 2 years), and complete histological examinations were not conducted.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES

In developmental toxicity studies, naphthalene was administered by gavage to pregnant animals during gestation, and little evidence was found of naphthalene fetal developmental toxicity. Signs of maternal toxicity (e.g., decreased body weight gain, lethargy) without fetal effects were found in a rat study (NTP, 1991) and a rabbit study (NTP, 1992b). Other studies were conducted at dose levels that either produced increased maternal mortalities (mice: Plasterer et al., 1985; rabbits: NTP, 1990) or no maternal or fetal effects (rabbits: Naismith and Matthews, 1985).

In a developmental study in Sprague-Dawley CD rats, naphthalene in corn oil was administered by gavage to groups of 25-26 pregnant females at daily dose levels of 0, 50, 150, or 450 mg/kg on gestational days 6-15 (NTP, 1991). Dams were examined daily for clinical signs of toxicity until sacrifice at gestation day 20. Fetuses were examined on gestation day 20 for gross, visceral, and skeletal malformations. Fetal development was essentially unaffected, but significant maternal toxicity was observed. Two dams died in the low-dose group, but no deaths occurred in the higher dose groups. Exposed dams displayed generalized depression in their activities during the first 5 days of dosing. This behavior was evident in 81% of the dams in the low-dose group and 96% of mid- and high-dose dams on the first day of dosing. By the third day of dosing, this behavior had disappeared in the low-dose group suggesting that tolerance to these effects had developed. The mid-dose group no longer displayed signs of central nervous system depression on the sixth day of dosing. The incidence of naphthalene-induced lethargy and slow breathing in the high-dose group declined with continued exposure, but never dropped below 15%. Rooting behavior, noted by the authors to be commonly observed in rodents after gavage administration of agents with noxious odor or local irritation activity, occurred in less than 10% of exposed animals during the first few days of dosing, but gradually increased as the study progressed. By gestation day 15, rooting behavior was observed in 92%, 24%, 0%, and 0% of the rats in the 450, 150, 50, and 0 mg/kg groups, respectively.

Statistically significant decreased body weight and body weight gain were observed in dams of the two highest dose groups; the effects on body weight were associated with decreased food and water consumption. No significant differences were observed between exposed groups and controls in maternal liver weights and gravid uterine weights. No treatment-related effects were noted in the number of corpora lutea per dam, the number of implantation sites per litter, the number of live fetuses per litter, the incidences of resorption or the incidence of late fetal deaths. Statistically significant tests for trends with increasing dose were demonstrated for adversely affected implants per litter and for average fetal body weight per litter, but an analysis of variance

did not detect a significant overall effect of dose on these parameters. No unequivocal exposure-related effects on visceral or skeletal fetal development were found. The authors concluded that the highest dose (450 mg/kg) was a NOAEL for fetal developmental effects and that the lowest dose level in the study (50 mg/kg) was a LOAEL for maternal toxicity; reductions in maternal weight gain and food consumption were observed at 150 and 450 mg/kg.

In a range-finding developmental toxicity study in New Zealand white rabbits, groups of 9-10 does were given gavage daily doses of 75, 150, 300, or 500 mg/kg naphthalene in corn oil from gestation days 6-18 (NTP, 1990). Maternal mortalities occurred in the 500 mg/kg (approximately 78%), 300 mg/kg (approximately 40%), and 150 mg/kg (approximately 40%) dose groups. Clinical signs of maternal toxicity were reported to occur in all dose groups, including decreased weight, bloody vaginal discharge, diarrhea, abortions, and lethargy. Gross examination of litters in surviving does on gestation day 30 showed no signs of fetal toxicity. Further details for this study are not available currently.

Subsequently, NTP (1992b) administered naphthalene in corn oil by gavage to groups of 25-27 pregnant New Zealand white female rabbits at daily dose levels of 0, 20, 80, or 120 mg/kg on gestational days 6-19 (NTP, 1992b). Dams were examined daily for clinical signs of toxicity until sacrifice at gestation day 30. Fetuses were examined on gestation day 30 for growth status, viability, and morphological development (gross, visceral, and skeletal malformations or variations). No maternal mortality was found in the control or treated groups, and each group contained 20-23 pregnant does at necropsy. Clinical observation revealed no consistent treatment-related signs of maternal toxicity. A comparable occurrence of diarrhea, observed in control and treated groups, was attributed to the corn oil vehicle. No statistically significant differences between the control and treated groups were found in maternal body weight at all gestational ages, maternal corrected gestational weight gain, or maternal food consumption. Likewise, no statistically significant differences between the control and treated groups were found in average live litter size, average fetal body weight, or incidence of malformations or variations on a per fetus or litter basis. The highest dose in this study, 120 mg/kg, was a NOAEL for both maternal and fetal developmental toxicity in New Zealand white rabbits.

In a range-finding developmental toxicity study in New Zealand white rabbits, groups of four does were given gavage daily doses of 0, 50, 250, 630, or 1,000 mg/kg naphthalene in 1% methylcellulose from gestation days 6-18 (Naismith and Matthews, 1985). All of the high-dose does died. Mortality and decreased weight gain occurred in 2/4 does at 630 mg/kg; survivors at this dose aborted their pregnancies. At the two lower dose levels, no exposure-related changes were observed in numbers of early resorption, postimplantation losses, number of corpora lutea, fetal survival, or gross fetal structural development.

In the subsequent developmental toxicity study in New Zealand white rabbits, groups of 18 does were given gavage daily doses of 0, 40, 200, or 400 mg/kg naphthalene in 1% methylcellulose from gestation days 6-18 (Naismith and Matthews, 1986). Cesarean sections were performed on does at gestational day 29. Maternal survival, body weights, and body weight gains were not affected by exposure. Treatment-related clinical signs observed in the 200 and 400 mg/kg groups included dyspnea, cyanosis, body drop, and decreased activity and salivation

(incidences were not reported). Reproductive and developmental parameters (including number of corpora lutea, total number of implantations, viable or nonviable fetuses, pre- or postimplantation loss, fetal body weights, and fetal sex distribution) were not affected by exposure. Examination of fetuses for visceral and skeletal abnormalities revealed no exposure-related effects on fetal development.

In a developmental toxicity study in CD-1 mice, pregnant females were given eight consecutive daily gavage doses of 0 or 300 mg/kg naphthalene in corn oil starting on day 7 of gestation (Plasterer et al., 1985). The control and exposed groups contained 40 and 33 mice, respectively. Deaths occurred in 5/33 exposed dams during the dosing period; all control dams survived and delivered litters. Clinical observations for signs of toxicity were not reported. Natural deliveries occurred in 26/28 exposed dams that survived the treatment. Average weight gain during gestation was significantly decreased in exposed dams compared with controls. The average number of live pups per litter in the exposed group was significantly smaller than that in the control group, but average body weight of the pups was not affected by exposure. Examination of the pups for gross structural abnormalities revealed no exposure-related effects. The 300 mg/kg level appeared to produce frank maternal (deaths and decreased weight gain in survivors) and fetal (decreased number of live pups per litter) effects.

4.4. OTHER STUDIES

4.4.1. Cancer/Noncancer Studies Related to Lung

Subacute repeated injections do not appear to cause bronchiolar epithelial damage in mice. O'Brien et al. (1989) reported that, following administration of seven daily injections of 0, 50, 100, or 200 mg/kg naphthalene to mice, no significant morphological changes were found in the lungs of exposed mice compared with controls. In this study, a single 300 mg/kg dose of naphthalene produced substantial bronchiolar epithelial damage. Seven daily doses of 200 mg/kg provided what was only described as "significant protection" against a single 300 mg/kg intraperitoneal injection given on day 8. Further details were not provided regarding the extent of histological examination or the results in this study. Buckpitt and Franklin (1989) suggested that these results are consistent with the development of tolerance with repeated exposure to low doses of naphthalene. Lakritz et al. (1996) extended the work of Buckpitt and Franklin (1989) on Clara cell tolerance to multiple doses of naphthalene. The study quantitatively defined alterations in the epithelial populations and expression of cytochrome P-450 monooxygenases in distal airways that are associated with the tolerance resulting from repeated exposure of mice to the acute Clara cell toxicant, naphthalene. The author concluded that after repeated exposure to short-term cytotoxic doses of naphthalene, distal bronchioles of tolerant mice are lined by epithelium that resembles that of controls; have reduced levels of naphthalene monooxygenase activity, but only at less than saturating concentrations; and have no decrease in covalent binding of reactive metabolites to proteins. Last, a study on the cellular response in naphthalene-induced Clara cell injury and bronchiolar epithelial repair in mice by Van Winkle et al. (1995) concluded that bronchiolar epithelial repair after naphthalene injury involves distinct phases of proliferation

and differentiation, proliferation of cells that are not differentiated Clara cells, and interaction of multiple cell types, including nontarget cells.

4.4.2. Studies With Methylnaphthalene

Results from mouse studies with orally administered 1- or 2-methylnaphthalene or a dermally applied mixture of 1-methylnaphthalene and 2-methylnaphthalene suggest that the lung is the target organ.

In a preliminary subchronic study, six groups of 10 B6C3F1 mice of each sex were given diets containing 0%, 0.0163%, 0.049%, 0.147%, 0.44%, or 1.33% 1-methylnaphthalene (> 97% pure) for 13 weeks (Murata et al., 1993). It was reported that no histopathological lesions were detected in any organs in any of the groups. Growth retardation associated with decreased food intake in mice of both sexes in the two highest groups was the only adverse effect noted in this 13-week study. Food intake data were not reported by Murata et al. (1993), but the following estimates of administered daily doses were calculated assuming an average daily dose of 142 mg/kg for the 0.147% group, based on the reported intake for the 0.15% group in the 81-week study and proportional changes with the other dose groups: 0, 16, 47, 142, 425, and 1,259 mg/kg. Actual doses for the two highest dose groups likely were lower than these estimates, since mice in these groups consumed food at a lower rate. Murata et al. (1993) did not specify the degree to which the food consumption rates were decreased.

Murata et al. (1993) gave groups of 50 male and 50 female B6C3F1 mice (6 weeks old at start) diets containing 0%, 0.075%, or 0.15% 1-methylnaphthalene (> 97% pure) for 81 weeks. After 81 weeks, all surviving mice were sacrificed. Major organs and tissues were weighed and fixed for histological examination. No exposure-related changes in growth or survival were found. From average 81-week total intakes reported by the authors (41.6 and 80.5 g/kg body weight), average daily doses were calculated to be 73 mg/kg for the 0.075% groups and 142 mg/kg for the 0.15% groups.

For the 81-week study, statistically significant increases in incidence of mice with bronchiolar/alveolar adenomas or combined adenomas and adenocarcinomas were found in the exposed male groups, but not in female groups, compared with control incidences. Incidences for adenomas were 4/50, 2/50, and 4/49 for control, 0.075%, and 0.15% females, respectively, and 2/49, 13/50, and 12/50 for the respective male groups. Adenocarcinomas were found in an additional three males in the 0.15% group, one additional female in the 0.15% group, and one additional female in the control group. No statistically significant exposure-related increases in tumor incidence were found at other sites. Adenomas were described as “clearly demarcated nodules consisting of relatively uniform cuboidal atypical cells forming either papillary patterns or solid nests”; adenocarcinomas were “noncircumscribed nodular lesions,” sometimes showing “cuboidal or columnar cells forming either tubular or papillary patterns” (Murata et al., 1993). Statistically significant increased incidences of pulmonary alveolar proteinosis were found in exposed groups of both sexes compared with controls.

Histological descriptions of the pulmonary alveolar proteinosis noted that "the alveolar lumens were filled with acidophilic amorphous material, foamy cells, and cholesterol crystals. Alveolar walls and epithelial cells were generally intact and no prominent edema, alveolitis, lipidosis, or fibrosis was observed in the interstitium" (Murata et al., 1993). Incidences for pulmonary alveolar proteinosis were 5/50, 23/50, and 17/49 for control, 0.075%, and 0.15% females and 4/49, 23/50, and 19/50 for control, 0.075%, and 0.15% males (Murata et al., 1993). No other statistically significant exposure-related changes in incidence of nonneoplastic lesions were found in other organs or tissues.

Murata et al. (1997) gave groups of 50 male and 50 female B6C3F1 mice (6 weeks old at start) diets containing 0%, 0.075%, or 0.15% 2-methylnaphthalene (> 97% pure) for 81 weeks. After 81 weeks, all surviving mice were sacrificed. Major organs and tissues were weighed and fixed for histological examination. No exposure-related changes in growth or survival were found. From average 81-week total intakes reported by the authors (29.65 and 62.75 g/kg body weight), average daily doses were calculated to be 52.3 mg/kg-day for the 0.075% groups and 110.7 mg/kg-day for the 0.15% groups.

For the 81-week study, statistically significant increases in incidences of mice with bronchiolar/alveolar adenomas and adenocarcinomas were found in the exposed male groups, but not in female groups, compared with control incidences. The only site where the tumor incidence exhibited a significant increase compared with controls was the lung of 2-methylnaphthalene-treated males. The incidence of total lung tumors, including adenomas and adenocarcinomas, was significantly increased in male mice given 0.075%, but not 0.15%, 2-methylnaphthalene in the diet ($p < 0.05\%$). The incidence for total lung tumors (adenomas and adenocarcinomas) were 2/49 (4.1%), 10/49 (20.4%), and 6/49 (12.2%) for control, 0.075%, and 0.15% males. No statistically significant exposure-related increases in tumor incidence were found at other sites. The histological finding of adenomas and adenocarcinomas were similar to those reported previously (Murata et al., 1993).

Macroscopic and histological findings of pulmonary alveolar proteinosis were similar to those reported previously (Murata et al., 1993). Pulmonary alveolar proteinosis developed in mice fed 0.075% and 0.15% 2-methylnaphthalene at incidences of 55.1% and 45.8%, respectively, in females and 42.9% and 46.9% in males. Other nonneoplastic lesions were only observed at very low incidences, and no significant differences were observed between 2-methylnaphthalene-treated and control groups.

Pulmonary alveolar proteinosis was induced in all mice in a group of 30 female B6C3F1 mice given twice weekly dermal applications on their backs of 119 mg/kg methylnaphthalene in acetone for 30 weeks (Murata et al., 1992). Fifteen control mice treated with acetone showed no signs of pulmonary alveolar proteinosis. The test material was specified as a mixture of 1-methylnaphthalene and 2-methylnaphthalene, but the relative proportions of the two isomers in the mixture were not reported. Gross examination of the lungs of exposed animals revealed "multiple, grayish white soft spots or nodules sharply demarcated from the pinkish-white surrounding normal tissue, without specific localization"; such gross lesions were not found in control lungs. Light microscopy showed the alveoli to be "filled with amorphous eosinophilic

material, many mononucleated cells with abundant foamy cytoplasm, and many clefts corresponding to cholesterol crystals separating the intra-alveolar materials and the lining cells." Alveolar walls were thickened without prominent fibrosis. Electron microscopy revealed that alveolar spaces contained extracellular membranous material ("myelinoid structures") and mononucleated giant cells ("balloon cells") containing myelinoid structures, lipid droplets, and amorphous crystals. Terminal bronchioles were not markedly affected. Based on these findings, Murata et al. (1992) hypothesized that initial damage to type I pneumocytes by methylnaphthalenes or metabolites was followed by compensatory hyperplasia and hypertrophy of type II pneumocytes and the eventual development of balloon cells that liberated numerous myelinoid structures when they ruptured.

The mouse noncancer lung response to chronically inhaled naphthalene (pulmonary inflammation) (NTP, 1992a) and the response to chronically ingested 1- or 2-methylnaphthalene (alveolar proteinosis) have similarities and differences in histological features. The main similarity between pulmonary inflammation from inhaled naphthalene and alveolar proteinosis from ingested naphthalene is that descriptions of both include signs of cell exudation into intra-alveolar regions, accompanied with the presence of large foamy macrophages and giant cells; however, the giant cells were multinucleated with inhaled naphthalene and mononucleated with 1-methylnaphthalene. Other differences include the findings that inflammation from inhaled naphthalene showed interstitial fibrosis and foci of alveolar epithelial hyperplasia, whereas proteinosis from ingested 1- or 2-methylnaphthalene showed no "prominent" interstitial fibrosis. The descriptions of alveolar proteinosis from ingested 1- or 2-methylnaphthalene or a dermally applied mixture of both isomers do not mention alveolar epithelial hyperplasia; however, Murata et al. (1992, 1993) proposed that hyperplasia of type II pneumocytes was involved in the response. Both compounds likely produce their effects via reactive metabolites, but it is uncertain if the noted differences in histological response are associated with differences in route of exposure or metabolic profiles or in chronic inflammatory responses to different "internal doses."

The present results, that 2-methylnaphthalene exhibited no clear carcinogenic potential to the lung in spite of inducing slightly higher incidences of pulmonary alveolar proteinosis compared with 1-methylnaphthalene (Murata et al., 1993), suggest that proteinosis is not a risk factor for the genesis of lung cancer.

Further research is needed to determine how and if the chronic noncancer lung effects of ingested 1-methylnaphthalene and 2-methylnaphthalene (alveolar proteinosis with no signs of epithelial necrosis or fibrosis), the chronic noncancer lung effects from inhaled naphthalene (alveolar inflammation and alveolar epithelial hyperplasia), and cancer lung effects of 1-methylnaphthalene and naphthalene are mechanistically related to each other and to the acute response to intraperitoneal injection with 1-methylnaphthalene and naphthalene (bronchiolar epithelial necrosis and hyperplasia).

4.4.3. Other Cancer Studies

Schmähl (1955) reported that naphthalene repeatedly administered by subcutaneous or intraperitoneal injection did not produce tumors in rats (in-house strains BDI and BDIII). Groups of 10 rats were given either subcutaneous or intraperitoneal weekly injections of naphthalene in oil (20 mg/rat per injection) starting at 100 days of age and continuing for 40 weeks (the total doses were 820 mg/rat). Rats were maintained until they died naturally. Lifespans were reported to be 700 or 900 days for rats with subcutaneous or intraperitoneal doses, respectively. Autopsies were performed on dead animals, and organs that appeared unusual were examined histologically (the report did not specify which organs were examined, if any). The author reported that no toxic effects were found with parenteral administration of naphthalene. No tumors developed in either group. Reported information on control rats was restricted to the statement that lifespan for exposed rats was similar to lifespan for control rats (700 days with subcutaneous doses and 900 days with intraperitoneal doses).

4.4.4. Genotoxicity Studies

Naphthalene and its metabolite has been extensively evaluated for potential genotoxicity. Negative results were found in many studies. The available data suggest that the genotoxic potential of naphthalene and/or metabolites is weak, at best.

Naphthalene was not mutagenic in *Salmonella typhimurium* assays in the presence or absence of liver metabolic preparations (Bos et al., 1988; Connor et al., 1985; Florin et al., 1980; Godek et al., 1985; McCann et al., 1975; Nakamura et al., 1987; Narbonne et al., 1987; NTP, 1992a; Sakai et al., 1985). Naphthalene did not damage DNA (as assayed by the induction of the SOS-repair system) in *Escherichia coli* PQ37 (Mersch-Sundermann et al., 1993).

NTP (1992a) found that naphthalene induced, in cultured Chinese hamster ovary cells, sister chromatid exchanges within a concentration range of 27-90 μg/mL in the presence or absence of metabolic activation and chromosomal aberrations within a range of 30-67.5 μg/mL only in the presence of metabolic activation.

Naphthalene was mutagenic in the marine bacterium *Vibrio fischeri* (Arfsten et al., 1994) and in the *Drosophila melanogaster* wing somatic mutation and recombination test (Delgado-Rodriguez et al., 1995). Culture of mouse embryos in medium containing 0.16 mM naphthalene produced a 10-fold increase in chromosomal damage compared with untreated controls; the genotoxic response to naphthalene was amplified by the inclusion of a hepatic metabolic activation system in the medium (Gollahon et al., 1990).

Incubation of human peripheral lymphocytes in medium containing naphthalene and a human liver metabolic activation system did not produce increased frequency of sister chromatid exchanges compared with controls (Tingle et al., 1993; Wilson et al., 1995). Naphthalene did not induce unscheduled DNA synthesis in cultured rat hepatocytes (Barfknecht et al., 1985) or increased numbers of micronuclei in bone marrow cells of mice following intraperitoneal injection

of single 250 mg/kg doses (Sorg et al., 1985). Single oral doses of naphthalene as high as 500 mg/kg did not increase the frequency of micronucleated erythrocytes in exposed mice compared with untreated control mice (Harper et al., 1984). Naphthalene did not induce in vitro transformations of Fischer rat embryo cells (Freeman et al., 1973) or Swiss mouse embryo cells (Rhim et al., 1974). Sina et al. (1983) reported that naphthalene did not induce single-strand DNA breaks in cultured rat hepatocytes as detected by alkaline elution.

Naphthalene metabolites, 1-naphthol and 2-naphthol, were not mutagenic in *S. typhimurium* with or without metabolic activation (Florin et al., 1980; McCann et al., 1975; Narbonne et al., 1987). Another proposed naphthalene metabolite, naphthoquinone, was not mutagenic in several strains of *S. typhimurium* with or without metabolic activation (Sakai et al., 1985), but Flowers-Geary et al. (1994) reported that naphthalene-1,2-dione was mutagenic in strains of *S. typhimurium* without metabolic activation. The naphthalene metabolite 1-naphthol failed to produce positive results in several other genotoxicity assays, including tests for sex-linked recessive lethal mutations in *D. melanogaster* (Gocke et al., 1981), mutations in mouse L5178Y cells (Amacher and Turner, 1982), unscheduled DNA synthesis in cultured rat hepatocytes (Probst and Hill, 1980), and induction of micronuclei in bone marrow cells of mice (Gocke et al., 1981) and rats (Hossack and Richardson, 1977) after acute in vivo exposure.

Tsuda et al. (1980) found no evidence for neoplastic transformation of liver cells in a group of 10 young adult Fischer 344 rats (sex not specified) treated with single gavage doses of 100 mg/kg naphthalene in corn oil compared with a group of 10 vehicle control rats. Rats were given gavage doses of naphthalene or vehicle following partial hepatectomy, but before dietary treatment with 2-acetylaminofluorene and another carcinogen, carbon tetrachloride. Gamma-glutamyl transpeptidase foci (observed following the dietary treatments of exposed and control rats) were used as an indicator of neoplastic transformation. In contrast to naphthalene, a single gavage dose of 200 mg/kg benzo[a]pyrene induced significant increases in the number, area, and size of gamma-glutamyl transpeptidase foci. (Time of study after treatment was not given.)

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

4.5.1. Inhalation

In humans, naphthalene has been found to cause hemolyte anemia and cataract formation at high concentrations (from accidental exposure and occupational exposure). However, information is not available regarding dose-response relationships for these effects in humans with either acute, subchronic, or chronic exposure by any route.

Studies in which animals were exposed to naphthalene by inhalation are restricted to a 2-year mouse study (NTP, 1992a), a 6-month mouse study (Adkins et al., 1986), and an acute (4-hour) mouse study (Buckpitt, 1982). As reviewed earlier, nasal and pulmonary lesions were

the only noncancer effects found in these studies. No exposure-related histological changes in other tissues (including cataracts) were found in the 2-year study. Thus, nasal and pulmonary effects are identified as the critical effects, at least in mice, from chronic inhalation exposure to naphthalene. Cataracts and hemolytic anemia were not found in exposed mice.

As discussed earlier in this Toxicological Review, health hazards of concern from exposure to naphthalene include hemolytic anemia, cataracts, and respiratory toxicity towards the respiratory tract (both noncancer and cancer effects). An overview of current understanding of the mechanisms involved in these effects follows. In general, naphthalene toxicities are thought to involve steps of bioactivation leading to electrophilic and/or reactive free radical reactive intermediates.

Naphthalene metabolites are generally thought to be involved in naphthalene-induced hemolytic anemia, but molecular mechanisms specific to naphthalene-induced hemolysis are not clearly understood (U.S. EPA, 1987; ATSDR, 1993). Humans experience hemolysis after naphthalene exposure by inhalation, oral, and dermal routes. In general, animals are less susceptible than humans. There are no reports of naphthalene-induced hemolysis in either rats or mice; however, hemolysis has been observed in dogs. Persons deficient in glucose-6-phosphate dehydrogenase (G6PDH) are particularly sensitive to naphthalene hemolysis due to the fact that G6PDH-deficient cells cannot quickly replenish the reduced glutathione (Dawson et al., 1958; Gosselin et al., 1984). One mechanistic hypothesis for this effect proposes that the decreased ability to maintain cellular nicotinamide adenine dinucleotide phosphate (NADPH) levels decreases the availability of reduced glutathione. Deficits in reduced glutathione levels are thought to decrease the rate of conjugation and excretion of naphthalene metabolites, thereby leading to increased levels of toxic naphthalene metabolic intermediates (U.S. EPA, 1987). The continued glutathione depletion weakens cell membranes and causes hemoglobin to become unstable and ultimately leads to hemolysis of red blood cells. Other possible causes of hemolysis include possible inhibition of either glutathione peroxidase or glutathione reductase by a naphthalene metabolite (Rathbun et al., 1990; Tao et al., 1991b). Neonates are also sensitive to naphthalene-induced hemolysis. Valaes et al. (1963) proposed that their sensitivity is due to immaturity of the detoxification pathways responsible for the conjugation and excretion of naphthalene metabolites.

Wells et al. (1989) proposed that naphthalene cataractogenesis in C57BL mice (from intraperitoneal injection) requires cytochrome P-450-catalyzed bioactivation to a reactive intermediate. Furthermore, the authors proposed that naphthoquinones or free radical derivatives are the proximate cataractogens. This hypothesis is based on observations that the incidence of naphthalene-induced cataracts in animals exposed to naphthalene was decreased by pretreatments with either cytochrome P-450 inhibitors, antioxidants, a glutathione precursor, or a free radical spin trapping agent. In addition, molar potencies for inducing cataracts in C57BL mice by 1,2- and 1,4-naphthoquinones were about 10-fold higher than naphthalene. No cataracts were formed in DBA/2 mice by treatments (e.g., naphthalene, naphthoquinones) that produced cataracts in C57BL mice. Wells et al. (1989) proposed that species and strain differences in susceptibility to naphthalene cataractogenicity may involve differences in naphthalene metabolism or differences in biochemical pathways of cytoprotection, repair, or other responses. Based on

results from in vivo experiments with rats given gavage doses of naphthalene and in vitro experiments with lenses of rats incubated with naphthalene and various naphthalene metabolites, Xu et al. (1992a,b) hypothesized that naphthalene dihydrodiol formed in the liver from naphthalene in the liver reaches the aqueous humor of the eye and penetrates into the lens where it is metabolized to form the putative proximate cataractogen, naphthoquinone.

Damage to nasal and/or alveolar/bronchiolar epithelial cells has been observed after acute intraperitoneal injection of naphthalene or methyl naphthalenes (1-methylnaphthalene or 2-methylnaphthalene) in mice, chronic inhalation exposure of mice to naphthalene, and chronic oral exposure of mice to 1- and 2-methylnaphthalenes. In addition, a predominantly benign tumorigenic response in the alveolar/bronchiolar region has been found in female mice and in male mice after chronic dietary exposure to 1-methylnaphthalene, but not 2-methylnaphthalene. (see Section 4.2 for review of studies).

Differences in sensitivity to naphthalene-induced respiratory effects have been found among animal species. Mice appear to be much more sensitive to naphthalene-induced pulmonary cytotoxicity than hamsters or rats (Buckpitt and Franklin, 1989; Buckpitt et al., 1992; Plopper et al., 1992a,b). Animal studies have linked naphthalene and methylnaphthalene toxicity in the lung to cytochrome P-450-mediated metabolism (Warren et al., 1982; O'Brien et al., 1985; Rasmussen et al., 1986; Buckpitt and Franklin, 1989). The proximate toxicants involved in naphthalene nasal and pulmonary toxicity have not been definitively identified, but enantiomeric epoxide intermediates, naphthoquinones, and free radical reactive intermediates have been proposed to be involved (Buckpitt and Franklin, 1989). Kanekal et al. (1991) suggested that the epoxides may be primarily responsible, based on the findings that, in perfused mouse lung preparations, racemic mixtures of naphthalene epoxides produced cytotoxicity at concentrations 10-fold lower than naphthalene. Due to its chemical reactivity, the epoxide also can bind covalently to cellular macromolecules, thereby potentially leading to cellular dysfunction. This characteristic and the knowledge that other aromatic hydrocarbon epoxides are cytotoxic, genotoxic, and/or carcinogenic have led to the hypothesis that 1,2-naphthalene epoxide is, at least partly, responsible for naphthalene toxicities. However, other metabolites such as 1-naphthol, 1,2-naphthoquinone or 1,4-naphthoquinone were not cytotoxic at concentrations approximately equal to effective naphthalene concentrations. Buckpitt et al. (1992) found that mouse lung microsomes metabolized naphthalene at rates approximately 92-fold greater than rates measured with rhesus monkey lung microsomes and that the predominant enantiomeric naphthalene epoxide formed by monkey (1S,2R-naphthalene oxide) was different from that formed by mouse (1R,2S-naphthalene oxide). Rat and hamster lung microsomes showed results for naphthalene metabolic rates and stereochemistry that were intermediate between those found with mice and monkey microsomes. Buckpitt and Bahnson (1986) found earlier that human lung microsomes showed metabolic activities similar to those measured with monkey lung microsomes (i.e., a slow rate and predominant formation of the 1S,2R-epoxide enantiomer). Buckpitt et al. (1992) suggested that these results are consistent with the hypothesis that primates may not be as susceptible as the mouse to the pulmonary toxicity of naphthalene, but they cautioned that "additional studies are needed to either support or refute the view that the rate and stereochemistry of naphthalene epoxidation is a critical factor in determining cell- and species-dependent naphthalene cytotoxicity."

Administration of naphthalene produces a dose-dependent and pulmonary region- (the most susceptible site is the mouse distal bronchioles), species- (mice > hamsters and rats), and cell-selective lesion of murine Clara cells. Buckpitt et al. (1995) studied correlation of Clara cell cytotoxicity with the metabolism in different airway regions of different rodents. This study reported that the rate and stereoselectivity of naphthalene metabolism by microsomal preparations correlate with pulmonary tissue and species differences in cytotoxicity. Obtained by microdissection, specific subcompartments of the pulmonary system were used to study the cytochrome P-450-dependent metabolism of naphthalene and the epoxide hydrolase/glutathione transferase-dependent metabolism of naphthalene oxide. The study reported that the rates of naphthalene metabolism were substantially higher in mouse airways than in comparable airways of hamsters or rats. Rates of metabolism were higher in distal airways than in the trachea of all species studied. Metabolism in mouse airways was highly stereoselective, whereas that in hamster and rat tissues was not. Nonciliated cells at all airway levels in mice were heavily labeled with an antibody to cytochrome P-450 2F2; little labeling was observed in any portion of rat and hamster lungs. Postmitochondrial supernatants prepared from mouse and hamster airways metabolized racemic naphthalene oxide to diol and glutathione adducts at substantially higher rates than did comparable preparations from rats. This study supports the view that the rate and stereoselectivity of naphthalene metabolism to naphthalene 1R,2S-oxide catalyzed by cytochrome P-450 2F2 are critical determinants in the species-specific and region-selective cytotoxicity of naphthalene in mice, with the most susceptible site as the mouse distal bronchioles.

The observation that intraperitoneal administration of either naphthalene, 2-methylnaphthalene, or 1-methylnaphthalene leads to Clara cell necrosis in the bronchiolar epithelium of mice (Buckpitt et al., 1992; Rasmussen et al., 1986) supports the assumption that the mouse lung can be the target from naphthalene and methylnaphthalenes exposures via noninhalation routes. Studies of the metabolism of naphthalene and 2-methylnaphthalene indicate that both are metabolized via ring epoxidation pathways involving cytochrome P-450 monoxygenases; however, 2-methylnaphthalene can also be metabolized via methyl group oxidation (see Buckpitt and Franklin, 1989). Studies of the metabolism of 1-methylnaphthalene were not located in the literature searched, but it seems reasonable to assume that, like 2-methylnaphthalene metabolism, both ring-epoxidation and methyl-group-oxidation pathways are involved in 1-methylnaphthalene metabolism. Buckpitt and Franklin (1989) reported that pretreatment of mice with inhibitors of cytochrome P-450 (piperonal butoxide, SKF 525-A) protected against pulmonary damage from intraperitoneal injection of naphthalene, but not against damage from 2-methylnaphthalene. Comparable experiments with 1-methylnaphthalene were not located. Further research is necessary to (1) determine how and if differences in metabolism between naphthalene and methylnaphthalenes influence their relative cytotoxic and carcinogenic potency with oral exposure and (2) clarify the similarities and differences in the mechanisms by which naphthalene and methylnaphthalenes produce pulmonary toxic effects.

The extensive research on the rodent pulmonary response to parenteral administration of naphthalene or methylnaphthalenes suggests that the observed responses to chronic naphthalene inhalation exposure are due to reactive metabolic intermediates that covalently modify cellular macromolecules leading to cellular dysfunction and/or that are involved in the production of tissue-damaging oxidative stress. Results from in vitro metabolic studies with lung microsomes

suggest that naphthalene metabolic rates in mice may be greater than those in primates (or other rodent species, hamsters and rats, that are less sensitive to naphthalene pulmonary toxicity) and that the types of metabolites may differ between mice and primates (Buckpitt and Bahnson, 1986; Buckpitt et al., 1992). These results provide suggestive evidence that primates may be less sensitive to naphthalene pulmonary toxicity than mice. Nevertheless, the proximate nasal or pulmonary toxicants have yet to be identified definitively, and no in vivo studies exist that allow a comparison of primate and murine sensitivities to naphthalene nasal or pulmonary toxicity.

In vitro studies by Kawabata and White (1990) on the effect of naphthalene and metabolites on the antibody-forming cells response of splenic cell cultures to sheep red blood cells did not demonstrate an immunosuppressive effect by naphthalene. This study, along with an in vivo study that screened the ability of 15 polycyclic aromatic hydrocarbons separately to suppress antibody response in C57B1/6 ($Ah_{+/+}$) mice immunized after a single oral dose (Silkworth et al., 1995), demonstrated that naphthalene had little or no immunosuppressive effect and supports the contention by Shopp et al. (1984) that naphthalene is not immunosuppressive.

4.5.2. Oral

Available data regarding naphthalene-induced noncancer effects in orally exposed animals and associated dose levels are summarized in Table 1. A notable deficiency in the animal database is the lack of any adequate chronic (lifetime) oral exposure studies for naphthalene. In the only lifetime oral study available, Schmähl (1955) reported that no toxic effects occurred in rats exposed for 2 years to approximate daily doses of 42 mg/kg naphthalene. However, as discussed earlier, inadequacies in reporting of experimental details and results limit the conclusions that can be drawn from this study regarding the toxicity of naphthalene.

Distinct noncancer effects found in animals following acute or subchronic oral exposure to fairly high oral doses (> 200-700 mg/kg) include hemolytic anemia (only in dogs) and cataracts (in rats and rabbits). Three 90-day exposure studies that administered lower doses of naphthalene (< 200-400 mg/kg) found less distinct effects in rats and mice. These were body weight decreases, depression of the central nervous system, and organ weight changes. There were no histological changes in major organs or tissues; neither hemolytic anemia nor cataracts were found. In several developmental studies in which pregnant animals were exposed to gavage doses of naphthalene during gestation, signs of maternal toxicity (e.g., decreased weight gain, clinical signs of nervous system depression) were observed without distinct fetal developmental effects.

Naphthalene doses of 150 mg/kg and 300 mg/kg produced 40% and 15% maternal mortality in respective studies with New Zealand white pregnant rabbits (NTP, 1990b) and CD-1 pregnant mice (Plasterer et al., 1985). Although dose levels as high as 450 mg/kg did not affect survival in pregnant rats, doses as low as 50 and 150 mg/kg administered during pregnancy produced signs of nervous system depression and decreased weight gain, respectively (NTP, 1991). The rat data provide evidence supporting the mouse and rabbit evidence that pregnant

Table 1. Effects and associated dose levels observed in animals after subacute or subchronic oral exposure to naphthalene

Effect	Species	Dose (mg/kg) and duration (d = days)	Reference
Hemolytic anemia			
Effect observed	Dogs	about 262; 7 d	Zuelzer and Apt, 1949
No effect observed	Rats	400; 90 d	BCL, 1980a
	Mice	133-200; 90 d	BCL, 1980b; Shopp et al., 1984
Cataracts			
Effect observed	Rats, rabbits	700-2,000; 3-102 d	Van Heyningen, 1979; Fitzhugh and Buschke, 1949; Xu et al., 1992a; Tao et al., 1991a,b
No effect observed	Rats	400; 90 d	BCL, 1980a
	Mice	120-200; 60-90 d	Shichi et al., 1980; BCL, 1980b; Shopp et al., 1984
Decreased body weight			
Effect observed	Rats	200; 90 d	BCL, 1980a
	Pregnant rats	150; 10 d	NTP, 1991
No effect observed	Rats	100; 90 d	BCL, 1980a
	Mice	133-200; 90 d	BCL, 1980b; Shopp et al., 1984
	Pregnant rats	50; 10 d	NTP, 1991
	Pregnant rabbits	400; 12 d	Naismith and Matthews, 1985
	Pregnant rabbits	120; 13 d	NTP, 1992b

Table 1. Effects and associated dose levels observed in animals after subacute or subchronic oral exposure to naphthalene (continued)

Effect	Species	Dose (mg/kg) and duration (d = days)	Reference
Nervous system depression			
Effect observed (clinical signs including lethargy)	Rats	400; 90 d	BCL, 1980a
	Mice	200; 90 d	BCL, 1980b
	Pregnant rats	50; 13 d	NTP, 1991
	Pregnant rabbits	200; 12 d	Naismith and Matthews, 1986
No effect observed	Rats	200; 90 d	BCL, 1980a
	Mice	100-133; 90 d	BCL, 1980b; Shopp et al., 1984
	Pregnant rabbits	40; 12 d	Naismith and Matthews, 1986
Organ weight changes			
Effect observed, absolute (decreased brain, liver, and spleen, females only)	Mice	133; 90 d	Shopp et al., 1984
No effect observed	Mice	53; 90 d	Shopp et al., 1984
Fetal developmental toxicity			
Effect observed (decreased maternal/fetal survival)	Pregnant mice	300; 8 d	Plasterer et al., 1985
	Pregnant rabbits	150; 13 d	NTP, 1990b
	Pregnant rabbits	630; 12 d	Naismith and Matthews, 1985
No effect observed	Pregnant rabbits	400; 12 d	Naismith and Matthews, 1986
	Pregnant rabbits	120; 13 d	NTP, 1992b
	Pregnant rats	450; 10 d	NTP, 1991

animals are particularly sensitive leading to a general toxic response to oral naphthalene that is poorly understood mechanistically. However, data for rabbits are not consistent across studies. The rabbit data collected by NTP (1990, 1992b) suggest that the dose-response curve is steep. Whereas doses of 150 mg/kg produced 40% maternal mortality in a range-finding study, 120 mg/kg was without effect on maternal survival, weight gain, fetal development, or fetal survival (NTP, 1990, 1992b). Earlier studies, using the same strain of pregnant rabbits, found no adverse maternal or fetal effects with gestational doses as high as 400 mg/kg; increased maternal mortalities were found ($\geq$ 50%) with doses $\geq$ 630 mg/kg (Naismith and Matthews, 1985, 1986). The basis for the apparent difference between the two rabbit gestational exposure studies is not known.

In general, results from the oral developmental studies in rats and rabbits are consistent with the conclusion that exposure to doses $\leq$ 100 mg/kg during pregnancy produces no toxic effects in developing fetuses and no maternal effects that are unequivocally adverse. Gestational-exposure doses $\leq$ 100 mg/kg did not produce any fetal or maternal toxic effects in rabbits at 40 mg/kg (Naismith and Matthews, 1986) or in rabbits at 20, 80, or 120 mg/kg (NTP, 1992b). The only exposure-related effects noted in pregnant rats exposed to 50 mg/kg were clinical signs of central nervous system depression (i.e., lethargy, shallow respiration) that occurred only during the first 3 days of dose administration (NTP, 1991). Rooting behavior and body weight changes associated with decreased food consumption were observed in pregnant rats at higher doses ($\geq$ 150 mg/kg). Studies exposing pregnant mice to doses lower than 300 mg/kg were not available.

In the absence of adequate chronic oral data for naphthalene, adequate dose-response data for hemolytic anemia and cataracts in appropriately sensitive animal species, and consistent data regarding mortality from unspecified causes in pregnant rabbits, the available animal data in Table 1 identify body weight decreases as the most appropriate critical effect for the purpose of RfD derivation. Mean body weight decreases greater than 10% compared with control values were found in male rats following 90-day gavage exposure to 200 mg/kg-day (BCL, 1980a). The NOAEL for terminal body weight changes in this study was 100 mg/kg-day. Nervous system depression in pregnant rats observed at 50 mg/kg-day (NTP, 1991) was considered to be nonadverse due to its transient nature.

Chronic dietary exposure to 1- or 2-methylnaphthalene produced high incidence of pulmonary alveolar proteinosis in mice (Murata et al., 1993) at doses as low as approximately 73 mg/kg-day 1-methylnaphthalene or 52 mg/kg-day 2-methylnaphthalene (Murata et al., 1997).

Given that both naphthalene and the methylnaphthalenes are lung injurants following acute parenteral administration (Buckpitt and Franklin, 1989), data from the chronic methylnaphthalene studies provide limited suggestive evidence that chronic oral exposure to naphthalene may produce similar effects on the lung. The metabolic differences between methylnaphthalenes and naphthalene (see Section 3.3), and the absence of lung effects in subchronic oral studies in rats (BCL, 1980a) or mice (BCL, 1980b; Shopp et al., 1984), however, lend sufficient uncertainty to preclude derivation of an RfD for naphthalene based on the results from the studies of mice exposed chronically to 1- or 2-methylnaphthalene.

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION

As reviewed earlier, human data are inadequate to evaluate the causal association between cancer occurrence and exposure to naphthalene. East German investigators (Wolf, 1976; Kup, 1978) reported several cases of laryngeal cancer in patients occupationally exposed to naphthalene, but most of these patients were tobacco smokers. No large-scale epidemiologic studies were located examining the possible association between naphthalene exposure and cancer.

The NTP (1992a) mouse inhalation study is the only available animal bioassay adequately designed to examine the carcinogenicity of lifetime naphthalene exposure. A statistically significant tumorigenic response (elevated incidence of alveolar/bronchiolar adenomas and carcinoma) was found in female B6C3F1 mice, but not in males, exposed by inhalation to naphthalene at 30 ppm for 6 hours/day, 5 days/week for 2 years (NTP, 1992a). NTP (1992a) concluded that the study provided "some evidence" of carcinogenicity in female mice, as opposed to "clear evidence" because all but one of the observed tumors in females were benign.

A 6-month inhalation study with female A/J strain mice exposed to similar naphthalene concentrations found a statistically significant increased number of lung tumors (alveolar adenomas) per tumor-bearing lung, thus providing some support to the weight of evidence that naphthalene induces lung tumors in mice (Adkins et al., 1986).

No carcinogenic responses were observed in studies with rats treated with naphthalene in the diet for more than 2 years or with repeated intraperitoneal or subcutaneous injections (Schmähl, 1955). The Schmähl (1955) study, however, has reporting and design limitations that make it inadequate as a cancer bioassay. No marked carcinogenic responses were found in animals exposed to naphthalene by other exposure routes in several other studies (skin-painting: Kennaway, 1930; Schmeltz et al., 1978; subcutaneous injection: Knake, 1956; bladder implantation: Boyland et al., 1964; intraperitoneal injection: La Voie et al., 1988); however, as discussed earlier, each of these studies has at least one weakness making it inadequate for assessing the potential for lifetime naphthalene exposure to produce cancer.

Naphthalene was not genotoxic in many short-term genotoxicity assays (in both eukaryotic and prokaryotic systems), but genotoxic responses to naphthalene were found in five different short-term bioassays. The available data suggest that the genotoxic potential of naphthalene and/or its metabolites is weak, at best.

Exposure of mice to 1- or 2-methylnaphthalene in the diet for 81 weeks produced a statistically significant incidence of alveolar/bronchiolar adenomas and carcinomas in male, but not female, mice exposed to 1-methylnaphthalene, but no significant tumorigenic response in males or females exposed to 2-methylnaphthalene (Murata et al., 1993, 1997). The tumorigenic response in male mice to 1-methylnaphthalene was predominantly benign.

In summary, human data are inadequate to evaluate a plausible association with cancer, and the observations of benign respiratory tumors in mice exposed to naphthalene via inhalation and

to 1-methylnaphthalene via the diet are limited in nature. Overall, naphthalene is classified as a possible human carcinogen (inadequate human and limited animal data); specifically a classification of Group C using criteria of the 1986 Guidelines for Carcinogenic Risk Assessment. Using the 1996 Proposed Guidelines for Carcinogenic Risk Assessment, the human carcinogenic potential of naphthalene via the oral or inhalation routes "cannot be determined" at this time based on human and animal data; however, there is suggestive evidence of potential human carcinogenicity based on increased lung tumors incidence in one species and one sex at the high dose only. Additional support includes increased respiratory tumors associated with 1-methylnaphthalene.

The mechanism whereby naphthalene or methylnaphthalenes produce benign respiratory tract tumors and cytotoxic effects are not fully understood, but are hypothesized to involve oxygenated reactive metabolites produced via cytochrome P-450 monooxygenase system (Buckpitt and Franklin, 1989). Given that in most genotoxicity tests with naphthalene, negative results have been obtained, it appears unlikely that naphthalene represents a genotoxic carcinogen. Even though mice appear to be much more sensitive than rats to lung injurants in general and specifically to naphthalene, the National Toxicology Program initiated a 2-year inhalation bioassay with rats in March of 1996. The results should be forthcoming approximately 1999.

4.7. SUSCEPTIBLE POPULATIONS

4.7.1. Possible Childhood Susceptibility

Children, especially neonates, appear to be more susceptible to acute naphthalene poisoning based on the number of reports of lethal cases in children and infants (Gerarde, 1960; U.S. EPA, 1987). Neonates are believed to be more susceptible to naphthalene-related hemolytic anemia, because their livers are not yet fully developed and the ability of the livers to conjugate naphthalene metabolites responsible for hemolysis is limited.

4.7.2. Possible Gender Differences

Persons who are deficient in G6PDH have a deficit in reducing power (NAPDH) and in levels of reduced glutathione, which is involved in conjugation of naphthalene metabolites. These deficits make cells, particularly erythrocytes, more vulnerable to reactive naphthalene metabolites (U.S. EPA, 1987). Male members of subpopulations of Asians, Arabs, Caucasians of Latin ancestry, African Americans, and Africans have an increased probability of G6PDH deficiency and are expected to have an increased susceptibility to naphthalene-induced hemolytic anemia (U.S. EPA, 1987).

Consistent gender differences in susceptibility to other toxic effects have not been found in available animal studies with naphthalene or methylnaphthalenes. Males and females displayed equivalent incidences of nontumorous nasal and pulmonary tract lesions in mice exposed by inhalation to naphthalene for 2 years (NTP, 1992a) and of pulmonary tract lesions in mice

exposed to methylnaphthalenes in the diet (Murata et al., 1993, 1997). Statistically significant increased incidence of alveolar/bronchiolar adenomas was found in female, but not male, mice exposed to naphthalene by inhalation (NTP, 1992a). In mice exposed to 1-methylnaphthalene, alveolar/bronchiolar adenoma incidence was significantly increased in males, but not in females (Murata et al., 1993). Male and female rats both showed a dose-dependent decrease in body weight gain and terminal body weight following 90-day gavage exposure to naphthalene in corn oil, but the decrease, based on terminal body weight, became biologically significant at 200 mg/kg-day in males and 400 mg/kg-day in females.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Study and Critical Effect

As discussed in Section 4.5.2, there are no adequate chronic oral dose-response data for naphthalene in humans or animals. Case reports of acute poisonings identify hemolytic anemia and ocular effects (e.g., cataracts) as effects of concern in humans exposed orally to naphthalene, but adequate dose-response data for these effects in appropriately sensitive animal species are not available. Decreased body weight in rats appears to be the most appropriate critical effect for deriving a chronic oral RfD for naphthalene. A NOAEL/LOAEL pair of 100 and 200 mg/kg-day was found for terminal body weight decreases greater than 10% of control values in male rats exposed by gavage to naphthalene for 90 days (BCL, 1980a).

Nervous system depression in pregnant rats (NTP, 1991), occurring at a lower dose (50 mg/kg-day), was judged to be nonadverse because it was transient. Data from studies of mice exposed acutely to injections of naphthalene or 1- or 2-methylnaphthalene (Buckpitt and Franklin, 1989) or chronically to 1- or 2-methylnaphthalene in the diet (Murata et al., 1993, 1997) provide suggestive evidence that chronic oral exposure to naphthalene at low doses may produce lung injury. However, deriving an RfD for naphthalene based on the methylnaphthalene data was judged to be too uncertain because of metabolic differences between naphthalene and methylnaphthalene and because of the absence of lung injury in subchronic oral studies in rats (BCL, 1980a) and mice (BCL, 1980b; Shopp et al., 1984).

5.1.2. Methods of Analysis

5.1.2.1. *Dose Conversion*

Fischer 344 rats (10/sex/dose) received gavage doses of naphthalene in corn oil at 0, 25, 50, 100, 200, or 400 mg/kg for 5 days/week for 13 weeks (BCL, 1980a). The NOAEL/LOAEL pairs for decreased terminal body weight (> 10% decrease compared with controls) were 200/400

mg/kg-day in females and 100/200 mg/kg-day in males. Duration adjustment of the doses arrived at a critical NOAEL/LOAEL pair of 71/143 mg/kg-day for decreased body weight in male rats.

5.1.2.2. *Dose-Response Modeling*

A NOAEL/LOAEL approach for RfD derivation was taken using the male rat body weight data. Several benchmark dose approaches were examined (see Appendix B, Benchmark Dose Calculations), but these approaches do not markedly decrease uncertainty or provide a significant advantage in deriving an RfD for naphthalene based on the male rat data from the BCL (1980a) study in which both a NOAEL and LOAEL were identified.

5.1.3. RfD Derivation

The duration-adjusted NOAEL for terminal body weight decrease (> 10% of control) in male rats from the BCL (1980a) 90-day gavage study, 71 mg/kg-day, was divided by an uncertainty factor of 3,000 (10 to extrapolate from rats to humans; 10 to protect sensitive humans; 10 to extrapolate from subchronic to chronic exposure; and 3 for database deficiencies, including the lack of chronic oral exposure studies and two-generation reproductive toxicity studies) to arrive at a chronic RfD for naphthalene of 2E-2 mg/kg-day.

5.1.4. Major Alternatives for Deriving RfD

A benchmark dose approach to modeling the male rat body weight data fit mathematical models for a continuous variable to the data using maximum likelihood methods (see Appendix B). In this approach, maximum likelihood estimates of dose (with no duration adjustment) associated with a 10% decrease in mean body weight compared with nonexposure conditions were 171 and 172 mg/kg-day using a polynomial and power model, respectively; respective 95% confidence lower limits on these doses, taken as benchmark doses (BMD), were 130 and 135 mg/kg-day. Assuming that either of these BMDs are surrogates for NOAELs, as suggested by the analysis of developmental toxicity data by Allen et al. (1994a,b) and Kavlock et al. (1995), making duration adjustments (BMD × 5/7), and applying the same 3,000 uncertainty factor used for the NOAEL/LOAEL approach arrives at a prospective RfD for naphthalene, 3E-2 mg/kg-day, that is very similar to the RfD derived with the NOAEL/LOAEL approach.

Benchmark dose approaches to deriving a chronic RfD for naphthalene were also examined using data for maternal body weight decreases in the NTP (1991) rat developmental toxicity study and data for lung proteinosis in mice exposed for 81 weeks to 1-methylnaphthalene in the diet (Murata et al., 1993). Decreased maternal body weight was not selected as the basis of chronic RfD derivation, because the pregnant rats were exposed for only a small percentage of their lives. As discussed earlier, deriving the naphthalene RfD based on 1-methylnaphthalene data was judged to be too uncertain because of metabolic differences between naphthalene and methylnaphthalenes

and because of the absence of lung injury in rats and mice orally exposed to naphthalene for subchronic periods.

5.1.5. Confidence in the Chronic RfD

The principal study was given a high confidence rating because adequate numbers of animals were included and experimental protocols were adequately designed, conducted, and reported. Confidence in the database was rated low, because of the lack of adequate chronic oral data for naphthalene; the lack of any dose-response data for naphthalene-induced hemolytic anemia, probably the most well-known health hazard to humans exposed to naphthalene; and the lack of two-generation reproductive toxicity studies. Infants deficient in G6PDH are thought to be especially sensitive to naphthalene-induced hemolytic anemia. Resulting confidence in the RfD is low.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

5.2.1. Choice of Principal Study and Critical Effect

As discussed in Section 4.5.1., human experience with acute inhalation exposure and occupational exposure to naphthalene has identified hemolytic anemia and cataracts as effects of concern, but there are inadequate human data to describe dose-response relationships for these effects. Animal inhalation studies are restricted to three studies of mice: a 2-year study (NTP, 1992), a 6-month study (Adkins et al., 1986), and a 4-hour study (Buckpitt, 1982). Results from the chronic study, supported by the subchronic and acute studies, identify nasal and pulmonary injuries as critical effects from chronic inhalation exposure to naphthalene; effects in other organs or tissues were not found. Incidence data for male and female mice with hyperplasia of the nasal respiratory epithelium, metaplasia of the nasal olfactory epithelium, and chronic pulmonary inflammation clearly show that the nose is more sensitive than the lung to chronic inhalation exposure to naphthalene (see Table 2). At both exposure levels (10 and 30 ppm for 6 hours/day, 5 days/week), > 95% of mice of either sex showed nasal lesions, whereas pulmonary lesions were found in < 1/3 and < 1/2 of mice exposed at 10 and 30 ppm, respectively (Table 2). Nasal lesions in the respiratory and olfactory epithelium in mice found in the NTP (1992a) study were therefore selected as the critical effects for the purpose of RfC derivation.

5.2.2. Methods of Analysis

5.2.2.1. *Calculation of the Human Equivalent Concentration (HEC)*

5.2.2.1.1. ***Dose conversion.*** Due to naphthalene's low water solubility and low reactivity, naphthalene-related effects on the nasal epithelium are expected to result following absorption of naphthalene and metabolism to reactive oxygenated metabolites, rather than being a result of direct contact. This hypothesis is supported by data on naphthalene metabolism indicating that

Table 2. Incidence of nonneoplastic respiratory lesions in B6C3F1 mice exposed by inhalation to naphthalene for 6 hours/day, 5 days/week for 2 years

Exposure level/sex (ppm)	Respiratory lesion		
	Inflammation, lung	Hyperplasia, nasal respiratory epithelium	Metaplasia, nasal olfactory epithelium
0/Male 0/Female	0/70 3/69	0/70 0/69	0/70 0/69
10/Male 10/Female	21/69 13/65	66/69 65/65	66/69 65/65
30/Male 30/Female	56/135 52/135	134/135 135/135	134/135 135/135

Source: NTP, 1992a.

toxic effects on the respiratory tract are due to a naphthalene metabolite that may be formed either in the liver or in the respiratory tract. For example, necrosis of bronchial epithelial (Clara) cells in mice (O'Brien et al., 1985, 1989; Tong et al., 1981) and necrosis of olfactory epithelium in mice, rats, and hamsters (Plopper et al., 1992a) occurs following intraperitoneal injection of naphthalene. The nasal effects from inhalation exposure to naphthalene were considered to be extrarespiratory effects of a category 3 gas, as defined in the U.S. EPA guidance for deriving RfCs (U.S. EPA, 1994b). Following this guidance, experimental exposure concentrations were adjusted to a mg/m^3 basis (0, 52, and 157 mg/m^3), adjusted to a continuous exposure basis ($mg/m^3 \times 6h/24h \times 5d/7d = mg/m^3 \times 0.1786$: 0, 9.3, and 28 mg/m^3), and converted to HECs by multiplying the adjusted concentrations by the ratio of mouse:human blood/gas partition coefficients. Because the blood/gas coefficients for naphthalene were not available, the default ratio of 1 was used.

5.2.2.1.2. ***Dose-response modeling.*** Whereas the data from the NTP (1992a) study show nasal effects to be the most sensitive effects from chronic inhalation exposure to naphthalene, they provide no indication of the shape of the dose-response curve because the incidence of nasal lesions at the lowest exposure level was 100% in females and nearly 100% in males (see Table 2). In this case, application of a BMD approach, in which quantal mathematical models are fit to the incidence data for nasal effects, does not sensibly assist in extrapolating to a NOAEL, and a NOAEL/LOAEL approach was taken for deriving an RfC for naphthalene.

5.2.3. RfC Derivation

The adjusted LOAEL(HEC) of 9.3 mg/m^3 for nasal effects (hyperplasia in respiratory epithelium and metaplasia in olfactory epithelium) was divided by an uncertainty factor of 3000 (10 to extrapolate from mice to humans; 10 to protect sensitive humans; 10 to extrapolate from a LOAEL to a NOAEL; and 3 for database deficiencies, including the lack of a two-generation reproductive toxicity study and chronic inhalation data for other animal species) to arrive at a chronic RfC for naphthalene of 3E-3 mg/m^3.

5.2.4. Alternatives for RfC Derivation

Benchmark dose approaches to deriving an RfC based on lung inflammation in mice were explored (see Appendix B for details); basing the RfC on lung effects, however, was judged to be inappropriate because of the evidence in the NTP (1992a) study that the nose was a more sensitive target than the lung.

5.2.5. Confidence in the RfC

The principal study was given medium confidence because adequate numbers of animals were used, and the severity of nasal effects increased at the higher exposure concentration. However, the study produced high mortality: < 40% survival in the male control group because of wound trauma and secondary lesions resulting from increased fighting. In addition, the hematological evaluation was not conducted beyond 14 days. The database was given a low-to-medium confidence rating because there are no chronic or subchronic inhalation studies in other animal species, and there are no reproductive or developmental studies for inhalation exposure. In the absence of human or primate toxicity data, the assumption is made that nasal responses in mice to inhaled naphthalene are relevant to humans; however, it cannot be said with certainty that this RfC for naphthalene based on nasal effects will be protective for hemolytic anemia and cataracts, the more well-known human effects from naphthalene exposure. Medium confidence in the RfC follows.

5.3. CANCER ASSESSMENT

As discussed in Section 4.6, data for humans are inadequate to evaluate a plausible association with cancer. Observations of predominantly benign respiratory tumors in mice exposed to naphthalene by inhalation for 2 years (NTP, 1992a) or to 1-methylnaphthalene in the diet for 81 weeks (Murata et al., 1993) provide suggestive evidence for the carcinogenicity of naphthalene, but the evidence is insufficient to assess the carcinogenic potential of naphthalene in humans. No quantitative cancer dose-response assessments (dose conversion, extrapolation methods, oral slope factor, or inhalation unit risk) for naphthalene are presented at this time due to the weakness of the evidence that naphthalene may be carcinogenic in humans.

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

6.1. HUMAN HAZARD POTENTIAL

Naphthalene (CAS no. 91-20-3) is a bicyclic aromatic hydrocarbon with the chemical formula $C_{10}H_8$ and a molecular weight of 128.16. Pure naphthalene is a white, water-insoluble solid at room temperature with a vapor pressure of 0.087 mmHg (U.S. EPA, 1987; ATSDR, 1993). It is produced by the distillation and fractionation of either petroleum or coal tar and is used principally as an intermediate in the production of phthalic anhydride. In addition, it is important in the manufacture of phthalate plasticizers, resins, dyes, and insect repellents. Naphthalene is know by the average citizen as a moth repellent in the form of balls or flakes.

Based on the occurrence of adverse effects following exposure, naphthalene is expected to be absorbed via the gastrointestinal tract, the respiratory tract, and the skin (U.S. EPA, 1987; ATSDR, 1993); however, no studies were located measuring the rate or extent of absorption in animals or humans. Once absorbed, naphthalene and/or its metabolites are expected to be distributed by the blood throughout the body. In humans, little information is available pertaining to the metabolism of naphthalene (ATSDR, 1995); however, it has previously been shown that the two principal stable metabolites formed by human hepatic microsomes are 1-naphthol and naphthalene 1,2-dihydrodiol (Tingle et al., 1993). Limited information from orally exposed animals (pigs, chickens, cows) indicate the highest concentrations of naphthalene or its metabolites occurred in the following tissues, listed in order of decreasing concentrations: lung, liver, kidney, heart, and spleen (Eisele, 1985). The initial step in the metabolism of naphthalene is ring oxidation via the cytochrome P-450 oxygenases to produce an arene epoxide intermediate, 1,2-naphthalene oxide. Such in vivo and in vitro metabolism of naphthalene in mammalian systems is further depicted in Figure 1. Whereas the highest cytochrome P-450 specific activities in mammalian tissues are normally found in the liver, naphthalene metabolism also has been demonstrated to occur in other tissues (notably eye and lung tissues; see Wells et al., 1989; Xu et al., 1992a; Buckpitt and Franklin, 1989). In the case of methylnaphthalenes, both ring and side chain oxidation occur via cytochrome P-450 monooxygenases. At comparable molar doses, the cytotoxicity of 1-methylnaphthalene in mice appears to be much less (~ 75%) compared with naphthalene and 2-methylnaphthalene (Rasmussen et al., 1986; ATSDR, 1995).

Health hazards of concern from exposure to naphthalene include hemolytic anemia, cataracts, and respiratory toxicity towards the respiratory tract (both noncancer and cancer effects). Mechanism of action is not known but it is generally thought to involve steps of bioactivation leading to electrophilic and/or reactive free radical reactive intermediates.

Naphthalene metabolites are generally thought to be involved in naphthalene-induced hemolytic anemia, but molecular mechanisms specific to naphthalene-induced hemolysis are not clearly understood (U.S. EPA, 1987a; ATSDR, 1993). Humans experience hemolysis after naphthalene exposure by the inhalation, oral, and dermal routes, and persons who are deficient in G6PDH have a deficit in reducing power (NADPH) and in levels of reduced glutathione, which is

involved in conjugation of naphthalene metabolites. These deficits make cells, particularly erythrocytes, more vulnerable to reactive naphthalene metabolites (U.S. EPA, 1987). Various male members of subpopulations (Asians, Arabs, Caucasians of Latin ancestry, African Americans, and Africans) have an increased probability of G6PDH deficiency (Dacie, 1967) and are expected to have an increased susceptibility to naphthalene-induced hemolytic anemia (U.S. EPA, 1987). Neonates are also sensitive to naphthalene-induced hemolysis. Valaes et al. (1963) proposed that their sensitivity is due to immaturity of the detoxification pathways responsible for the conjugation and excretion of naphthalene metabolites. In general, animals are less susceptible than humans. There are no reports of naphthalene-induced hemolysis in either rats or mice; however, hemolysis has been observed in dogs.

6.2. DOSE RESPONSE

6.2.1. Noncancer/Oral

There are no adequate chronic oral dose-response data for naphthalene in humans or animals. Case reports of acute poisonings identify hemolytic anemia and ocular effects as effects of concern in humans orally exposed to naphthalene, but adequate dose-response data for these effects in a sensitive animal species are not available. The limited subchronic oral animal data identify decreased body weight in rats as the most appropriate critical effect for deriving a chronic oral RfD for naphthalene. The chronic RfD for naphthalene, 2E-2 mg/kg-day, was derived by dividing a duration-adjusted NOAEL, 71 mg/kg-day, for mean terminal body weight decrease (> 10% of control) in male rats exposed to naphthalene in corn oil by gavage, 5 days/week for 90 days (BCL, 1980a) by an uncertainty factor of 3,000 (10 to extrapolate from rats to humans; 10 to protect sensitive humans; 10 to extrapolate from subchronic to chronic exposure; and 3 for database deficiencies, including the lack of chronic oral exposure studies and two-generation reproductive toxicity studies). Confidence in the RfD was given a low rating, predominantly because of deficiencies in the database, including the lack of chronic oral data and the lack of dose-response data for hemolytic anemia in a sensitive animal model. Dose-response data for dogs exposed to oral doses of naphthalene are needed to reduce uncertainty that the derived chronic RfD will be protective for naphthalene-induced hemolytic anemia in humans.

6.2.2. Noncancer/Inhalation

Hemolytic anemia and cataracts are effects of concern in humans exposed to naphthalene, but adequate exposure-response data are not available for these effects in humans or animals. Studies of mice exposed by inhalation identify nasal lesions (hyperplasia of respiratory epithelium and metaplasia of olfactory epithelium) as critical effects for RfC derivation (NTP, 1992a). A chronic RfC of 3E-3 mg/m^3 was derived by dividing an adjusted LOAEL(HEC) of 9.3 mg/m^3 for nasal effects by an uncertainty factor of 3,000 (10 to extrapolate from mice to humans; 10 to protect sensitive humans; 10 to extrapolate from a LOAEL to a NOAEL; and 3 for database deficiencies, including the lack of a two-generation reproductive toxicity study and chronic inhalation data for other animal species). Confidence in the RfC was given a medium rating, due

to medium confidence in the principal study (NTP, 1992a), but low-to-medium confidence in the database. As with the RfD, confidence in the RfC should increase with future research to obtain exposure-response data for hemolytic anemia in dogs, which appear to be more sensitive to hemolytic anemia than mice.

6.2.3. Cancer/Oral and Inhalation

Data for humans are inadequate to evaluate a plausible association between naphthalene and cancer. Observations of predominantly benign respiratory tumors in mice provide suggestive evidence for the carcinogenicity of naphthalene, but the evidence is insufficient to assess the carcinogenic potential of naphthalene in humans. Benign tumorigenic responses in mice to naphthalene likely involve a nongenotoxic mechanism involving cytotoxicity and subsequent regeneration. No quantitative cancer dose-response assessments for naphthalene were derived due to the weakness of the evidence that naphthalene may be carcinogenic in humans. NTP started an inhalation cancer bioassay with rats in March 1996. Results from this study will add significant information to the database regarding the possible carcinogenicity of naphthalene.

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APPENDIX A. EXTERNAL PEER REVIEW—SUMMARY OF COMMENTS AND DISPOSITION

The support document and IRIS summary for naphthalene 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, 1994a). 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 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.

A. GENERAL QUESTIONS

The four external reviewers offered editorial comments and many minor but valuable suggestions, all of which have been incorporated into the text to the extent feasible. Substantive scientific comments are addressed below.

Question 1. Are there other studies that should be included as additional or supporting studies for the RfD and cancer assessments?

Comments:

Animal studies for the RfD and cancer assessments. The external peer review draft of the Toxicological Review cited the literature published by Murata et al. (1993) on 1-methylnaphthalene, which showed an increased incidence in alveolar/bronchiolar adenomas and alveolar proteinosis. A recent publication (Murata et al., 1997) essentially replicated these studies with 2-methylnaphthalene. The incidence of adenomas and carcinomas was 20.4% and 12.2% in male mice given 0.075% and 0.15% 2-methylnaphthalene in the diet, respectively, compared with an incidence of 4.1% for historical controls used in this study. With respect to noncancer effects, 50% of the female and male mice treated at both doses had alveolar proteinosis.

PBPK Models. Some additional studies could possibly provide useful toxicokinetic information for revising the online database in the future. The most recently cited PBPK model study is by Sweeney et al. (1996), which is presently cited in the review; however, one study not cited is Butler et al., 1990. It is clear that validation of the published PBPK models for naphthalene would be helpful in evaluating the cross-species comparisons as well as differences in route of administration. Specifically, tissue and blood level data in both mice and rats after both inhalation and oral administration may be available in reports submitted to NTP. Although it is too late to obtain and use these data for the current Toxicological Review, EPA should attempt to obtain this information and revise the online database as appropriate after reviewing the kinetic data.

Susceptibility of the Young. A very recent study in mice showing that 7-day-old animals were considerably more susceptible than adults to the lung toxicity of naphthalene suggests that this may represent a highly susceptible population. Bronchioles were severely injured at the lowest dose tested (25 mg/kg), whereas only minimal injury was noted at 50 mg/kg in the adults (Fanucchi et al., 1997). The apparent high susceptibility of young animals should be addressed in a section entitled Supporting/Additional Studies.

Tolerance. While highly speculative, the finding that multiple doses of naphthalene result in tolerance may have relevance for the current assessment. While this issue is mentioned in the Toxicological Review, its potential impact is not explored. Briefly, seven daily treatments with an intraperitoneal dose of 200 mg/kg naphthalene results in lungs that are histologically and morphologically similar to vehicle-treated animals (O'Brien et al., 1989). Moreover, the effects of treatment with a very large challenge dose (300 mg/kg) are blunted by this pretreatment regimen of naphthalene. This is certainly consistent with the findings of Shopp et al. (1984), showing no gross abnormalities of the lung after 90 days' exposure at doses up to 133 mg/kg. Assuming that there were no significant differences in the bioavailability of naphthalene after oral (Shopp et al., 1984) and intraperitoneal administration, the finding that daily doses at this level produce moderate toxicity in the distal airways (Plopper et al., 1992) suggests that daily administration of this compound may be far less significant than intermittent administration. There are no data of which this reviewer is aware, however, that speak to this issue.

Developmental Effects. Harris et al. (1979) observed retarded cranial ossification and heart development in fetuses of dams treated intraperitoneally with 395 mg/kg-day naphthalene in corn oil from days 1-15 of gestation and sacrificed 1 day prior to anticipated parturition. However, the degree of maternal toxicity during exposure was not reported. In another study (Matorova, 1982), it was reported that exposure of mice, rats, and guinea pigs to naphthalene in drinking water can suppress spermatogenesis and progeny development.

Immunosuppression.. Two recent studies were cited (by one reviewer) that support the contention of Shopp et al. (1984) that naphthalene is not immunosuppressive. In vitro studies by Kawabata and White (1990) on the effect of naphthalene and metabolites on the antibody-forming cells' response of splenic cell cultures to sheep red blood cells did not demonstrate an immunosuppressive effect by naphthalene. An in vivo study by Silkworth et al. (1995), which screened the ability of 15 polycyclic aromatic hydrocarbons separately to suppress antibody response in C57B1/6 ($Ah_{+}/_{+}$) mice immunized after a single oral dose, demonstrated that naphthalene had little or no immunosuppressive effect.

Metabolism. As pointed out in the Toxicological Review, the methylnaphthalenes are not metabolized only to arene oxides, but the side chain methyl group is susceptible to oxidation by the cytochrome P-450 monooxygenases. This is indeed a significant metabolic pathway in dissected airways from mouse lung, according to unpublished data by Buckpitt (personal communication, A.R. Buckpitt, University of California, Davis, June 23, 1997). The possible significance of this is related to the finding that aldehyde dehydrogenase activities are low (or nondetectable) in lung (Patel et al., 1979). The potential toxicity of the aldehyde raises the possibility that there are distinct differences between naphthalene and the methylnaphthalenes that

are related to differences in metabolism. Nevertheless, as argued in the external peer review draft of the Toxicological Review, there are numerous similarities in the lesions arising from naphthalene and 1-methylnaphthalenes (notably the species and cell specificity) that suggest that in the absence of appropriate data on naphthalene, the published information on 1- and 2-methylnaphthalenes should be used to derive the RfD.

Human Studies/Children. As pointed out in the external peer review draft of the Toxicological Review, there are virtually no reliable epidemiology studies that can guide in the assessment of toxicity. The published case reports, for the most part, do not provide information on levels of exposure or do not describe any lesions histopathologically. The single exception is a report in the Japanese literature of a child poisoned with approximately 5 g moth balls (Ijiri et al., 1987). At autopsy, there was congestion, edema, and hemorrhage of the lungs. In addition, histopathology of the liver showed infiltration of polymorphonuclear leucocytes and lymphocytes as well as fatty changes. Edema and hemorrhage of the lungs are not prominent findings in animals, and with an N of 1 it is difficult to know whether these findings are significant. Blood levels of naphthalene were reported at 0.55 mg/L. Nevertheless, the potential of a lung lesion as well as changes in the liver, albeit at high doses (estimated to be 1 g/kg), suggests that lesions in these two organs are possible in the human. The time to death was short (1 hour), and tissue injury may have been considerably greater had the child lived a few more hours. In mice, lung lesions are observed as early as 1 hour after treatment but are not maximal until 4-8 hours.

EPA Response to Comments: Relevant case study information has been added to the Toxicological Review as appropriate. Relevant studies cited above under "comments" pertaining to animal studies for the RfD and cancer assessments, PBPK models, susceptibility of the young, tolerance, developmental effects, immunosuppression, metabolism and human studies/children have been included in the Toxicological Review.

Question 2. Was the RfD based on the most critical effect and study (studies)?

Comments: One reviewer was very much in favor of using 1-methylnaphthalene to derive the RfD for naphthalene rather than the critical effect based on a 10% decrease in mean body weight (BCL, 1980a). Three reviewers did not support using 1-methylnaphthalene to derive the RfD due to concerns about the lack of pharmacokinetic data to elucidate similarities and differences between naphthalene and 1-methylnaphthalene. One of these reviewers preferred using the subchronic oral rat study (BCL, 1980a) based on a 10% decrease in mean body weight in males as the alternate method for deriving the RfD. One reviewer recommended delaying a decision on any RfD until a better animal model is available to quantify the effects (hemolytic anemia and cataracts) observed in humans, while another reviewer recommended waiting until the NTP rat inhalation study is available to the Agency for route-to-route extrapolation in December 1999. One reviewer, who has been involved in naphthalene research for many years, was very supportive of using 1-methylnaphthalene as a surrogate for naphthalene in deriving the RfD based on the following reasons:

-- The differences in dose required to produce toxicity from naphthalene and 2-methylnaphthalene is less than twofold based on acute dosing experiments with lung lesions as an endpoint (unpublished data by one reviewer).
-- 2-Methylnaphthalene is more slowly metabolized than naphthalene and is not as good a glutathione depletor as naphthalene; nevertheless, these differences are relatively small (unpublished data by one reviewer).
-- These relatively small differences between naphthalene and 2-methylnaphthalene are reinforced by the fact that there are numerous similarities in the lesions arising from naphthalene and 1-methylnaphthalene (notably, species and cell specificity) and most recently 2-methylnaphthalene (Murata et al., 1997) as cited in the Toxicological Review.
-- Unlike the mouse lung, which is extremely sensitive to naphthalene as compared with other species and especially humans, the rat is not susceptible to a number of acute lung injurants, including the naphthalenes and several of the chlorinated ethylenes (TCE, DCE). Moreover, there is weak epidemiologic evidence of pulmonary effects of vinyl chloride in the human, and again, mice respond to this compound while rats do not (Suzuki, 1981). In addition, other publications were cited, namely, neoplastic and nonneoplastic effects of vinyl chloride in mouse lung and observations of the site-specific carcinogenicity of vinyl chloride to humans (Infante, 1981). Furthermore, numerous studies on mainstream and sidestream tobacco smoke, a well-known human carcinogen, have failed to demonstrate any significant carcinogenic effect of tobacco smoke with the exception of laryngeal lesions in dogs and rats and adenomas in mice (Witschi et al., 1997). Only recently have studies demonstrated a tumorigenic effect of tobacco smoke, and these were conducted in A/J mice, a strain with relatively high background incidence of adenomas. More than 80% of the tumors observed were adenomas, and the remainder were adenocarcinomas. The inhalation studies with naphthalene, specifically, the chronic mouse bioassay (NTP, 1992a) used to derive the RfC, demonstrate a slight increase in adenomas in female mice but in a strain (B6C3F1) that is far less susceptible to lung tumors than are A/J mice, a strain with relatively high background incidence of adenomas.
-- Hemolytic anemia and cataracts are easily observed in the human, and it appears that the background incidences of these diseases is sufficiently small so that if this were a major problem with naphthalene as a result of environmental or industrial exposures, it would have been identified in prior epidemiologic studies, especially in the case of hemolytic individuals deficient in glucose-6-phosphate. Even if high-dose subchronic studies had been conducted in the rabbit or dog (the two species in which cataracts and hemolytic anemia have been observed at high doses with naphthalene), there are few relevant comparative in vitro data in humans. Thus, extrapolation would still be problematic.

EPA Response to Comments: EPA considered the peer reviewer comments carefully, considered an alternative study by Shopp et al. (1984) for deriving the RfD, and considered the alternative of using 1-methylnaphthalene as a surrogate for naphthalene. EPA ultimately decided to base the RfD on the Battelle (1980a) study. The reasons were as follows:
-- The verification of the chemical dose, animal maintenance and study design (10 rats/sex/dose group for 5 dose groups and 1 control group) are consistent with GLP guidelines submitted for 90 day studies, unlike the Shopp et al. (1984) study in which the numbers of animals actually evaluated compared to those exposed for most endpoints (organ weights, clinical chemistry and immunological testing) were small.

-- The decrease in mean terminal body weight in the BCL (1980a) study was not a result of decreased food consumption and was accompanied by clinical signs (diarrhea, lethargy and rough coats) consistent with sick animals.
-- Decreases in mean terminal body weight of at least 10% were observed in females and males in the case of the BCL (1980a) study, unlike the Shopp et al. (1984) study, in which no significant changes in body weight were reported at any dose level.
-- The statistically significant alterations ($p<0.05$) observed in the absolute (brain, liver and spleen) and relative weight (spleen) of some organs in the absence of any decrease in body weight (Shopp et al., 1984) is not consistent with the absence of lesions and the lack of significant alterations in the clinical chemistry data, hematology, mixed-function oxidate activity and the immunotoxicity assays for either sex.
-- Although the gross and histopathological examination was limited to the control and high dose group in the BCL (1980a) study, renal lesions of low incidence were observed in the kidneys (focal cortical lymphocytic infiltration, focal and diffuse tubular regeneration) and thymus (lymphoid depletion) in males and females, respectively, at 100 mg/kg (71 mg/kg/day), unlike the Shopp et al. (1984) study in which gross necropsy (no histopathological examination of tissues) on a randomly selected number of animals revealed no lesions.

Question 3. Was the RfC based on the most critical effect and study (studies)?

Comments: Three of the four reviewers agreed with the draft selection of the NTP (1992a) chronic mouse inhalation study for derivation of the RfC using lung inflammation as the critical effect rather than nasal effects, specifically, nasal inflammation or hyperplasia of respiratory epithelium of nose of severity equal to or greater than 3. One reviewer was not convinced that mouse lung lesions are relevant to humans, and therefore in the absence of benchmark concentration (BMC) model restrictions (due to high response rates of ~ 100%), the nasal effects would have been the more relevant endpoints to use rather than lung gland inflammation, even accepting portal-of-entry effects. However, if the Agency is going to use the NTP (1992a) bioassay to derive an RfC, then it was recommended by two reviewers that the uncertainty factor of 10 associated with lung gland inflammation should be changed to 1 to account for the greater sensitivity of the mouse to lung lesions induced by naphthalene compared with any other species, including humans. The critical endpoint should first be based on the toxicology (i.e., choice of most appropriate endpoint) and subsequently on the models if appropriate data exist. It appears that the mouse lung is more relevant than the nose for the critical effect.

EPA Response to Comments: EPA agrees that the critical effect should be based on the toxicology and not driven by choice of the best model fit. Therefore, the three different endpoints (nasal inflammation, hyperplasia of the respiratory epithelium of the nose with severity equal to or greater than 3, and lung gland inflammation) were reviewed to define the critical effect and subsequently see if the response data are appropriate to fit the model. In this situation, based on the response rates of the three different endpoints, lung gland inflammation is the only endpoint that one can model via benchmark methodology.

EPA ultimately decided to base the RfC on the critical effect of nasal hyperplasia and metaplasia in respiratory and olefactory epithelium, respectively and the RfC should be derived

from the chronic inhalation mouse study (NTP, 1992a) utilizing the traditional LOAEL/NOAEL approach rather than the Benchmark approach. The use of the traditional approach permitted the derivation of an RfC for nasal and lung lesions. Due to the fact that the nose is the most sensitive target organ in the absence of portal-of-entry effects, it was decided that the RfC (3E-3 mg/m^3, UF 3000) be based on nasal lesions; namely, hyperplasia and metaplasia in respiratory and olfactory epithelium.

Question 4. Were the most appropriate cancer studies selected for development of the qualitative and quantitative aspects of the risk assessment of naphthalene?

Comments: Inhalation. The external peer review draft included a qualitative and quantitative inhalation cancer assessment. Three of the four reviewers were comfortable with the draft selection of the NTP (1992a) chronic mouse inhalation study, since this was the only complete study where exposures and effects (alveolar/bronchiolar adenomas and carcinomas in female mice) were well characterized and where animals were held for sufficient periods to observe effects. However, one reviewer felt that the available inhalation cancer data are not adequate to predict carcinogenesis in humans for the following reasons: (1) Tumors, not carcinomas, have been identified in only one species (B6C3F1 mice), and the lung has not been identified as the target organ in humans; (2) the effects observed in humans (hemolytic anemia) have not been reproduced in animals under chronic exposure conditions; and (3) there is the possibility of a mouse-specific response, similar to the species response of tumorigenesis unique to male rats (as a result of the interaction of hydrocarbons with alpha-2-microglobulin) and not humans and considered irrelevant by EPA (1991). Therefore, according to the proposed new cancer guidelines (U.S. EPA, 1996a), naphthalene should be classified as "cannot be determined" based on inadequate data. Further discussions should not commence until the NTP rat inhalation oncogenicity study has been completed and is available in December 1999. However, if EPA decides to generate a unit risk estimate at this time, one reviewer recommended the use of a threshold model in view of the fact that the mode of action appears to be epigenetic in nature and not genotoxic.

EPA Response to Comments: **Inhalation:** The Agency considered but could not reach consensus on the use of this study for estimating an inhalation unit risk. Problems using the study for quantitative purposes due to weak tumor data (with exception of one carcinoma, all benign tumors), and a decision to delay the derivation until NTP completes their naphthalene inhalation bioassay in rats. Ultimately, EPA determined that the available chronic inhalation studies in mice (NTP, 1992a) should not be used to generate a unit risk for naphthalene at this time, due to the weakness of the evidence that naphthalene may be carcinogenic in humans.

Comments: Oral. the external peer review draft propsed using 1-methylnaphthalene (Murata et al., 1993) as a surrogate for the development of a slope factor (mg/kg-day) for naphthalene, two reviewers agreed with this approach and further recommended that if this approach is taken, then the RfD also should be based on the use of this surrogate study. The opinion of one reviewer who supported the use of the Murata et al. (1993) study was based on the following: (1) The mouse, as compared with the rat, is the most appropriate animal model for naphthalene and other

acute lung injurants; (2) a recent publication (Murata et al., 1997) essentially replicates the previous cancer findings (increased incidence in alveolar/bronchiolar adenomas) and noncancer findings (alveolar proteinosis) using 2-methylnaphthalene; and (3) there are numerous similarities in the lesions arising from naphthalene and 1-methylnaphthalene (notably, the species and cell specificity) and most recently 2-methylnaphthalene. One reviewer also cited the same reasons as cited in the preceding comments on inhalation for not utilizing the Murata et al. (1993) oral study for deriving a slope factor for naphthalene. As in the inhalation comments above, one reviewer recommended the use of a threshold model in generating slope factors due to the epigenetic mode of action of naphthalene.

One reviewer recommended that one derive the slope factor for naphthalene via route-to-route extrapolation from the NTP (1992a) inhalation study and compare this estimate with that derived from the Murata et al. (1993) study using 1-methylnaphthalene. Such a comparison would either support or negate the use of 1-methylnaphthalene as a surrogate for naphthalene.

EPA Response to Comments: **Oral:** The qualitative and quantitative basis for deriving a slope factor for naphthalene from 1-methylnaphthalene (Murata et al., 1993) was considered because no oral naphthalene studies are available to support this endeavor. The reasons mentioned above provide an approach for using methylnaphthalene as a surrogate to derive a slope factor for naphthalene. Further, route-to-route extrapolation from the NTP (1992a) inhalation study yielded an oral slope factor of 1E-2 per mg/kg-day. This is similar to the oral slope factor (3.5E-2 per mg/kg-day) derived from the Murata et al. (1993) study using 1-methylnaphthalene as a surrogate for naphthalene. Despite these points, the Agency ultimately decided not to derive a slope factor due to the potential differences between naphthalene and 1- and 2-methylnaphthalene with respect to pharmacokinetics and cancer. Problems with using the surrogate approach included the lack of oral studies for naphthalene for comparison, and differences in tumor (adenomas and adenocarcinomas) incidence data.

Question 5. For the noncancer assessments, are there other data that should be considered in developing the uncertainty factors or modifying factor?

Comments: Regarding the RfC based on lung gland inflammation, as proposed in the external peer review draft, two reviewers suggested that the uncertainty factor of 10 be reduced to 1 due to the sensitivity of the mouse to lung lesions induced by naphthalene compared with any other species, including humans. This would result in an RfC more consistent with those calculated for nasal lesions and still be protective of sensitive subpopulations. One reviewer also suggested that benchmark methodology guidelines should be provided to determine whether an LEC_{10} 95% confidence internal lower boundary on concentration exists at an extra risk level of 0.1. LED_{10} 95% confidence interval lower boundary on dose at extra risk levels of 0.10, 0.05, or 0.01 (or corresponding maximum likelihood estimates) is considered a NOAEL or a LOAEL as far as adding an uncertainty factor. With respect to the RfD based on the use of 1-methylnaphthalene as a surrogate for naphthalene, one reviewer felt that an uncertainty factor of 100 was appropriate to protect against other noncancerous effects even in potentially more sensitive populations such as children.

EPA Response to Comments: There is an extensive database (Buckpitt et al., 1987, 1992; Buckpitt and Bahnson, 1986) showing indirectly that mice are more sensitive than humans and other primates, as well as more sensitive than rats, to the effects of naphthalene on the respiratory tract. Therefore, an uncertainty factor of 1 rather than 10 was suggested for extrapolation from mice to humans. However, this reduction in uncertainty is not warranted based on the in vitro data since this may not occur under in vivo conditions. If the benchmark approach were used, the LEC_{10} or LED_{10} would be used rather than the NOAEL; thus in deriving the uncertainty factors, there would be no need to use 10 to account for use of a LOAEL. (Note: Use of the benchmark methodology is described in: (1) *Framework for Human Health Risk Assessment Colloquia Series* (U.S. EPA, 1997), (2) EPA/630/P-96/011 *Report on the Benchmark Dose Peer Consultation Workshop* (U.S. EPA, 1996b), and (3) EPA/600/P-96/002A *Benchmark Dose Technical Guidance Document* (external review draft) (U.S. EPA, 1996c)). However, the Agency ultimately decided to use the LOAEL/NOAEL approach because the nose was identified as the most sensitive target organ in the absence of portal-of-entry effects. Due to the exceedingly high response in nasal lesions observed at the lowest dose compared to lung lesions, application of the benchmark dose methodology was not deemed possible.

Question 6. Do the confidence statements and weight-of-evidence statements present a clear rationale and accurately reflect the utility of the studies chosen, the relevancy of the effects (cancer and noncancer) to humans, and the comprehensiveness of the database? Do these statements make sufficiently apparent all the underlying assumptions and limitations of these assessments? If not, what needs to be added?

Comments: According to one reviewer, the confidence statements are conservative, and the most relevant studies were identified along with the appropriate critical evaluation of the studies. Key experimental data that would be useful in establishing the RfD and RfC are not available in the literature, and areas where data are lacking have been identified clearly in the review. The animal-to-human extrapolation, exposure route differences, and lack of any significant epidemiologic studies in the human decrease the confidence of the RfD/RfC assessments.

One reviewer regarded the review to be very comprehensive and clear in presenting most of the available data on naphthalene and in describing the process of selecting studies and developing reference values. Limitations of the data were fully presented with the exception of the limitations of the cancer determination.

Two of the reviewers suggested reworking the weight-of-evidence statements and taking a bit more conservative approach with regard to the carcinogenic endpoint for naphthalene.

EPA Response to Comments: In the external peer review draft, the Agency had proposed using the chronic mouse feeding study with pulmonary proteinosis as the critical effect for the RfD using 1-methylnaphthalene (Murata et al., 1993) as a surrogate for naphthalene. Due to potential differences in the pharmacokinetics of these PAHs, it was later decided to base the RfD (2E-2 mg/kg/day) on an NTP sponsored study which was conducted by Battelle Columbus Laboratories (BCL, 1980a) in which a 10% or greater decrease in mean terminal body weight in males was identified as the critical effect. Ultimately, the Agency decided that the overall

confidence using this approach is high for the principal study, low for the data base, and low for the RfD.

For the RfC, the choice of the principal study (NTP, 1992a) remained the same as in the external peer review draft; however, the critical effect was changed from lung to nasal lesions based on the fact that nasal effects were the most sensitive critical effects observed (incidence of nasal lesions at the lowest exposure level of 100% in females and nearly 100% in males) and the fact that such lesions were not portal-of-entry effects. Ultimately, the Agency decided to downgrade the confidence from high for the principal study, low for the data base and medium for the RfD, to medium, low-to-medium, and medium, respectively. The downgrade in confidence in the study was based on issues inherent in the conduct of the study (high mortality and limited hematological evaluation).

In the carcinogenicity assessments, the Agency ultimately decided that the chronic inhalation study in mice (NTP, 1992a) not be used to generate an inhalation unit risk for naphthalene at this time due to the weakness of the evidence (with the exception of 1 carcinoma, all benign tumors) that naphthalene may be carcinogenic in humans. Based on the potential differences between naphthalene and 1-and 2-methylnaphthalenes (Murata et al., 1993, 1997) with respect to pharmacokinetics and cancer (lack of oral studies for naphthalene and different cancer results), the Agency decided that an oral slope factor for naphthalene should not be developed at this time. The weight-of-evidence statement reflects the finding of limited animal evidence that naphthalene is a human carcinogen.

B. CHEMICAL-SPECIFIC QUESTIONS

Question 1. Is the application of benchmark methodology in the external peer review draft appropriate in general and specifically in the case of naphthalene with respect to deriving an RfD and RfC based on the 95% lower confidence limit of the maximum likelihood estimate and in extrapolating from the LED_{10} to 10^{-6} risk in the case of cancer? Explain.

Comments: Benchmark methodology offers the opportunity to more accurately perform low-dose extrapolation of data for significant toxic endpoints in order to develop safe exposure levels. The two reviewers addressing this question were of the opinion that the most important decision in deriving an RfD/RfC is to first identify that endpoint which is the most toxic (i.e., lowest LOAEL, critical effect) and relevant to humans and then determine if the model fits the data, not force the data to fit the model. If the data does not fit the model (e.g., excessive response rate), then use the traditional approach rather than force the data to fit the model and choose the incorrect critical effect.

EPA Response to Comments: EPA agrees with two of the reviewers in that the critical toxicological endpoint should first be determined and subsequently see if the dose-response data fits the model, rather than the model driving the critical effect. The Agency ultimately decided not to use the benchmark approach because it offered no advantage over the LOAEL/NOAEL approach. For the carcinogenicity assessment, the question of whether to use the benchmark

methodology to extrapolate to low doses became irrelevant due to the decision to not provide quantitative cancer assessments.

Question 2. As identified in Appendix B, the benchmark RfC for naphthalene was based on nasal effects (nasal inflammation and hyperplasia of respiratory epithelium of the nose of severity greater than 3). Due to the extremely high response rates, these endpoints were replaced with a lower response rate, namely, lung lesions. Do you agree with this approach?

Comments: One reviewer agreed with the approach due because the high response rates and lack of data at lower concentrations left few options but to select an endpoint where fewer of the animals responded. However, two reviewers stated emphatically that the choice of the most sensitive endpoint or critical effect should not be driven by the model fit (e.g., in the case of the choice of lung gland inflammation over nasal effects [nasal inflammation or hyperplasia of respiratory epithelium of nose of severity greater than 3]), but should be based first on the toxicology and relevancy to humans. If the model does not fit the data for deriving an RfC based on the critical effect chosen, then the RfC should be derived using the traditional LOAEL/NOAEL approach. One reviewer was not confident that the lung lesions in the mouse were relevant to human toxicity, while another reviewer felt that the RfC should be based on lung lesions using the traditional rather than the benchmark approach. The basis for the traditional approach was that the NTP (1992a) study had only two nonzero dose groups and it was felt that the endpoints of concern did not provide data that are conducive to modeling.

EPA Response to Comments: The Agency agrees that the most appropriate methodology is to first determine the critical effect and subsequently fit these data to the model. Mice, unlike rats, are the only species that have contracted lung cancer due to the inhalation of cigarette smoke. This supports the sensitivity of the species and the organ to the most simple polynuclear aromatic hydrocarbons, such as naphthalene. With respect to nonzero dose groups, two such groups do not appear to be a limiting factor in using the benchmark approach, provided the response rate of the high nonzero dose group is not exceedingly high. The Agency ultimately decided that nasal lesions should be the critical effect and a LOAEL/NOAEL approach be used. In this case, the application of benchmark methodology was not possible due to the extremely steep dose-response data at the lowest dose.

Question 3. In deriving the RfC, is the LOAEL/NOAEL approach more appropriate compared with the benchmark approach? Explain.

Comments: One reviewer did not feel qualified to comment. Two of the reviewers were of the opinion that the traditional LOAEL/NOAEL approach should be used in deriving the RfC. Another reviewer was of the opinion that neither model assumes an appropriate uncertainty factor for extrapolation between species given the apparent greater sensitivity of mice to nasal and lung lesions from exposure to naphthalene.

EPA Response to Comments: With respect to the uncertainty factor used in deriving the RfD via either the benchmark or LOAEL/NOAEL approach, the interspecies (animal to human) value of 10 will not be decreased to account for the apparent greater sensitivity of mice to lung lesions. There is an extensive in vitro metabolic database (Buckpitt et al., 1987, 1992; Buckpitt and Bahnson, 1986) showing that mice are more sensitive than humans and other primates, as well as more sensitive than rats, to the effects of naphthalene on the respiratory tract. However, an uncertainty factor of 10 was used for extrapolation from mice to humans due to the fact that pharmacokinetic data are still lacking for different species and different organs. The Agency ultimately used the LOAEL/NOAEL approach, as explained above.

Question 4. In the external peer review draft, the inhalation chronic mouse bioassay (NTP, 1992a) was used in deriving the inhalation unit risk from either the two-stage polynomial or direct extrapolation from the LED_{10} to 10^{-6} risk. Was this the most appropriate study to use for deriving this inhalation unit risk estimate? Explain.

Comments: Three reviewers were of the opinion that the NTP (1992a) chronic mouse inhalation study was the appropriate study (because it was the only complete study where exposures and effects were well characterized and where animals were held for sufficient periods to observe effects) to be used in deriving the inhalation unit risk. The extrapolation from the alveolar/bronchiolar adenomas and carcinoma in female mice was thought rational and justified. On the other hand, one reviewer was of the opinion that there is not yet sufficient data to consider naphthalene a likely human carcinogen based on one mouse oncogenicity study that did not produce a significant incidence of carcinomas in an organ system demonstrably relevant to humans. However, the reviewer stated that if EPA uses this study (NTP, 1992a) to generate a unit risk estimate, a nonlinear model of effect with a threshold of response should be employed because the majority of the genotoxicity data supports naphthalene as being epigenetic.

EPA Response to Comments: The majority of reviewers supported using the chronic mouse inhalation study in deriving the inhalation unit risk based on a statistically significant increase in the incidence of alveolar/bronchiolar adenomas observed in female mice at 30 ppm (28/135) relative to the female control group (5/69). The observation of only one lung carcinoma was considered significant in that the alveolar/bronchiolar adenomas and carcinoma constitute a morphological continuum in a species and in an organ that has been shown to be sensitive to a source of many polynuclear aromatic hydrocarbons, including naphthalene. The Agency ultimately decided not to derive an inhalation unit risk, however, because of the weakness of the tumor incidence data and weakness of the evidence that naphthalene may be carcinogenic to humans.

Question 5. The benchmark RfD was first derived from an oral study of 1-methylnaphthalene as a surrogate for naphthalene (Murata et al., 1993). Is too little is known about the pharmacokinetics of either compound via different routes of exposure to make this extrapolation? Alternatively, EPA is proposing that the benchmark RfD based on pulmonary proteinosis from 1-methylnaphthalene be deleted in favor of the RfD based on the Battelle subchronic rat study (BCL, 1980a) with a 10% decrease in mean male body weight as the critical effect using either the benchmark or the LOAEL/NOAEL approach. Which approach can be best scientifically supported?

Comments: Three of four reviewers agreed that the Murata et al. (1993) oral chronic study using B6C3F1 mice should not be used in deriving the RfD based on the potential difference in pharmacokinetic data between naphthalene and 1-methylnaphthalene. Although two reviewers were in favor of using effects associated with exposure to naphthalene rather than 1-methylnaphthalene, one reviewer was not comfortable with using decreased male body weight (BCL, 1980a) as a significant toxic finding and suggested waiting until better data are available from the chronic rat inhalation study by NTP via route-to-route extrapolation. One reviewer felt that neither the traditional LOAEL/NOAEL or benchmark approaches were scientifically defensible.

EPA Response to Comments: The Agency chose to use the BCL (1980) study for derivation of the RfD, as described in response to General Question #2.

Question 6. Do you consider a 10% decrease in mean body weight significant in terms of toxicity (i.e., the critical effect in terms of deriving an RfD)?

Comments: Three of four reviewers felt that it was inappropriate to use the 10% decrease in male body weight as a significant endpoint. One reviewer was of the opinion that such an endpoint was nothing more than an indication that the material had been tested at the maximum tolerated dose. One reviewer maintained that the mouse and the lung were the most appropriate animal model and endpoint, respectively, to support derivation of an RfD, unlike the rat, based on a decrease in male body weight.

EPA Response to Comments: The Agency decided to use the BCL study (1980) and the critical effect of 10% decrease in mean body weight. EPA considers a 10% decrease in mean terminal body weight as a significant toxic endpoint especially when the weight loss is not associated with decreased food consumption and was accompanied by clinical signs (diarrhea, lethargy and rough coats) consistent with sick animals.

Question 7. In deriving the RfD, is the LOAEL/NOAEL approach more appropriate compared with the benchmark approach?

Comments: Opinions of reviewers were quite varied, from having no comment, to suggesting the use of one or the other or either approach. (See earlier discussion of comments.)

EPA Response to Comments: The primary theme regarding the choice of BMD versus LOAEL/NOAEL approaches should be, first, the identification of the critical effect, and subsequently the fit of the data to such a model. If the data are not appropriate to derive a BMD (e.g., too high a response rate, only one nonzero data point, difficulty in identifying the LOAEL), then the LOAEL/NOAEL approach should be considered. In essence, the toxicology—not the model fit—should be the driver with respect to deriving the critical effect. As described above, the Agency ultimately decided to use the LOAEL/NOAEL approach for deriving the RfD.

Question 8. In the external peer review draft, the oral slope factor was derived from alveolar/bronchiolar adenomas (single carcinoma among female rats with adenomas) using the oral study of 1-methylnaphthalene (Murata et al., 1993) as a surrogate for naphthalene. Due to potential pharmacokinetic differences with respect to species and exposure routes, the Murata et al. (1993) study was dropped for quantitative consideration (development of oral slope factor) and used only as supportive evidence. At the present time, no oral cancer quantitation appears possible. Do you think this hypothesis was appropriate, and if so, are there other studies that could be used for quantitative estimation of a slope factor?

Comments: Three reviewers were not in favor of using the 1-methylnaphthalene study (Murata et al., 1993) as a surrogate for naphthalene in deriving the oral slope factor based on a statistically significant increase in alveolar/bronchiolar adenomas and one carcinoma. One reviewer was of the opinion that such benign tumors (adenomas) and one carcinoma in a single species (B6C3F1) of mice were not relevant to humans. A decision on the carcinogenic status of naphthalene, in particular, the oral slope factor, should be deferred until the NTP inhalation rat study is available in December 1999. Based on EPA's 1996 proposed cancer guidelines (U.S. EPA, 1996a), naphthalene should be characterized as "cannot be determined" category until the NTP rat oncogenicity study (route-to-route extrapolation) is completed. One reviewer also suggested using the cancer inhalation data for naphthalene to calculate an oral cancer potency factor (route-to-route extrapolation) based on the justification in the document for considering the lung effects for naphthalene as systemic effects. Moreover, if the results from the cross route and 1-methylnaphthalene extrapolations are very different, they could provide an additional reason for not providing an oral cancer estimate. One reviewer felt that no study was appropriate for deriving the oral slope factor at the present time and did not understand how the Murata et al. (1993) study could be used as supportive evidence. The opinion of one reviewer in support of the use of the Murata et al. (1993) study was discussed above.

EPA Response to Comments: The majority of reviewers were not supportive of using the chronic feeding study of 1-methylnaphthalene as a surrogate for developing a naphthalene oral slope factor because pharmacokinetic differences were probably quite significant. The Agency agrees and has therefore not developed an oral slope factor.

Question 9. Considering that the existing rodent data for naphthalene appears inadequate for quantifying the effects that have been observed in humans, namely, hemolytic anemia and cataract formation, and because of concern in using 1-methylnaphthalene as a surrogate for naphthalene in risk assessment, is it appropriate to derive an RfD in the absence of better animal models?

Comments: One reviewer was of the opinion that it was appropriate to derive an RfD considering the fact that appropriate animal models have not been developed for naphthalene with respect to hemolytic anemia and cataracts. In addition, this reviewer was of the opinion that hemolytic anemia and cataracts are easily observed in the human, and it appears that the background incidences of these diseases is sufficiently small so that if this were a major problem with naphthalene as a result of environmental or industrial exposures, it would have been identified in prior epidemiologic studies, especially in the case of hemolytic individuals deficient in glucose-6-phosphate. Even if high-dose subchronic studies had been conducted in the rabbit or

dog (the two species in which cataracts and hemolytic anemia have been observed at high doses with naphthalene), there are few relevant comparative in vitro data in humans. Thus, extrapolation would still be problematic. Unlike one reviewer who favored using the 1-methylnaphthalene as a surrogate for naphthalene (Murata et al., 1993) in deriving the RfD, another reviewer, although favoring the chronic oral rat study (BCL, 1980a), was not sure that an RfD based on a decrease in mean body weight in rats will protect against these two adverse effects. Two reviewers unequivocally stated that an RfD should not be derived in the absence of better animal models.

EPA Response to Comments: The Agency decided not to use 1-methylnaphthalene as a surrogate for naphthalene (Murata et al., 1993) in the development of the RfD because of the potential difference in the pharmacokinetics of these polycyclic aromatric hydrocarbons (PAHs).

Question 10. In view of the fact that hemolytic anemia and cataracts appear to form at high exposure levels and the fact that animals are more sensitive than humans, does the recommended RfD based on a decrease in body weight protect against such critical effects?

Comments: One reviewer was of the opinion that this RfD (based on using 1-methylnaphthalene as a surrogate for naphthalene and not the Battelle study [BCL, 1980a]) should be protective of hemolytic anemia (even in G6PDH-deficient individuals), and cataracts are not a likely a problem at the naphthalene levels to be encountered in industrial or environmental settings. This is not, of course, the case with large doses of naphthalene encountered in poisoning. One reviewer thought that the RfD based on decreased body weight in male rats appears overly protective against critical effects of naphthalene from lifetime exposure. In the rat subchronic study (BCL, 1980a), changes in hemoglobin, hematocrit, and red blood cells that might be significant toxic effects relevant to humans were not observed until 400 mg/kg was reached. Decreased body weight was observed at doses as low as 50 mg/kg. With an uncertainty factor of 3000, the RfD is certainly low enough to protect against oral naphthalene hazard. One reviewer felt that data do not exist to show that animals are more sensitive to these effects (hemolytic anemia and cataract formation); moreover, the limited human data suggest that humans may be more sensitive to these effects. Therefore, no one can be sure that an RfD based on a decrease in body weight in rats will protect against these two adverse effects. In addition, a comparison of the RfD with the admittedly limited dose data (large doses encountered in poisoning) in humans can provide some degree of comfort that exposure at the RfD is unlikely to lead to these adverse effects. One reviewer was of the opinion that the RfD, based on a 10% decrease in body weight, would not protect against these effects, since this loss in weight in animal studies is due to cytotoxic response at high doses.

EPA Response to Comments: There were a variety of peer reviewer responses as to the toxicological significance of deriving an RfD, based on a 10% mean decrease in body weight as the critical effect, and whether this RfD will be protective against the development of cataracts and hemolytic anemia in humans. Overall, it appears that this RfD should be protective against humans developing hemolytic anemia and cataracts from environmental or industrial levels of naphthalene. In addition, a comparison of the RfD with the admittedly limited dose data (large doses encountered in poisoning) in humans can provide some degree of comfort that exposure at the RfD is unlikely to lead to these adverse effects. The Agency ultimately decided that the BCL

(1980a) study, using a 10% mean decrease in terminal body weight as the critical effect, should be used in deriving the RfD; however, it concluded that in the absence of appropriate dose-response data in the appropriate animal models, one cannot say whether the RfD will be protective of hemolytic anemia and cataracts.

APPENDIX B. BENCHMARK DOSE CALCULATIONS

This report on Benchmark Dose Calculations for naphthalene was prepared as background material in the development of the Toxicological Review and IRIS Summary.

The information contained within was carefully reviewed and considered in the development of the health assessment for naphthalene. Although a decision was made to use the LOAEL/NOAEL approach rather than the benchmark methodology in the derivation of the RfD/RfC, this appendix provides the reader with the background material that was used to make this judgment.

A. NAPHTHALENE TOXICITY AND CHOICE OF STUDIES TO MODEL

Quantitative data on naphthalene are limited; many of the studies were conducted at high naphthalene doses in order to investigate the mechanism of action. Studies appropriate for benchmark modeling were limited to a chronic inhalation study (NTP, 1992a), a subchronic oral study (BCL, 1980a), and an oral developmental toxicity study (NTP, 1991).

The inhalation study was conducted with B6C3F1 mice exposed to naphthalene at target concentrations of 0, 10, or 30 ppm (0, 52, or 157 mg/m^3) for 6 hours/day, 5 days/week for 103 weeks (NTP, 1992a). The duration-adjusted levels were 0, 9.3, and 28 mg/m^3, respectively. No hematological effects were observed at 2 weeks, the only time they were assessed. Lung and nasal inflammation was observed in males and females. Inflammation, metaplasia of the olfactory epithelium, and hyperplasia of the respiratory epithelium were reported in the noses of virtually all exposed mice of both sexes, but in only one control female mouse. These effects were slightly more severe in the high-concentration group. Lung lesions included inflammation, histiocytic infiltration, and granulomatous infiltration. Lung lesions were observed in exposed and control animals but were more severe and had a higher incidence in the exposed animals. However, there was no clear concentration-response relationship for many of the lesions (e.g., histiocyte cellular infiltration in lungs of males, granulomatous inflammation in lungs of males). In addition, the incidence of the lung lesions was lower than that of the nasal lesions. The lung lesions were generally classified as mild, and the severity generally similar in both exposed groups. The average severity of the nasal lesions was mild to moderate, with the average severity at the high concentration tending to be higher than that at the low concentration. Therefore, modeling results reported here are limited to lung inflammation in males and females and to lymphocyte cellular infiltration of the lung in females; these were the only endpoints that showed a significant concentration-related increase with a monotonic concentration-response curve. Quantal (incidence) data were modeled for the histopathology endpoints for which significant increases were observed. In addition to nasal inflammation, both olfactory epithelium metaplasia and respiratory epithelium hyperplasia were modeled. These endpoints are considered to be part of a healing process, rather than preneoplastic lesions. In addition, there was no evidence of naphthalene-related nasal neoplasms; nasal neoplasms were limited to two nasal adenomas in females at 10 ppm. Our initial modeling included responses of all severities. As described in the

Results section, we also modeled the incidence of nasal lesions of severity greater than or equal to 3.

In a subchronic study (BCL, 1980a), Fischer 344 rats (10/sex/dose) received gavage doses of naphthalene in corn oil at 0, 25, 50, 100, 200, or 400 mg/kg-day for 5 days/week for 13 weeks. Duration-adjusted exposures are 0, 17.85, 35.7, 71.4, 142.8, and 285.6 mg/kg-day. (Note: Such adjustments were not made for NOAEL and LOAEL doses listed in Tables B-3 and B-4.) Two males in the high-dose group died, and clinical signs (diarrhea, lethargy, hunched position) were limited to the high-dose group, except for rough hair coats at week 10 at doses of ≥100 mg/kg-day. A dose-dependent decrease in body weight gain was observed in both males and females at ≥100 mg/kg-day. This decrease became biologically significant (> 10%) at 200 mg/kg-day in males and at 400 mg/kg-day in females. No eye lesions were reported at any dose. Hematological findings were observed only at 400 mg/kg-day and included marginal decreases in hemoglobin and hematocrit in both sexes, increased mature neutrophils, and decreased lymphocytes. The study authors did not conduct statistical analyses on hematology parameters. Using a pair-wise t-test, we found significant decreases in male and female hemoglobin (Hgb) and hematocrit (Hct) levels and in male red blood cells (RBCs) at the high dose. We did not conduct statistical tests on the neutrophil or lymphocyte levels. Histopathology revealed kidney lesions in two mid-dose males and one high-dose male and lymphoid depletion of the thymus in two high-dose females. Based on these data, decreased body weight gain was modeled in both sexes. Although the effects on anemia-related indices (Hgb, Hct, RBCs) were statistically significant, they were not of sufficient magnitude to be biologically significant. However, these data suggest that 400 mg/kg-day was near the threshold for clinically significant anemia in this study. We did not model these effects or the effects on neutrophils or lymphocytes, because effects were observed only at the high dose, and thus the BMDs would be higher than those for the body weight endpoint.

Other subchronic studies were not modeled because they did not observe significant adverse effects. In a study similar to the one conducted in rats, B6C3F1 mice received gavage doses of naphthalene at 0, 12.5, 25, 50, 100, or 200 mg/kg-day (BCL, 1980b). The only findings were transient signs of toxicity (lethargy, rough coat, decreased food consumption) at weeks 3-5 in males and females at the high dose and nonsignificant dose-related decreases in body weight gain in high-dose females. There were no naphthalene-related histopathological lesions or hematology changes. Shopp et al. (1984) conducted a subchronic study with CD-1 ICR mice administered 5.3, 53, or 133 mg/kg-day by gavage for 90 days. There was no evidence of hematological effects or cataracts and no biologically significant decreases in body weight. Although spleen weight was significantly decreased in high-dose females, there was no effect on cellular or humoral immune function.

The only animal study in which anemia was reported was a study in dogs by Zuelzer and Apt (1949). Single dogs received seven daily doses of about 290 mg/kg-day or individual doses of 410 or 1,525 mg/kg. All dogs developed anemia and became listless 2 or more days postexposure. Although the sample size was too limited for modeling, this study suggests that dogs are an appropriate animal model for the hemolytic effects of naphthalene.

In the developmental study (NTP, 1991), groups of 28 mated female Sprague-Dawley rats were administered 0, 50, 150, or 450 mg/kg-day naphthalene by gavage on gestation days 6-15. Two dams in the 50 mg/kg-day group died; the cause of death was not known, but it was not attributable to dosing errors (and thus could have been due to chemical exposure). However, no deaths were reported at higher levels. Dose-related clinical signs of central nervous system (CNS) depression (lethargy, slow breathing) and rooting were observed at all dose levels. However, these effects were transient, largely occurring only on dosing days 1 and 2 at 50 mg/kg-day, and on dosing days 1-5 at 150 mg/kg-day and declining in incidence at the high dose, from 90% to 15%. The CNS effects were not modeled, due to their transient nature. Maternal body weight gain was significantly decreased during the treatment period at 150 and 450 mg/kg-day and was modeled as a continuous effect. Although there was a significant trend for decreased average fetal weight in both male and female pups, the decrease was not significant at any single dose. Furthermore, the fetal weight at the high dose was 95% of the control value for both males and females. This indicates that the threshold for fetal body weight changes is higher than the threshold for malformations, and it suggests that a biologically significant decrease was not observed at the doses tested. Therefore, decreased fetal weight was not modeled.

The developmental toxicity study (NTP, 1991) also reported a dose-related, statistically significant trend of increased percentage of litters with malformed fetuses (a quantal endpoint), although pairwise comparisons found no significant effect. The study authors noted that most of this increase was attributable to a dose-dependent increase in the percentage of litters with enlarged lateral ventricles of the brain. They also noted that interpretation of the results for this endpoint was confounded by wide variability in the response observed in the control group. The study was conducted with two replicates; although the average incidence of fetuses per litter with enlarged ventricles at the high dose was similar in both replicates (~ 9%), there was a 10-fold difference between the control group replicates (6% vs. 0.6%). A similar wide variation for the control group was observed with this endpoint in another study conducted by this laboratory (Price et al., 1996). A final caveat about this endpoint is that the laboratory in which these studies were conducted now classifies enlarged lateral ventricles of the brain as a variation rather than as a malformation (see Allen et al., 1996).

The primary adverse effect associated with inhalation or oral exposure of humans to naphthalene is hemolytic anemia; neonates and people with a G6PDH deficiency are particularly susceptible. Neonates are believed to be more susceptible to naphthalene-related hemolytic anemia because their livers are not yet fully developed and the ability of the livers to conjugate the naphthalene metabolites responsible for hemolysis is limited. People who are G6PDH-deficient have a deficit in reducing power (reduced glutathione), making cells, particularly erythrocytes, more vulnerable to oxidative damage (U.S. EPA, 1987). Neither oral nor inhalation exposure levels were available in human studies reporting anemia (Melzer-Lange and Walsh-Kelly, 1989; Owa, 1989; Owa et al., 1993).

B. CALCULATION OF INHALATION HUMAN EQUIVALENT CONCENTRATIONS

In calculating the HECs for the NTP (1992a) study, we noted that although the observed effects were in the respiratory tract, naphthalene is neither water soluble nor highly reactive. Based on its chemical properties, naphthalene is a water-insoluble/perfusion-limited (Category 3) gas as defined in the inhalation RfC guidance (U.S. EPA, 1994b). This class of gases generally produces "extra respiratory" effects following the absorption of the gas. Respiratory effects are usually caused by Category 1 gases (highly water soluble/rapidly reactive) or Category 2 gases. Category 2 gases are water-soluble gases that can result in blood accumulation, due to being reversibly reactive or moderately to slowly metabolized in the respiratory tissue. According to the RfC guidance, Category 3 gases causing respiratory effects are treated as Category 2 gases for the purpose of calculating HECs. However, the respiratory effects observed in the NTP (1992a) study were considered extra respiratory effects of a Category 3 gas for the purpose of calculating HECs, based on the following rationale.

Due to its low water solubility and low reactivity, naphthalene-related effects on the nasal epithelium would be expected to result from absorbed naphthalene, rather than from direct contact. This hypothesis is supported by data on naphthalene metabolism indicating that toxic effects on the respiratory tract are due to a naphthalene metabolite that may be formed either in the liver or in the respiratory tract. For example, necrosis of bronchial epithelial (Clara) cells is seen in mice (O'Brien et al., 1985, 1989; Tong et al., 1981), and necrosis of the olfactory epithelium occurs in mice, rats, and hamsters injected intraperitoneally with naphthalene (Plopper et al., 1992). These data are discussed in more detail in the naphthalene RfC prepared under this contract (Clement International Corporation, 1996c). Thus, the HEC is calculated based on the ratio of partition coefficients in mice and humans. Because these data are not available, the default ratio of 1 was used.

C. METHODS FOR MODELING

The data sets available for analysis include both quantal and continuous endpoints. The quantal endpoints were modeled using the Weibull and polynomial models:

Weibull model: $P(d) = 1 - \exp\{-\alpha - \beta*(d-d_0)^{\gamma}\}$, Eq. 1

where P(d) is the probability of response at dose d and the four unknown parameters, α, β, d_0, and γ are estimated by maximum likelihood methods. The parameter γ is not constrained to be an integer, but it is generally constrained to be greater than or equal to 1. As described in more detail in Section F, Results, the parameter γ was not constrained in this manner for the modeling of nasal lesions of severity greater than or equal to 3 because the response for these endpoints plateaued at less than a 100% response. Such a dose-response relationship can only fit well by a Weibull model with γ allowed to be less than 1. In our applications, when removing the constraint on γ was necessary, the following two-step procedure was followed. First, the model was fit with γ constrained to be greater than 0.01; the maximum likelihood estimate (MLE) of γ was recorded. Second, the model was re-run with γ constrained to be greater than the recorded

MLE (a constraint that is imposed on both the MLEs and the bound calculations). From that second step, the MLE dose and BMC corresponding to the benchmark response (BMR) of interest were obtained. In this manner, γ was prevented from approaching 0 in the bound calculations, thereby preventing the BMC from approaching 0 as well. The tendency for the lower bound, defining the BMC, to approach 0 is well known for low-dose "supralinear" dose-response relationships. The "threshold" parameter, d_0, was included in the modeling only when a sufficient number of dose groups were available (at least four) and when the model without a threshold provided a relatively poor fit to the data.

The polynomial model can be described as

$$P(d) = 1- \exp\{-q_0 - q_1*(d-d_0) - q_2*(d-d_0)^2 - ... - q_k*(d-d_0)^k\}, \quad \text{Eq. 2}$$

where the parameters, the q's and d_0, are estimated by maximum likelihood methods. The degree of the polynomial was restricted to be no greater than the number of dose groups minus one. The same restrictions on estimation of the threshold parameter, d_0, were applied here as with the Weibull model. In the case of the polynomial model, the total number of parameters estimated was constrained to be no greater than the number of dose groups.

Three methods were used to model body weight, the only continuous endpoint modeled for naphthalene. The first two methods used the modeling approach described by Gaylor and Slikker (1990) and elaborated by Crump (1995). This approach uses all of the information contained in the original observations, but it defines BMDs/BMCs in terms of probability of response.

Use of such modeling requires definition of a background incidence of abnormality, p_0, or the specification of a level of response that can be considered the cut-point between normal and abnormal responses, x_0. Specification of p_0 (and of the type of distribution—assumed here to be normal for all endpoints) implicitly defines a cut-point, x_0, when the parameters for the background variability are estimated as part of the modeling. Similarly, specification of a cut-point determines the background incidence once the background variability is estimated. The BMD is then defined as the lower bound on dose at which the increased probability of an abnormal response is equal to 5% or 10% (see below). In the absence of endpoint-specific toxicology data to support a choice of a p_0 value, we used a range of p_0 values (0.001, 0.01, 0.05) that bracketed reasonable choices. For the body weight endpoint (from BCL, 1980a), BMRs were defined both based on specified p_0 values and with the cut-point, x_0, specified. The latter definition of BMR was used in an initial attempt to incorporate the common practice of considering a 10% decrease in mean body weight relative to controls to represent an adverse effect. (As discussed below, this method does not correspond well to a 10% decrease in mean, and another modeling approach was used to better reflect the 10% decrease in the mean.) In that case, the only other option investigated was for a single designation of p_0, i.e., $p_0 = 0.05$. Maternal body weight gain in the developmental toxicity study (NTP, 1991) was modeled using the range of p_0 values, because use of a 10% difference from the control is less established when the data are reported in terms of body weight *gain* (rather than mean body weight).

Two models are available to describe how the probability of response is assumed to vary with dose. The first is an adaptation of the Weibull model:

$$P(d) = p_0 + (1-p_0)\,[1 - \exp\{-(\beta * d)^{\gamma}\}, \qquad \text{Eq. 3}$$

where P(d) is the probability of response at dose d and the unknown parameters, β and γ, as well as a background mean response level and a fixed standard deviation estimate for all dose groups, are estimated by maximum likelihood methods. The parameter γ is not constrained to be an integer, but it is constrained to be greater than or equal to 1. If a normal distribution is assumed, the Weibull model can be expressed as the change in mean as a function of dose:

$$m(d) = m(0) + \sigma[N^{-1}(1-p_0) - N^{-1}((1-p_0)\exp\{-(\beta * d)^{\gamma}\})], \qquad \text{Eq. 4}$$

where N^{-1} is the inverse normal function, σ is the standard deviation (assumed constant for all doses), β and γ are as shown in Equation 1, and m(0) is the mean response level at zero dose. Note that the operand of the second inverse normal function in Equation 4 is equivalent to 1-P(d). Thus, Equation 4 shows how changes in the mean values for continuous data can be expressed in terms of probability of response.

The power model was also used to model continuous endpoints:

$$m(d) = \alpha + (\beta * d)^{k}, \qquad \text{Eq. 5}$$

where m(d) is the mean response at dose d and the three unknown parameters, α, β, and k, as well as the dose group standard deviations, are estimated by maximum likelihood methods. The dose group standard deviations estimated by the model account for both the variation in the data and for the difference between the observed mean and the mean estimated by the model. The parameter k is not constrained to be an integer, but it is constrained to be greater than or equal to 1.

Just as there was an equation specifying m(d) when P(d) was given by the Weibull model, so too are there equations giving the probabilities of response that correspond to changes in the means as given by the power model, i.e.,

$$P(d) = 1 - N[N^{-1}(1-p_0) - (\beta * d)^{k}/\sigma], \qquad \text{Eq. 6}$$

where N is the cumulative normal function, N^{-1} is its inverse, and σ is the standard deviation assumed to hold for the normal variation at all doses. This form is for those cases in which increased values of the endpoint are adverse When decreased values are considered adverse, the corresponding equation for probability of response is

$$P(d) = N[N^{-1}(p_0) + (\beta * d)^{k}/\sigma]. \qquad \text{Eq. 7}$$

While the continuous form of the Weibull model assumes that the standard deviation is constant for all dose groups, the power model can be run either using the same or different

standard deviations for each group. In Equations 6 and 7, a standard deviation common to all groups has been assumed. However, in addition to the fixed standard deviation case, we modeled each endpoint using the power model with group-specific (different) standard deviations. Although the standard deviations do not appear explicitly in the power model (Equation 5), they are also estimated in the modeling and affect estimates of the probability of response (see Equations 6 and 7). Because the standard deviations define the spread of the data around the means predicted by Equation 5, constraining the standard deviations may affect the model fit to the extent that the predictions of the means must be altered in order to accommodate the single fixed standard deviation for all groups.

The second method used to model body weight was designed to correspond to the historical and commonly accepted practice of considering a 10% change in mean body weight to represent an adverse effect. With this method, body weight was modeled using the power model (Equation 5) and a corresponding continuous polynomial model, with the BMR defined as a 10% change in mean weight. The appropriate choice of BMR for body weight change is discussed further in Section G, Discussion.

The BMR levels can be defined in terms of either additional or extra risk, for all the endpoints. Additional risk is defined as

$$P(d) - P(0),$$

and extra risk is defined as

$$[P(d) - P(0)] / [1 - P(0)].$$

In keeping with EPA practice, extra risk was generally used for these analyses as the more conservative choice in the absence of information on whether the background response occurs via the same mechanism as the chemical-induced response. The dose corresponding to a given extra risk will always be the same or lower than the dose corresponding to the same percent additional risk. There is very little difference between additional risk and extra risk for low values of P(0). The exceptions to this rule were the following. The software that is currently in use for applying the Weibull or power models for probability of response for continuous endpoints will calculate additional risk only. In addition, for the body weight change endpoints expressed as a change in the mean, only a specific “relative” change level (10%) was considered as the BMR.

It should also be noted that the comparisons of BMDs and NOAELs done by Allen et al. (1994a,b) and Kavlock et al. (1995) were done using additional risk; changing to extra risk might alter the relationships that were uncovered there and which, at this time, drive a number of decisions regarding the response levels to use for BMD definition.

For other endpoints, 5% and 10% risk were considered as the BMRs, as specified in the work assignment. These choices are consistent with experience with developmental toxicity endpoints (Allen et al., 1994a,b; Kavlock et al., 1995), where it was suggested that BMRs of 5% or 10% are appropriate if one desires BMDs that are similar, on average, to NOAELs. The

choice of 5% or 10% depended on the manner in which the data were treated. Based on the results of Allen et al. (1994a), the 10% response level may be expected to be only slightly conservative, on average, relative to corresponding NOAELs. This expectation represents a generalization from a quantal treatment of developmental toxicity endpoints to the other quantal endpoints considered in this analysis. In any case, the specific relationships between the calculated quantal BMDs and the corresponding NOAELs for naphthalene are presented below and could not have been predicted a priori.

The appropriate definition of the benchmark response is less understood for continuous endpoints than it is for quantal endpoints. For the modeling of continuous data based on probability of response, the most commonly used value of p_0 is 0.05 (5% of controls deficient); p_0 values of 0.05, 0.01, and 0.001 were used to evaluate the sensitivity of the predicted NOAEL to the selection of p_0. Using the power model, a p_0 of 0.05 and a BMR of 0.1 is equivalent to defining the BMD as the dose that results in change in the mean response equal to 0.6 times the standard deviation (Crump, 1995). Kavlock et al. (1995) found that, for a fetal weight endpoint, a BMR defined as $sd_0/2$ yielded BMDs that were on average similar to the corresponding NOAELs for a set of developmental endpoints. Thus, in the absence of additional information, the combination of $p_0 = 0.05$ and BMR = 0.1 appears to be an appropriate choice for the BMD using the continuous models that predict results in terms of the probability of response.

It should be noted that none of the treatments of the continuous endpoints considered in Allen et al. (1994a) or Kavlock et al. (1995) corresponds to the proposed approach for the continuous endpoints in this analysis. Because of that uncertainty, many combinations of BMR (5% and 10%), p_0 (0.05, 0.01, 0.001), and models (Weibull and power, the latter with and without a single standard deviation) have been explored.

D. DETERMINING GOODNESS OF FIT

For the quantal models, goodness of fit was determined by the modeling software using the chi-square test. For the continuous models, goodness of fit was determined on a "global" basis by comparing the model predicted mean responses for each dose group to the corresponding observed means and summing the squared differences. That sum of squares can be "normalized" and evaluated for significant differences by considering the variability of the observations within each dose group. More formally, an F-test was performed. This test automatically normalizes the differences between the observed and predicted means and accounts for the degrees of freedom associated with the predictions and those associated with the within-group variability.

The definition of degrees of freedom depends somewhat on the context. In cases where the experimental mean values for each dose group are compared with values predicted by the model (as is the case for the models considered here), the number of degrees of freedom is the difference between the number of dose groups and the number of parameters estimated in the model. In general, degrees of freedom specify how many residuals (differences between the model predictions and observations) are unconstrained by the model.

To assess the model-dependence of the fit of the models to the data sets, the best fitting Weibull model and power model with a single standard deviation were compared with one another for a continuous endpoint, and the best fitting polynomial and quantal Weibull model were compared in the case of a quantal endpoint. The maximized log-likelihoods, as well as the BMD estimates themselves, were compared. Because the two pairs of models have the same number of parameters, the one with the greater log-likelihood can be considered the better fitting model. No test of statistical significance is associated with this comparison. The power model that allowed for different standard deviations could not be compared with the other two continuous models in this manner. The additional parameters representing the separate standard deviations provide additional fit flexibility, so that allowing separate standard deviations for each experimental group will always result in a fit at least as good (i.e., likelihoods at least as large) as using a single standard deviation for a given choice of model. However, this generalization does not hold when comparing across different models.

E. CALCULATING SLOPES

Slopes were calculated for selected endpoints. The slopes were calculated as the derivatives of the functions of probability of response P(d), with respect to dose. Slopes were determined for the BMR equal to 10% additional risk. For the Weibull model, the slope has a relatively simple form:

$$P'(d) = (1-p_0)\,(\beta^{\gamma}\gamma d^{\gamma-1})\exp\{-(\beta d)^{\gamma}\}. \qquad \text{Eq. 8}$$

The slope for the power model is more complicated and depends, through Equations 6 or 7, on whether increases or decreases in the endpoint are considered to be adverse. When increases are considered to be adverse, the slope on the probability of response is

$$P'(d) = 1/\sigma^{*}\{N'[N^{-1}(1-p_0) - (\beta^{*}d)^{k}/\sigma] * k\beta^{k}d^{k-1}\}, \qquad \text{Eq. 9}$$

where N' is the derivative of the normal function. Similarly, when decreases are considered to be adverse,

$$P'(d) = 1/\sigma^{*}\{N'[N^{-1}(p_0) + (\beta^{*}d)^{k}/\sigma] * k\beta^{k}d^{k-1}\}. \qquad \text{Eq. 10}$$

Slopes were not calculated for the models applied to body weight endpoints expressed as a change in mean. Such slopes would be in terms of the change in body weight (rather than probability of response) as a function of dose and are not, therefore, comparable to the slopes derived for other endpoints.

F. RESULTS

F.1. Inhalation Data

Table B-1 shows the results of our initial modeling of the quantal data, including all severity grades for all endpoints. For these data sets, the response for all three nasal lesions was 100% in females and therefore could not be modeled. For nasal inflammation in males, the high observed and predicted response rate for both positive concentrations (Figure B-1, with confidence limits shown in Figure B-2 and a blow-up of the region of interest in Figure B-3) magnified the impact on the goodness-of-fit statistic of any deviation between observations and predictions. As a consequence, an unacceptable fit was obtained for both models ($p < 1E-6$). Therefore, the modeling was repeated using only the control and low-concentration groups. Since there were only two exposure groups, only two parameters could be used to fit the model. This constrained the equations to being linear in the exponent, which reduced the Weibull and polynomial models to identical forms. Using only the two groups, a perfect fit was obtained, and the BMC_{10} was 2.1 mg/m^3. A perfect fit was the expected result, since two points can be fit exactly with two parameters. The BMC_{10} estimated using all three exposure groups was of similar magnitude (3.7 mg/m^3) to that estimated using the better fitting procedure; this suggests that the high-exposure group and the apparently poor fit of the models to all exposure groups had little influence on the BMC estimates. Because the incidence of inflammation was almost 100% in the male mice, the calculated MLE and BMC are much lower than the LOAEL.

Acceptable fits were also obtained for lung inflammation in females and lymphocyte infiltration in females, but the MLEs and BMCs for these endpoints were much higher than those for nasal inflammation in males or females. This is consistent with what would be expected based on visual inspection of the data, since the response rate increases much more rapidly for the nasal endpoints. Compare, for example, the female mouse lung inflammation response rates (Figure B-4) with those for male nasal inflammation (Figure B-2). A poor fit was obtained for lung inflammation in males (Table B-1, Figure B-5). This was because the slope of the concentration-response curve between the controls and low-exposure group was higher than the slope between the low- and high-exposure groups (i.e., a concave downward curve). Because the models used are concave upward in the low-response region, the best fit was a line that splits the difference between the points. Since the response at both exposure levels was much higher for the nasal lesions than for the lung lesions, we focused our efforts to improve the modeling on the nasal endpoints.

In consultation with EPA, two issues became apparent with respect to the nasal endpoints. First, although all of the nasal lesions were clearly naphthalene related (the only nasal lesion in the control group was inflammation in one female), the adversity of the less severe lesions is unclear. While lesions of severity grades minimal and mild are visible histologically, it is unclear whether they correspond to any functional decrement. Second, while one of the strengths of the BMD methodology is that it uses relatively small extrapolations beyond the data, extrapolation from a response near 100% to a 10% response level introduces more uncertainty than smaller extrapolations. (For a more detailed discussion on this issue, see the paper in this series on benchmark modeling of nickel [Clement International Corporation, 1996b].) In light of these

issues, we repeated the modeling. All three nasal lesions (inflammation, metaplasia of olfactory epithelium, and hyperplasia of respiratory epithelium) were modeled for both sexes, and only severity grades of 3 or 4 (moderate or marked) were included.

As shown in Table B-2, unacceptable fits were obtained for most of the endpoints. Marginally acceptable fits were obtained for nasal inflammation in male mice ($p = 0.087$) and respiratory hyperplasia in female mice ($p = 0.049$). The corresponding BMCs were 3.7 and 2.4 mg/m^3, respectively. These values are about an order of magnitude higher than the BMC for nasal inflammation calculated including all severity grades. The poor fit can be attributed to the plateauing of response. Because the models are designed to reach 100% response at high doses, plateauing of response at lower levels is difficult to fit. Therefore, we attempted to improve the fit by allowing the power on the Weibull model to be less than 1, as described in Section C, Methods for Modeling. Although supralinear dose-response curves (i.e., power less than 1) can be very conservative for low-dose extrapolation, the added conservatism can be smaller at doses close to or within the experimental dose range. Because the benchmark dose approach is based on curve-fitting (representation of dose-response relationships at doses close to the experimental range), supralinear dose-response curves that provide improved fit may be appropriate and were considered here.

Table B-1. Results of initial BMC modeling for inhalation exposure to naphthalene, including all severity grades

		Polynomial model			Weibull model		
Endpoint	**BMR, extra risk**	**MLE concentration**	**Lower bound on concentration**	***P*-value**	**MLE concentration**	**Lower bound on concentration**	***P*-value**
Nasal inflammation in female mice—no NOAEL; LOAEL is 9.3 mg/m^3	0.10	4.55e-02	0.00	1.00e+00	4.27e-02	0.00	1.00e+00
	0.05	2.22e-02	0.00		2.08e-02	0.00	
	0.01	4.34e-03	0.00		4.07e-03	0.00	
Nasal inflammation in male mice—no NOAEL; LOAEL is 9.3 mg/m^3	0.10	4.29e-01	3.40e-01	0.00	4.29e-01	3.40e-01	0.00
	0.05	2.09e-01	1.65e-01		2.09e-01	1.65e-01	
	0.01	4.09e-02	3.24e-02		4.09e-02	3.24e-02	
Nasal inflammation in male mice, dropping high concentration group	0.10			[a]	2.77e-01	1.97e-01	1.00e+00
	0.05				1.35e-01	9.60e-02	
	0.01				2.64e-02	1.88e-02	

[a]The polynomial model was not run because using only two parameters (for the control and low-concentration exposure groups) results in a linear model, in which case the Weibull and polynomial models are identical.

Table B-1. Results of initial BMC modeling for inhalation exposure to naphthalene, including all severity grades (continued)

		Polynomial model			Weibull model		
Endpoint	**BMR, extra risk**	**MLE concen-tration**	**MLE concen-tration**	***P*-value**	**MLE concen-tration**	**Lower bound on concen-tration**	***P*-value**
Lung lesions, inflammation in female mice—no NOAEL; LOAEL is 9.3 mg/m^3	0.10	6.55e+00	5.14e+00	6.26e-01	6.55e+00	5.14e+00	6.26e-01
	0.05	3.19e+00	2.50e+00		3.19e+00	2.50e+00	
	0.01	6.25e-01	4.90e-01		6.25e-01	4.90e-01	
Lung lesions (infiltration, cellular lymphocyte) in female mice—no NOAEL; LOAEL is 9.3 mg/m^3	0.10	1.31e+01	7.85e+00	1.20e-01	1.31e+01	7.85e+00	1.20e-01
	0.05	6.36e+00	3.82e+00		6.36e+00	3.82e+00	
	0.01	1.25e+00	7.49e-01		1.25e+00	7.49e-01	
Lung lesions (inflammation) in male mice—no NOAEL: LOAEL is 9.3 mg/m^3	0.10	4.73e+00	3.93e+00	6.00e-03	4.73e+00	3.93e+00	6.00e-03
	0.05	2.30e+00	1.91e+00		2.30e+00	1.91e+00	
	0.01	4.51e-01	3.75e-01		4.51e-01	3.75e-01	

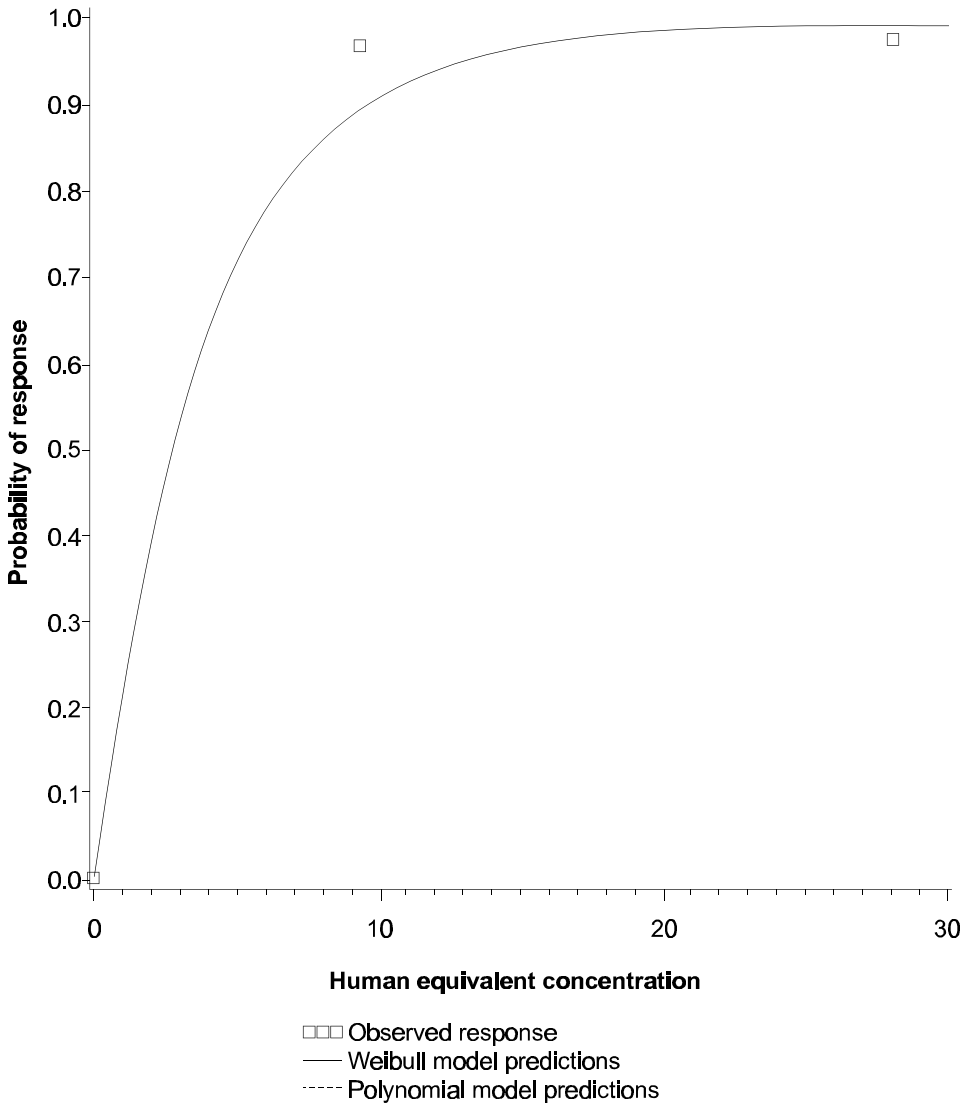

Figure B-1. Nasal inflammation in male mice, all severity grades.

Source: NTP, 1992a.

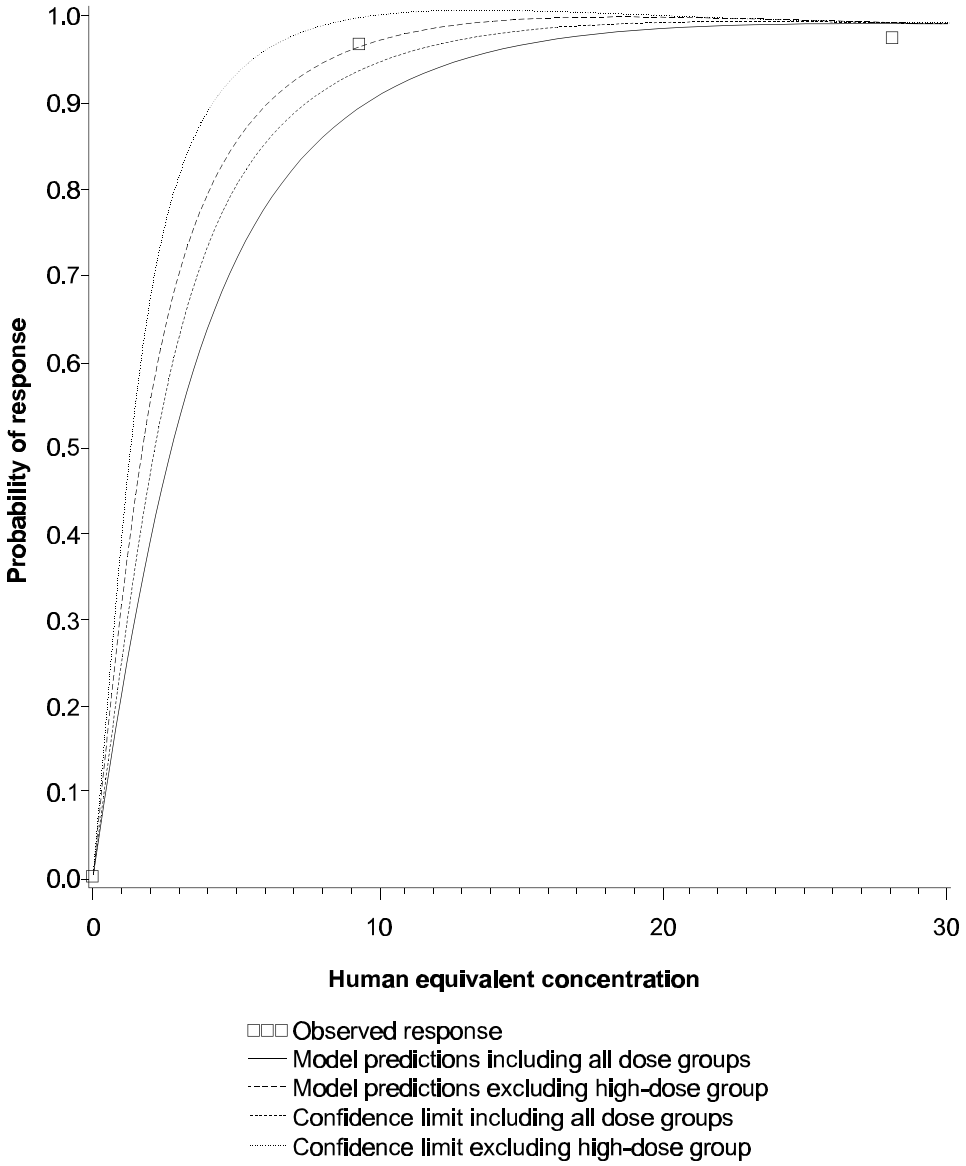

Figure B-2. Nasal inflammation in male mice, all severity grades, including confidence limits.

Source: NTP, 1992a.

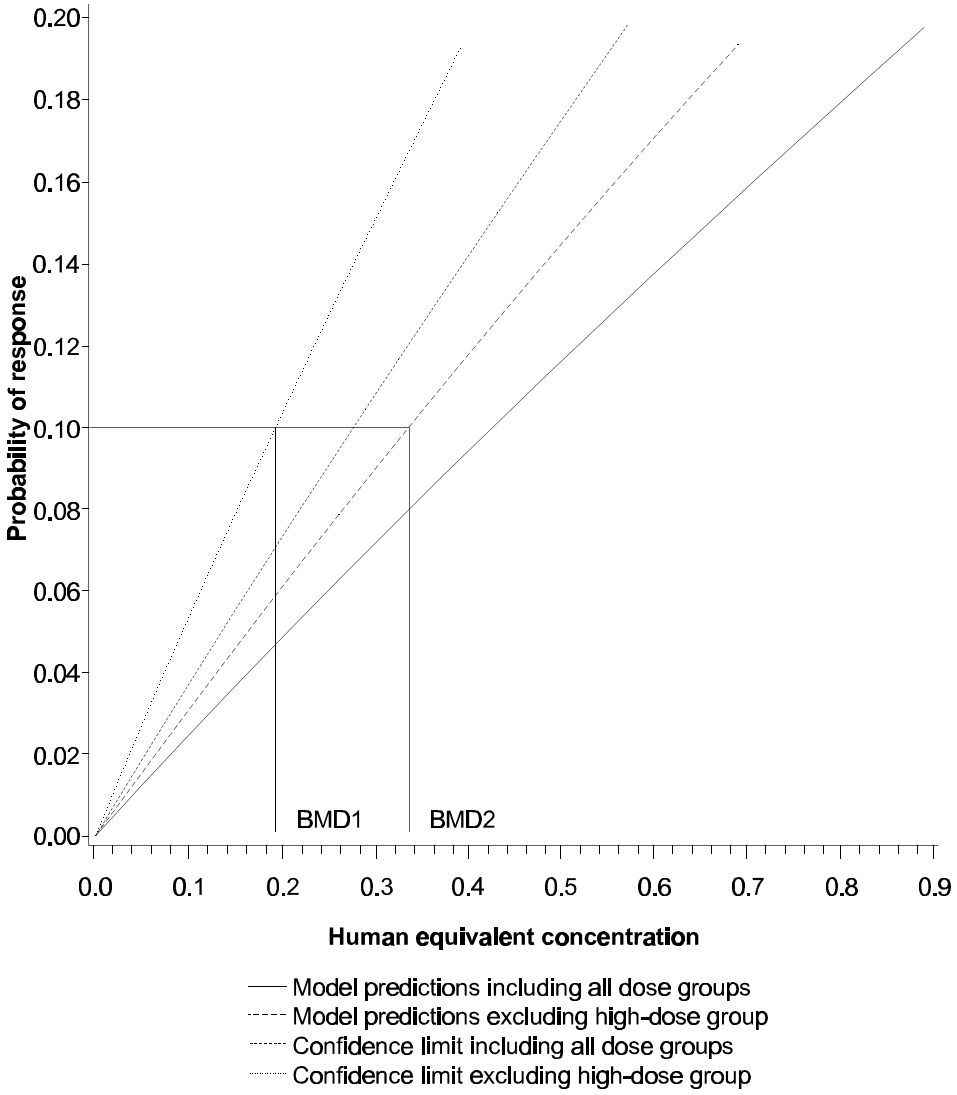

Figure B-3. Nasal inflammation in male mice, all severity grades, low-exposure region expanded.

Source: NTP, 1992a.

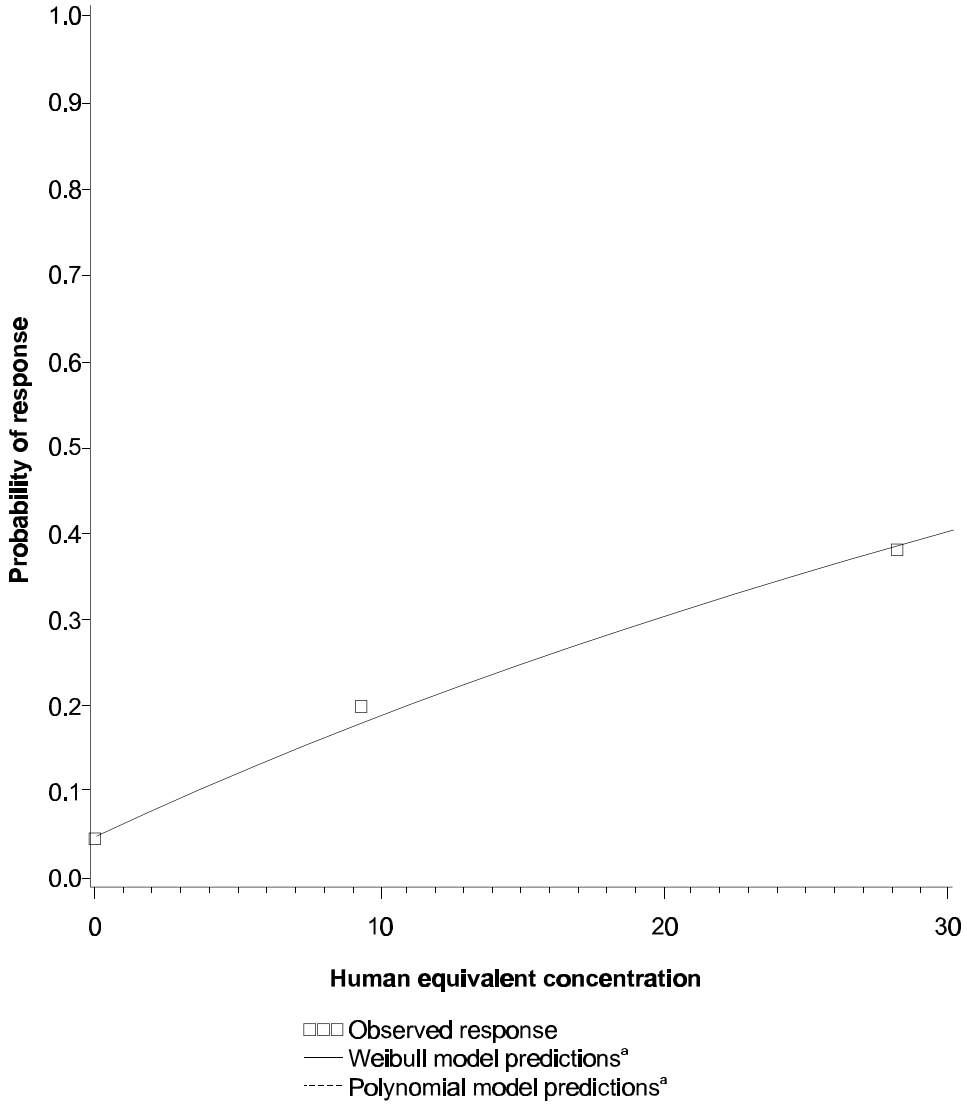

Figure B-4. Lung inflammation in female mice, all severity grades.

Source: NTP, 1992a.

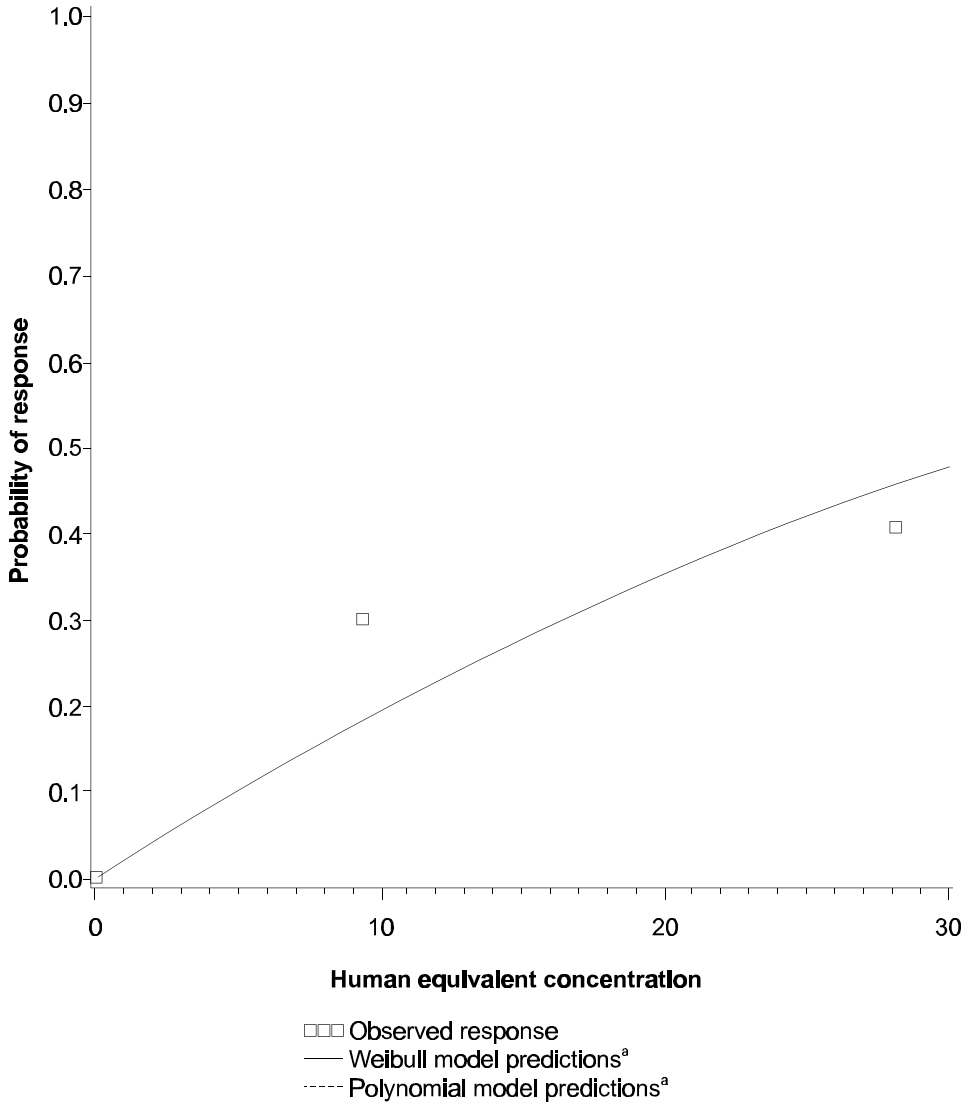

Figure B-5. Lung inflammation in male mice, all severity grades.

Source: NTP, 1992a.

Table B-2. Results of BMC modeling for inhalation exposure to naphthalene nasal lesions severity grade ≥ 3[a]

	Polynomial					Weibull				
BMR	**MLE**	**BMD**	**Log-likelihood**	**G-O-F[b] *P*-value**	**Chi-square**	**MLE**	**BMD**	**Log-likelihood**	**G-O-F[b] *P*-value**	**Chi-square**
Nasal inflammation in male mice										
1.00e-01	4.38e+00	3.66e+00	-1.35e+02	8.70e-02	2.93e+00	4.38e+00	3.66e+00	-1.35e+02	8.70e-02	2.93e+00
5.00e-02	2.13e+00	1.78e+00	-1.35e+02	8.70e-02	2.93e+00	2.13e+00	1.78e+00	-1.35e+02	8.70e-02	2.93e+00
Nasal inflammation in male mice (high dose dropped)										
1.00e-01	3.04e+00	2.13e+00	-4.06e+01	1.00e+00	8.95e-34	3.04e+00	2.13e+00	-4.06e+01	1.00e+00	9.17e-31
5.00e-02	1.48e+00	1.04e+00	-4.06e+01	1.00e+00	8.95e-34	1.48e+00	1.04e+00	-4.06e+01	1.00e+00	9.17e-31
Nasal inflammation in male mice (Weibull lower bound < 1)										
1.00e-01	N/A[c]					1.38e+00	1.02e+00	-1.34e+02	1.00e+00	4.30e-12
5.00e-02	N/A[c]					4.06e-01	2.99e-01	-1.34e+02	1.00e+00	4.30e-12
Nasal inflammation in female mice										
1.00e-01	6.54e+00	5.31e+00	-1.24e+02	6.63e-03	7.37e+00	6.54e+00	5.31e+00	-1.24e+02	6.63e-03	7.37e+00
5.00e-02	3.18e+00	2.58e+00	-1.24e+02	6.63e-03	7.37e+00	3.18e+00	2.58e+00	-1.24e+02	6.63e-03	7.37e+00
Nasal inflammation in female mice (high dose dropped)										
1.00e-01	3.47e+00	2.36e+00	-3.63e+01	1.00e+00	1.51e-29	3.47e+00	2.36e+00	-3.63e+01	1.00e+00	0.00
5.00e-02	1.69e+00	1.15e+00	-3.63e+01	1.00e+00	1.51e-29	1.69e+00	1.15e+00	-3.63e+01	1.00e+00	0.00

[a]For all endpoints in this table: no NOAEL; LOAEL is 9.3 mg/m^3.
[b]G-O-F = Goodness of fit.
[c]This modeling approach could be used only for the Weibull model.

Table B-2. Results of BMC modeling for inhalation exposure to naphthalene nasal lesions severity grade ≥ 3 (continued)

	Polynomial					Weibull				
BMR	**MLE**	**BMD**	**Log-likelihood**	**G-O-F[b] *P*-value**	**Chi-square**	**MLE**	**BMD**	**Log-likelihood**	**G-O-F[b] *P*-value**	**Chi-square**
Nasal inflammation in female mice (Weibull lower bound < 1)										
1.00e-01	N/A					2.64e-01	1.25e-01	-1.21e+02	1.00e+00	3.92e-13
5.00e-02	N/A					1.97e-02	9.29e-03	-1.21e+02	1.00e+00	3.92e-13
Olfactory metaplasia in male mice										
1.00e-01	3.07e+00	2.62e+00	-1.51e+02	0.00	2.08e+01	3.07e+00	2.62e+00	-1.51e+02	0.00	2.08e+01
5.00e-02	1.49e+00	1.27e+00	-1.51e+02	0.00	2.08e+01	1.49e+00	1.27e+00	-1.51e+02	0.00	2.08e+01
Olfactory metaplasia in male mice (high dose dropped)										
1.00e-01	1.44e+00	1.10e+00	-4.78e+01	1.00e+00	2.86e-31	1.44e+00	1.10e+00	-4.78e+01	1.00e+00	0.00
5.00e-02	7.03e-01	5.34e-01	-4.78e+01	1.00e+00	2.86e-31	7.03e-01	5.34e-01	-4.78e+01	1.00e+00	0.00
Olfactory metaplasia in male mice (Weibull lower bound < 1)										
1.00e-01	N/A					1.87e-07	4.32e-08	-1.41e+02	1.00e+00	5.66e-12
5.00e-02	N/A					1.99e-10	0.00	-1.41e+02	1.00e+00	5.66e-12
Olfactory metaplasia in female mice										
1.00e-01	3.63e+00	3.07e+00	-1.56e+02	0.00	4.40e+01	3.63e+00	3.07e+00	-1.56e+02	0.00	4.40e+01
5.00e-02	1.77e+00	1.49e+00	-1.56e+02	0.00	4.40e+01	1.77e+00	1.49e+00	-1.56e+02	0.00	4.40e+01

Table B-2. Results of BMC modeling for inhalation exposure to naphthalene nasal lesions severity grade ≥ 3 (continued)

	Polynomial					Weibull				
BMR	**MLE**	**BMD**	**Log-likelihood**	**G-O-F[b] *P*-value**	**Chi-square**	**MLE**	**BMD**	**Log-likelihood**	**G-O-F[b] *P*-value**	**Chi-square**
Olfactory metaplasia in female mice (high dose dropped)										
1.00e-01	1.21e+00	9.26e-01	-4.47e+01	1.00e+00	2.49e-31	1.21e+00	9.26e-01	-4.47e+01	1.00e+00	6.94e-27
5.00e-02	5.91e-01	4.51e-01	-4.47e+01	1.00e+00	2.49e-31	5.91e-01	4.51e-01	-4.47e+01	1.00e+00	6.94e-27
Olfactory metaplasia in female mice (Weibull lower bound < 1)										
1.00e-01	N/A					0.00	0.00	-1.38e+02	1.10e-01	2.56e+00
5.00e-02	N/A					0.00	0.00	-1.38e+02	1.10e-01	2.56e+00
Respiratory hyperplasia in male mice										
1.00e-01	2.28e+00	1.96e+00	-1.41e+02	2.25e-04	1.36e+01	2.28e+00	1.96e+00	-1.41e+02	2.25e-04	1.36e+01
5.00e-02	1.11e+00	9.56e-01	-1.41e+02	2.25e-04	1.36e+01	1.11e+00	9.56e-01	-1.41e+02	2.25e-04	1.36e+01
Respiratory hyperplasia in male mice (high dose dropped)										
1.00e-01	1.28e+00	9.77e-01	-4.76e+01	1.00e+00	6.90e-31	1.28e+00	9.77e-01	-4.76e+01	1.00e+00	2.94e-30
5.00e-02	6.21e-01	4.76e-01	-4.76e+01	1.00e+00	6.90e-31	6.21e-01	4.76e-01	-4.76e+01	1.00e+00	2.94e-30
Respiratory hyperplasia in male mice (Weibull lower bound < 1)										
1.00e-01	N/A					1.41e-02	8.65e-03	-1.34e+02	1.00e+00	1.30e-25
5.00e-02	N/A					1.34e-03	8.23e-04	-1.34e+02	1.00e+00	1.30e-25

Table B-2. Results of BMC modeling for inhalation exposure to naphthalene nasal lesions severity grade ≥ 3 (continued)

	Polynomial					Weibull				
BMR	**MLE**	**BMD**	**Log-likelihood**	**G-O-F[b] *P*-value**	**Chi-square**	**MLE**	**BMD**	**Log-likelihood**	**G-O-F[b] *P90*-value**	**Chi-square**
Respiratory hyperplasia in female mice										
1.00e-01	2.75e+00	2.35e+00	-1.35e+02	4.89e-02	3.88e+00	2.75e+00	2.35e+00	-1.35e+02	4.89e-02	3.88e+00
5.00e-02	1.34e+00	1.14e+00	-1.35e+02	4.89e-02	3.88e+00	1.34e+00	1.14e+00	-1.35e+02	4.89e-02	3.88e+00
Respiratory hyperplasia in female mice (high dose dropped)										
1.00e-01	1.92e+00	1.41e+00	-4.37e+01	1.00e+00	1.34e-31	1.92e+00	1.41e+00	-4.37e+01	1.00e+00	0.00
5.00e-02	9.34e-01	6.85e-01	-4.37e+01	1.00e+00	1.34e-31	9.34e-01	6.85e-01	-4.37e+01	1.00e+00	0.00
Respiratory hyperplasia in female mice (Weibull lower bound < 1)										
1.00e-01	N/A					6.26e-01	4.78e-01	-1.33e+02	1.00e+00	1.59e-12
5.00e-02	N/A					1.83e-01	1.40e-01	-1.33e+02	1.00e+00	1.59e-12

With one exception, excellent fits were obtained for all endpoints when the lower bound on the power was allowed to be less than 1 (Table B-2, Figures B-6 and B-7). Interestingly, the relative sizes of the BMCs for the different endpoints calculated with the power restricted to greater than or equal to 1 (and with the corresponding poor fits) were not predictive of the relative sizes of the BMCs calculated allowing the power to be less than 1. As expected, the BMCs were consistently smaller than those calculated with the power restricted to greater than or equal to 1. The BMCs calculated for nasal inflammation in male mice (1.0 mg/m^3) and respiratory hyperplasia in female mice (0.48 mg/m^3) were within a factor of 2-5 of the BMCs calculated with the restricted power, and the BMC for nasal inflammation in female mice was somewhat lower (0.12 mg/m^3). However, the lowest BMC calculated with the power unrestricted (0.008 mg/m^3 for respiratory hyperplasia in male mice) was more than an order of magnitude lower than the next higher BMC calculated using this method. This low value resulted from the low value estimated for the lower bound on the power and the associated, relatively large coefficient (multiplier) for the dose term in the equation. Note, however, that this is not a case in which the BMC is small because of wide confidence intervals. The MLE, as well as the BMC, was smaller than the BMCs from the other endpoints considered in this manner. The small values for the MLE and BMC simply result from the fit of the dose-response function that requires a steep increase in response rate at low doses. The BMC for olfactory metaplasia in males was very low (4E-8 mg/m^3, MLE = 2E-7) for similar reasons (see also Figure B-6). Olfactory metaplasia in females was the only lesion for which there was a poor fit, due to a nonmonotonic concentration-response (Figure B-8). Even with this decreased response at the high exposure level, the fit for this endpoint was better than in the original modeling ($p = 0.11$ vs. $p < 0.00001$). However, the estimated power was quite low (0.01), resulting in a steep concentration-response curve and very low MLE (5E-78). Thus, even when the lower bound on the power is restricted as described in Section C, apparently quite conservative numbers can result. Note that the apparent conservatism was greatest for the endpoint for which there was the highest response, and therefore, the greatest degree of extrapolation below the experimental doses was necessary. Due to this apparent high degree of conservatism, we also repeated the modeling for these endpoints with the power restricted to 1, dropping the high-exposure group.

The results obtained with the high-exposure level dropped are also shown in Table B-2. Because only one positive concentration was used, a perfect fit ($p = 1.0$) was obtained for all endpoints. The most sensitive endpoint was metaplasia of the olfactory epithelium in female mice, with a BMC of 0.93 mg/m^3. The BMC values calculated for hyperplasia of the respiratory epithelium in male mice (0.98 mg/m^3) and metaplasia of the olfactory epithelium in male mice (1.1 mg/m^3) were of a comparable size. Interestingly, olfactory metaplasia in males and females were also the most sensitive endpoints when the lower bound on the power was allowed to be less than 1; higher values compared with the other endpoints were obtained when all exposure levels were modeled, due to a greater degree of plateauing (males) or nonmonotonic response (females). However, dropping the high concentration resulted in a narrower range of BMC values across all endpoints (a factor of 2.5), compared with a factor of 5.4 when all exposure levels were modeled. Figures B-6 and B-8 compare the model fit for the three different methods used to model olfactory metaplasia in males and females, respectively; Figure B-7 shows the results for respiratory hyperplasia in males.

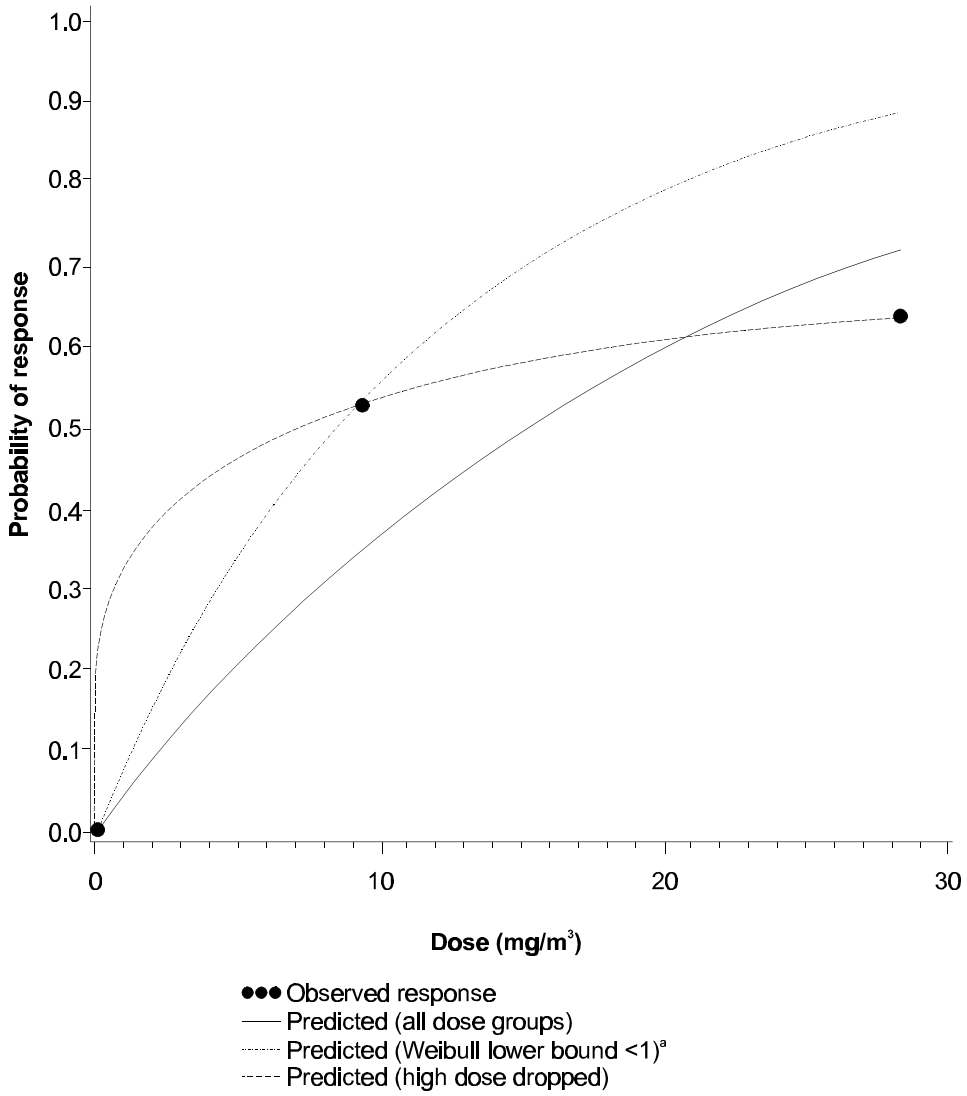

Figure B-6. Metaplasia of the olfactory epithelium in the nose of male mice, severity ≥ 3.

Source: NTP, 1992a.

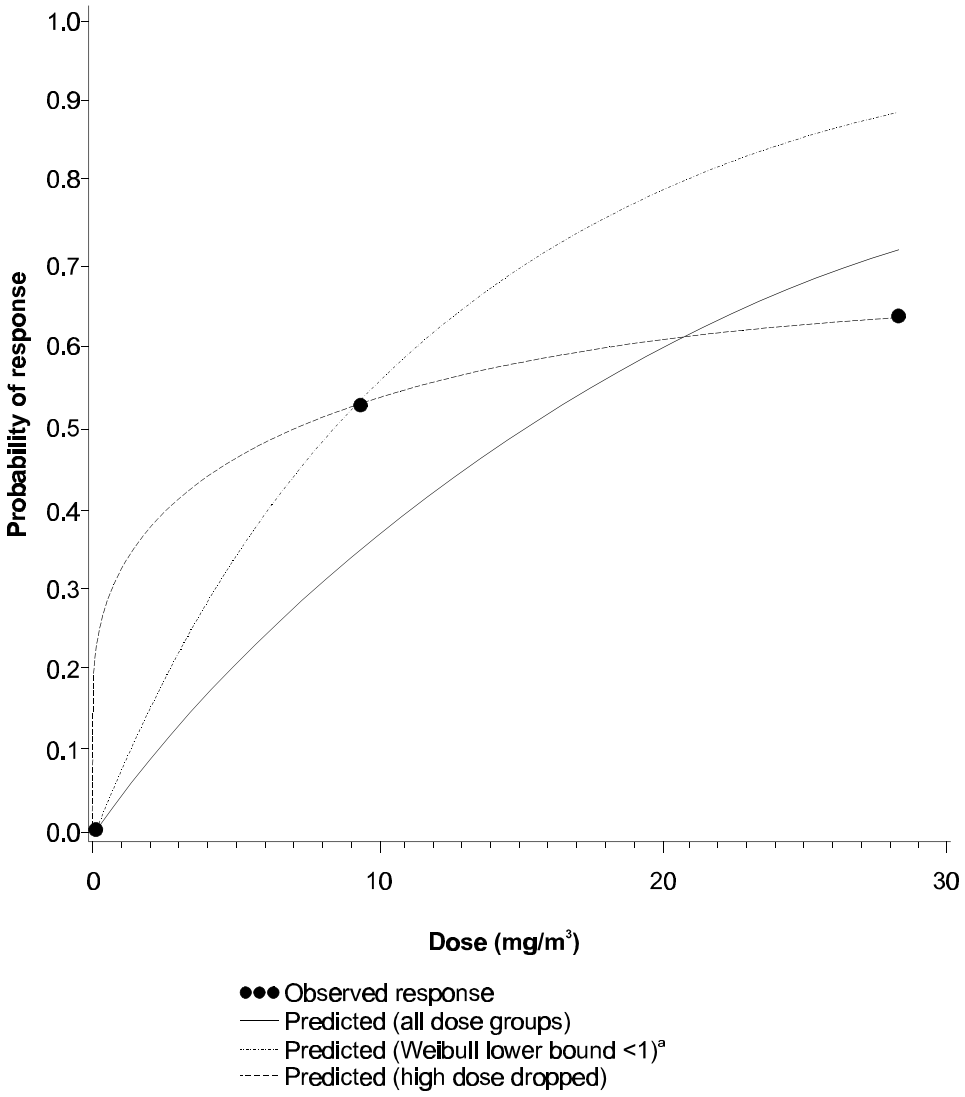

Figure B-7. Hyperplasia of the olfactory epithelium in the nose of female mice, severity ≥ 3.

Source: NTP, 1992a.

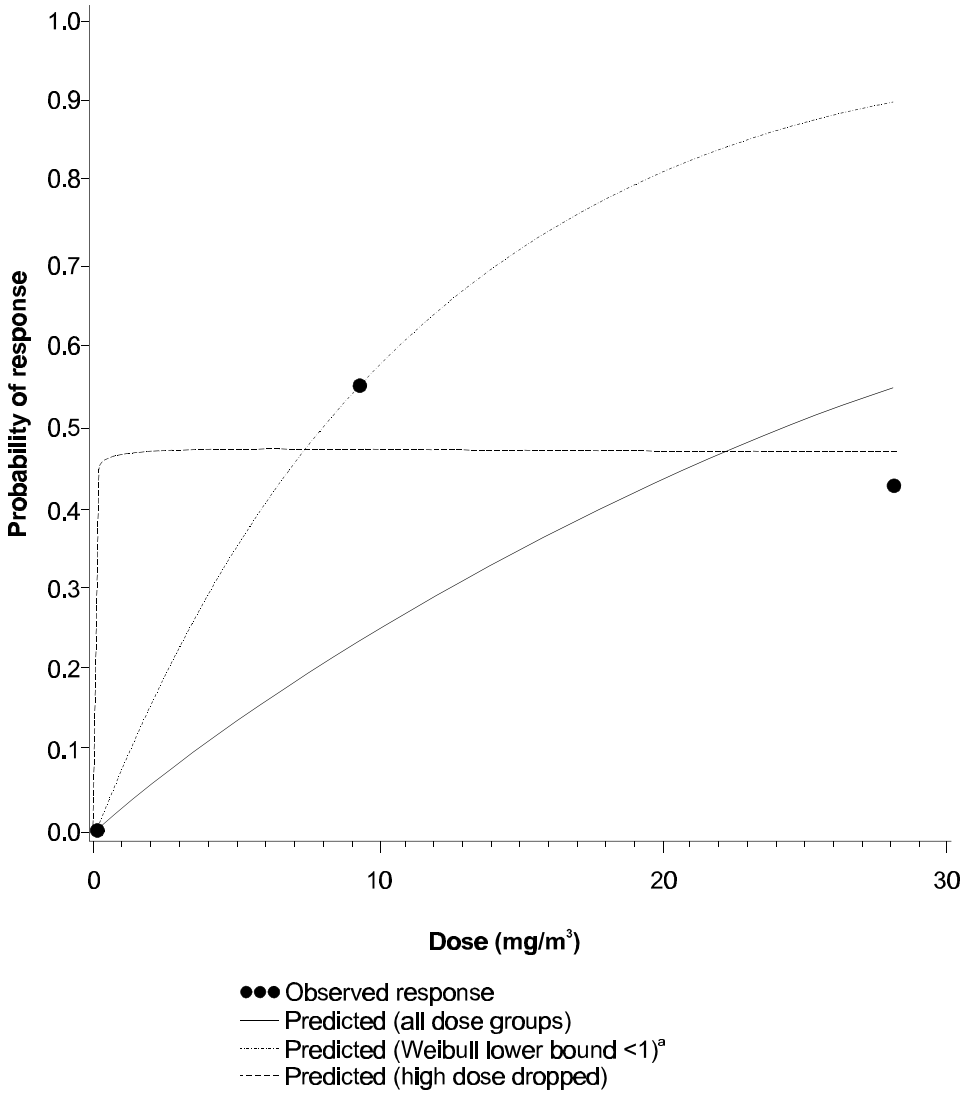

Figure B-8. Metaplasia of the olfactory epithelium in the nose of female mice, severity ≥ 3.

Source: NTP, 1992a.

Slopes were calculated for the three most sensitive endpoints, metaplasia of the olfactory epithelium in male and female mice and hyperplasia of the respiratory epithelium in male mice. The slope has been determined for each of the three approaches that were considered for this endpoint (see Figures B-6, B-7, and B-8) with the results summarized as follows:

Approach	**Slope Estimate**		
	Metaplasia		Hyperplasia
	Males	Females	Males
All three doses, power ≥ 1	3.6E-2	3.1E-2	4.8E-2
All three doses, any positive power	2.3E+5[a]	2.1E+74[b]	3.4
Control and low dose only	8.7E-2	1.0E-1	9.7E-2

[a]May be slightly inaccurate because of convergence problems in lower bound calculation.
[b]This slope is calculated at the MLE dose corresponding to 10% extra risk because the BMD estimate was 0 (which would give infinite slope). The value shown is an underestimate; even so, it is astronomical.

All of these slopes (with the exception noted) were determined at their respective BMDs (for a 10% response level). Note that the ordering of the slopes corresponds exactly with the graphical representation of the steepness of the curves in Figures B-6 to B-8 at relatively low doses; indeed, the slope is simply a quantitative expression of the steepness of a curve at a particular point.

F.2. Oral Data

Decreased male and female body weight relative to controls was modeled from the subchronic oral study in rats (BCL, 1980a) (Figures B-9 and B-10). As described in Section C, three definitions of the BMD were used. In the first definition, the BMR was defined as a 10% decrease in the mean body weight, in order to most closely approximate the definition generally used for adverse differences in body weight from the control. As shown in Table B-3, the BMDs (130 mg/kg-day for males, and 235 mg/kg-day for females) were in close agreement with corresponding NOAELs, although consistently slightly higher. Similar values were obtained using the polynomial and power models, and the fits were acceptable. The model fit is shown in Figures B-9 and B-10 for males and females, respectively.

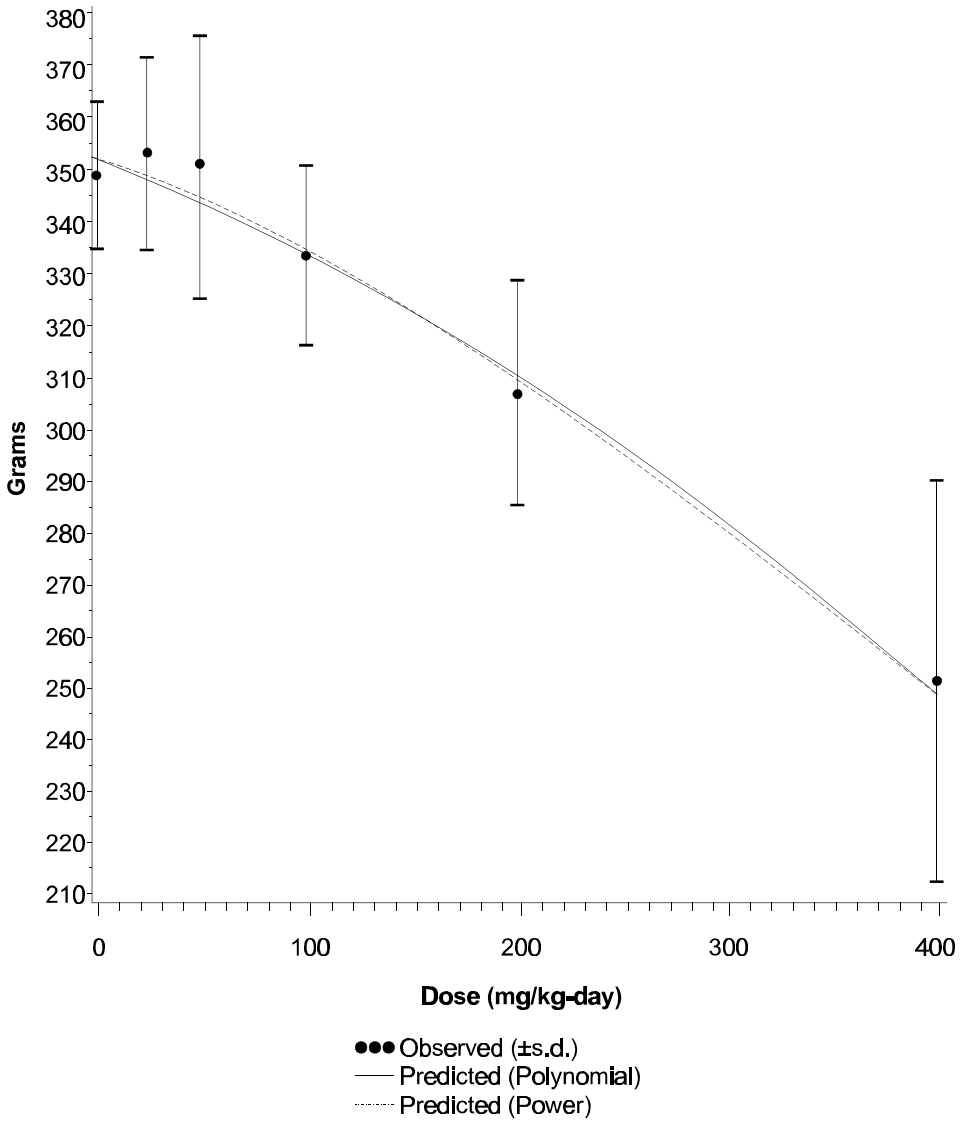

Figure B-9. Body weight in male rats.

Source: BCL, 1980a.

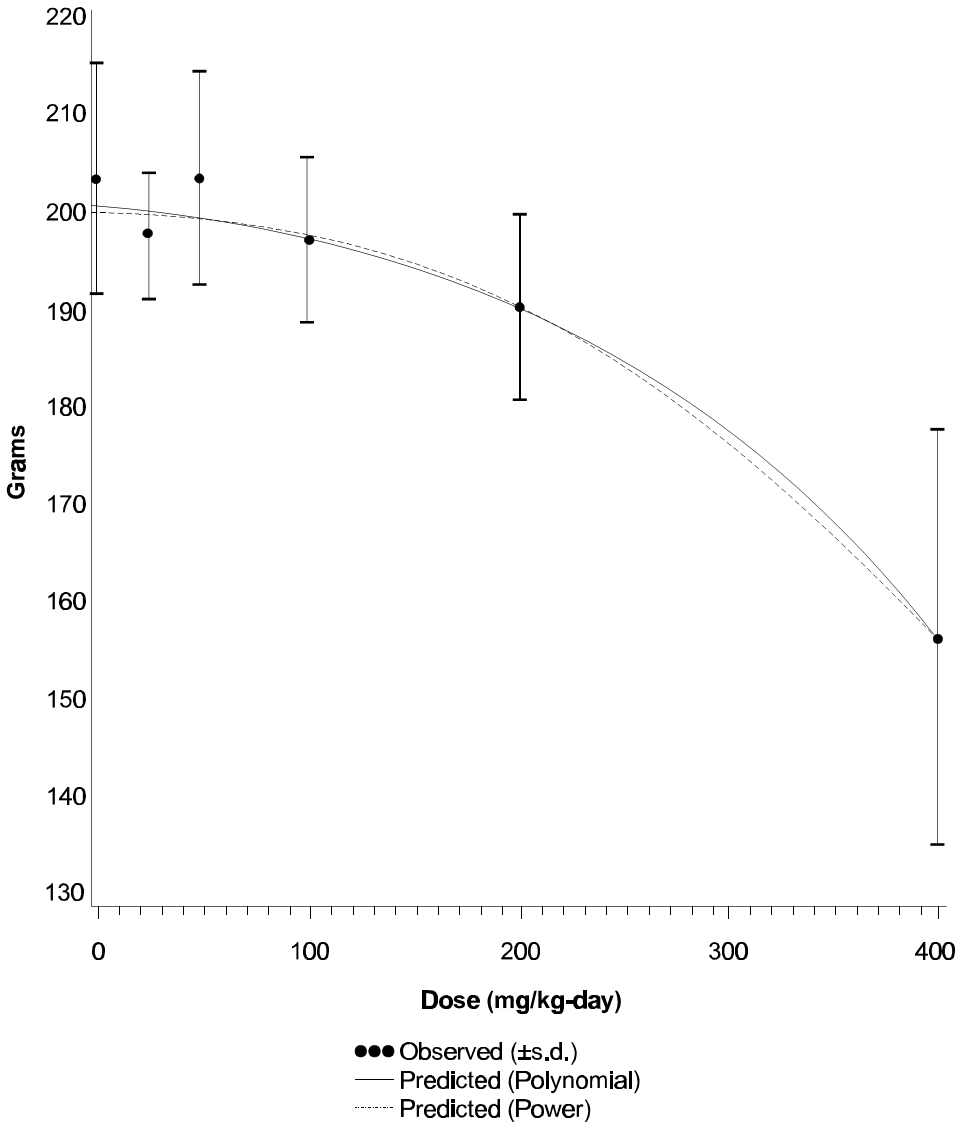

Figure B-10. Body weight in female rats.

Source: BCL, 1980a.

Table B-3. Quantal and body weight (expressed as change in mean) results for oral exposure to naphthalene

		Polynomial						Power				
Model	**BMR type**	**BMR**	**MLE**	**BMD**	**Log-likelihood**	**G-O-F[a] *P*-value**	**Chi-square**	**MLE**	**BMD**	**Log-likelihood**	**G-O-F[a] *P*-value**	**Chi-square**
Naphthalene female weight (BCL, 1980a); NOAEL is 200 mg/kg-day, LOAEL is 400 mg/kg-day												
Continuous	Relative	1.00e-01	2.85e+02	2.35e+02	-1.40e+02	3.49e-01	N/A	2.82e+02	2.36e+02	-1.40e+02	5.23e-01	N/A
Naphthalene male weight (BCL, 1980a); NOAEL is 100 mg/kg-day, LOAEL is 200 mg/kg-day												
Continuous	Relative	1.00e-01	1.71e+02	1.30e+02	-1.79e+02	5.85e-01	N/A	1.72e+02	1.35e+02	-1.78e+02	6.84e-01	N/A
Naphthalene maternal body weight gain; NOAEL is 50 mg/kg-day, LOAEL is 150 mg/kg-day												
Continuous	Relative	1.00e-01	8.03e+01	6.78e+01	-2.72e+02	5.56e-02	N/A	8.03e+01	6.78e+01	-2.72e+02	5.56e-02	N/A
		Polynomial						**Weibull**				
Model	**BMR type**	**BMR**	**MLE**	**BMD**	**Log-likelihood**	**G-O-F[a] *P*-value**	**Chi-square**	**MLE**	**BMD**	**Log-likelihood**	**G-O-F[a] *P*-value**	**Chi-square**
Number of litters with malformed fetuses (NTP, 1991); no LOAEL designated, see text												
Quantal	Extra	1.00e-01	1.10e+02	5.71e+01	-6.11e+01	9.80e-01	6.00e-04	1.10e+02	5.71e+01	-6.11e+01	1.00e+00	6.00e-04
Quantal	Extra	5.00e-02	5.37e+01	2.78e+01	-6.11e+01	9.80e-01	6.00e-04	5.37e+01	2.78e+01	-6.11e+01	1.00e+00	6.00e-04

[a]G-O-F = Goodness of fit.

For the second definition of the BMR, the incidence of an adverse effect in the control population (p_0) was defined, as was done for the maternal weight endpoint of the NTP (1991) study. The third definition was based on the toxicologist's rule-of-thumb that a 10% decrease in mean final body weight compared with the control mean is considered adverse. This defined a cut-point (x_0) for adversity for individual body weights. The BMD was then defined as the dose at which a defined percent of the population (e.g., 10%) had a body weight below this cut-point.

As shown in Table B-4, the BMDs calculated for male body weight using a BMR defined based on probability of response were consistently lower than the NOAEL. For fixed p_0, the BMDs ranged from 32 to 53 mg/kg-day, for a BMR of 10%. Somewhat higher BMDs (57-77 mg/kg-day for a BMR of 10%) were obtained with fixed x_0, because fixing x_0 resulted in lower p_0 values. This was especially true in the case of the power model with group-specific standard deviations, for which the control group standard deviation was less than that for the other groups. The smaller the standard deviation, the further out in the control group distribution is a fixed decrease in body weight (i.e., to 314 g). Because the position of a point with respect to the tail of the distribution determines its probability (in this case the value of p_0), the p_0 value in the case with smaller estimated control group standard deviation is less than in other cases. Lower p_0 values entail higher BMDs, all else being equal. The reason that smaller values of p_0 result in large BMDs (for fixed BMR) is related to the shape of the normal distribution assumed for the variability of the responses, the so-called "bell-shaped" curve. The p_0 value specifies how far out in the tail of the distribution the adverse response is: for smaller p_0, fewer unexposed animals are considered to be in the abnormal range. As dose changes, we are basically considering shifts in the distributions; the mean value for the endpoint being measured changes to reflect the shift to more adverse effects, and more of the tail of the distribution enters into the abnormal range. For a given change in the mean, there is a larger change in response for $p_0 = 0.05$ than for $p_0 = 0.001$ (because the slope of the bell curve is greater at larger p_0). Stated another way, a smaller change in the mean is necessary to produce a given increased risk (e.g., a BMR of 10%) for a larger p_0. Since the response is being measured relative to untreated animals, a small change in mean corresponds to a lower dose.

Somewhat higher BMDs were calculated for decreased female body weight, but the BMDs were still lower than the corresponding NOAEL by about a factor of 2, for BMR = 10%. In contrast to the male body weight response, the x_0 values were essentially the same, whether p_0 or x_0 was fixed prior to modeling. As a result, the differences in BMDs observed in the males, as a function of the models and of whether p_0 or x_0 was specified, did not occur for the females.

Decreased maternal body weight gain in the NTP (1991) study was also modeled using two different approaches. For a BMR defined in terms of the probability of response, 18 modeling variations (using BMRs of 5% and 10%, p_0 values of 0.001, 0.01, and 0.05, and three different models) were considered (Table B-5). A previous deliverable in this series (Clement International Corporation, 1996a) discussed a method for reducing the number of modeling runs, based on first identifying the most important endpoints, using a fixed combination of p_0 and BMR, and then running the remaining variations on the chosen endpoints. Since only one continuous endpoint from this study was of interest for modeling, no attempt was made to use a shortcut procedure.

Table B-4. Male and female body weight (expressed as a probability of response)

Dataset	Model	Standard deviation	P_0	X_0	BMR	MLE	BMD	Upper bound	Log-likelihood	G-O-F[a] *P*-value
Male body weight—NOAEL is 100 mg/kg-day, LOAEL is 200 mg/kg-day										
Male	Weibull model, P_0 fixed	One standard deviation for all groups	5.00e-02	314.87	5.00e-02	67.37	37.82	107.74	-213.84	0.91492
Male	Weibull model, P_0 fixed	One standard deviation for all groups	5.00e-02	314.87	1.00e-01	88.475	53.849	132.89	-213.84	0.91492
Male	Kpower model, P_0 fixed	One standard deviation for all groups	5.00e-02	316.3	5.00e-02	50.721	27.617	94.293	-214.27	0.73279
Male	Kpower model, P_0 fixed	One standard deviation for all groups	5.00e-02	316.3	1.00e-01	77.276	46.25	128.15	-214.27	0.73279
Male	Kpower model, P_0 fixed	Different standard deviations for each group	5.00e-02	329.74	5.00e-02	37.974	18.828	69.609	-207.98	0.71148
Male	Kpower model, P_0 fixed	Different standard deviations for each group	5.00e-02	329.74	1.00e-01	56.884	31.531	95.436	-207.98	0.71148
Male	Weibull model, X_0 fixed	One standard deviation for all groups	4.58e-02	314	5.00e-02	68.841	45.059	98.939	-213.84	0.91898

[a]G-O-F = Goodness of fit.

Table B-4. Male and female body weight (expressed as a probability of response) (continued)

Dataset	Model	Standard deviation	P_0	X_0	BMR	MLE	BMD	Upper bound	Log-likelihood	G-O-F[a] *P*-value
Male	Weibull model, X_0 fixed	One standard deviation for all groups	4.58e-02	314	1.00e-01	90.173	63.616	122.63	-213.84	0.91898
Male	Kpower model, X_0 fixed	One standard deviation for all groups	4.05e-02	314	5.00e-02	55.693	34.101	85.103	-214.27	0.73279
Male	Kpower model, x_0 fixed	One standard deviation for all groups	4.05e-02	314	1.00e-01	83.303	57.25	116.95	-214.27	0.73279
Male	Kpower model, X_0 fixed	Different standard deviations for each group	2.90e-03	314	5.00e-02	91.896	52.277	132.63	-207.89	0.68237
Male	Kpower model, X_0 fixed	Different standard deviations for each group	2.90e-03	314	1.00e-01	113.22	76.774	150.61	-207.89	0.68237
Female body weight—NOAEL is 200 mg/kg-day, LOAEL is 400 mg/kg-day										
Female	Weibull model, P_0 fixed	One standard deviation for all groups	5.00e-02	181.25	5.00e-02	141.51	85.808	211	-178.94	0.58143
Female	Weibull model, P_0 fixed	One standard deviation for all groups	5.00e-02	181.25	1.00e-01	168.73	110.91	236.29	-178.94	0.58143
Female	Kpower model, P_0 fixed	One standard deviation for all groups	5.00e-02	181.82	5.00e-02	122.78	62.797	210.6	-178.81	0.62302

Table B-4. Male and female body weight (expressed as a probability of response) (continued)

Dataset	Model	Standard deviation	P_0	X_0	BMR	MLE	BMD	Upper bound	Log-likelihood	G-O-F[a] *P*-value
Female	Kpower model, P_0 fixed	One standard deviation for all groups	5.00e-02	181.82	1.00e-01	159.41	93.886	244.04	-178.81	0.62302
Female	Kpower model, P_0 fixed	Different standard deviation for each group	5.00e-02	181.42	5.00e-02	135.66	74.651	215.33	-170.37	0.50152
Female	Kpower model, P_0 fixed	Different standard deviation for each group	5.00e-02	181.42	1.00e-01	173.16	106.28	251.16	-170.37	0.50152
Female	Weibull model, X_0 fixed	One standard deviation for all groups	6.40e-02	182.7	5.00e-02	135.16	86.541	197.76	-178.94	0.57442
Female	Weibull model, X0 fixed	One standard deviation for all groups	6.40e-02	182.7	1.00e-01	162.06	112.51	222.82	-178.94	0.57442
Female	Kpower model, X_0 fixed	One standard deviation for all groups	5.80e-02	182.7	5.00e-02	117.74	68.252	192.97	-178.81	0.62302
Female	Kpower model, X0 fixed	One standard deviation for all groups	5.80e-02	182.7	1.00e-01	154.08	101.45	226.84	-178.81	0.62302
Female	Kpower model, X_0 fixed	Different standard deviation for each group	7.21e-02	182.7	5.00e-02	127.13	81.173	190.29	-170.21	0.52705
Female	Kpower model, X_0 fixed	Different standard deviation for each group	7.21e-02	182.7	1.00e-01	163.51	117.14	224.23	-170.21	0.52705

Source: BCL, 1980a.

Table B-5. Maternal body weight gain (expressed as a probability of response)

Dataset	Model	Standard deviation	P_0	BMR	MLE	BMD	Upper bound	Log-likelihood	G-O-F[a] *P*-value
Maternal body weight gain—NOAEL is 50 mg/kg-day, LOAEL is 150 mg/kg-day									
Maternal body weight gain	Weibull model, P_0 fixed	One standard deviation for all groups	5.00e-02	1.00e-01	78.825	35.778	147.96	-327.16	0.11226
Maternal body weight gain	Weibull model, P_0 fixed	One standard deviation for all groups	5.00e-02	5.00e-02	45.559	17.392	98.491	-327.16	0.11226
Maternal body weight gain	Weibull model, P_0 fixed	One standard deviation for all groups	1.00e-02	1.00e-01	165.01	95.555	261.63	-327.02	0.13411
Maternal body weight gain	Weibull model, P_0 fixed	One standard deviation for all groups	1.00e-02	5.00e-02	105.18	53.443	180.81	-327.02	0.13411
Maternal body weight gain	Weibull model, P_0 fixed	One standard deviation for all groups	1.00e-03	1.00e-01	348.59	235.32	547.67	-326.83	0.17007
Maternal body weight gain	Weibull model, P_0 fixed	One standard deviation for all groups	1.00e-03	5.00e-02	244.84	157.17	376.39	-326.83	0.17007
Maternal body weight gain	Kpower model, P_0 fixed	One standard deviation for all groups	5.00e-02	1.00e-01	137.91	111.28	193.49	-328.81	0.05799

[a]G-O-F = Goodness of fit.

Table B-5. Maternal body weight gain (expressed as a probability of response) (continued)

Dataset	Model	Standard deviation	P_0	BMR	MLE	BMD	Upper bound	Log-likelihood	G-O-F[a] *P*-value
Maternal body weight gain	Kpower model, P_0 fixed	One standard deviation for all groups	5.00e-02	5.00e-02	82.349	66.45	126.44	-328.81	0.05799
Maternal body weight gain	Kpower model, P_0 fixed	One standard deviation for all groups	1.00e-02	1.00e-01	249.29	201.16	327.28	-328.81	0.05799
Maternal body weight gain	Kpower model, P_0 fixed	One standard deviation for all groups	1.00e-02	5.00e-02	174.89	141.13	236.78	-328.81	0.05799
Maternal body weight gain	Kpower model, P_0 fixed	One standard deviation for all groups	1.00e-03	1.00e-01	411.26	331.86	539.88	-328.81	0.05799
Maternal body weight gain	Kpower model, P_0 fixed	One standard deviation for all groups	1.00e-03	5.00e-02	329.8	266.13	432.95	-328.81	0.05799
Maternal body weight gain	Kpower model, P_0 fixed	Different standard deviation for each group	5.00e-02	1.00e-01	92.226	68.887	133.52	-323.77	0.05754
Maternal body weight gain	Kpower model, P_0 fixed	Different standard deviation for each group	5.00e-02	5.00e-02	55.07	41.134	83.978	-323.77	0.05754

[a]G-O-F = Goodness of fit.

Table B-5. Maternal body weight gain (expressed as a probability of response) (continued)

Dataset	Model	Standard deviation	P_0	BMR	MLE	BMD	Upper bound	Log-likelihood	G-O-F[a] *P*-value
Maternal body weight gain	Kpower model, P_0 fixed	Different standard deviation for each group	1.00e-02	1.00e-01	166.71	124.53	236.24	-323.77	0.05754
Maternal body weight gain	Kpower model, P_0 fixed	Different standard deviation for each group	1.00e-02	5.00e-02	116.96	87.36	166.64	-323.77	0.05754
Maternal body weight gain	Kpower model, P_0 fixed	Different standard deviation for each group	1.00e-03	1.00e-01	275.03	205.43	389.72	-323.77	0.05754
Maternal body weight gain	Kpower model, P_0 fixed	Different standard deviation for each group	1.00e-03	5.00e-02	220.55	164.74	312.53	-323.77	0.05754

Source: NTP, 1991.

Acceptable fits for maternal body weight gain were obtained for all of the combinations of model, p_0 value, and BMR. For $p_0 = 0.05$, BMR = 10%, a BMD of 36 mg/kg-day was calculated using the Weibull model with one standard deviation, while higher values were calculated under the same conditions using the power model with multiple standard deviations (69 mg/kg-day) or a single standard deviation (111 mg/kg-day). For this combination of p_0 and BMR, the first two models calculated BMDs comparable to the NOAEL (50 mg/kg-day); the BMD calculated using the power model with a single standard deviation is about twice the NOAEL. The interrelationships among the various factors contributing to this pattern are interesting. The Weibull model provides a slightly better fit to the mean values (note the larger goodness-of-fit *p*-value for that model in Table B-5), and that model was nonlinear, whereas the power models were linear. The overall slope of the Weibull model was steeper between 0 and 50 mg/kg-day than was that for the power models; hence, when the standard deviation estimates were the same, as was the case with the Weibull model and the power model with a single standard deviation, the steeper model predicted the lower BMD (36 vs. 111). However, the observed control group standard deviation was less than the standard deviations observed in the other groups, so that the model allowing separate standard deviations predicted a smaller control group standard deviation than did the model with a single standard deviation for all groups. This factor decreased the BMD (from 111 to 69). Although that decrease brought the BMD more in line with the NOAEL and the BMD from the Weibull model, it was not sufficient to decrease the BMD to the level of the Weibull model BMD, which remained the lowest because of the curvature predicted by the Weibull model. A Weibull model with group-specific estimates of standard deviation would be expected to predict an even smaller BMD. When the maternal body weight gain was modeled using the BMR defined as a 10% decrease in the mean, the result (678 mg/kg-day) was much higher than the NOAEL. As discussed further in the next section, this difference is probably related to the definition of the endpoint as body weight gain, rather than body weight.

Although the lowest oral BMD (57 mg/kg-day) was obtained for the percent litters with malformed fetuses in the oral developmental study in rats (NTP, 1991), we do not consider this to be a reliable endpoint. As discussed above in the study summaries, most of the increase in malformations was attributable to a malformation (enlarged lateral ventricle of the brain) for which there was considerable variability in the control group. If one were to consider this endpoint of concern, the BMD corresponding to a 10% BMR was 57 mg/kg-day for this endpoint (using both the Weibull and polynomial models), close to the low dose of 50 mg/kg-day (Table B-3). Although there was a significant trend test for this effect, the authors reported that an ANOVA test did not detect a significant effect of dose for percent malformed fetuses per litter, nor was there a significant difference among groups by a chi-square test. When we performed a Fisher exact test, the high-dose group incidence was determined to be significantly greater than the incidence in the control group ($p = 0.045$), although this test did not correct for multiple comparisons.

The slopes for the probabilities of response at the BMDs (corresponding to 10% additional risk and p_0 of 0.05) were all relatively small. For the power and Weibull models the slopes for male and female body weight (Figures B-9 and B-10) were as follows:

	Power model	Weibull model
Female body weight (BCL, 1980a)	1.9×10^{-3}	2.4×10^{-3}
Male body weight (BCL, 1980a)	3.1×10^{-3}	3.6×10^{-3}
Maternal body weight gain (NTP, 1991)	1.3×10^{-3}	2.6×10^{-3}

There was little model dependency in the slope estimates for any of these endpoints. For the developmental toxicity endpoint (percent of litters with malformed fetuses), the slope from either the Weibull or polynomial models was 1.4×10^{-3} at the BMD. This slope is very similar to that for the other oral endpoints. No slope was calculated for the models applied to body weight endpoints expressed as a change in mean, since such slopes would be in terms of the change in body weight (rather than probability of response) as a function of dose.

G. DISCUSSION

G.1. Choice of Endpoints to Model

Results are reported here for modeling the most sensitive endpoints from the NTP (1992a) inhalation study, as well as body weight and developmental toxicity endpoints from oral studies. As shown, modeling choices among multiple endpoints in a study can be made by comparing response levels for a given exposure level. For example, the response rate for lung inflammation in males rose from 0% in controls to 30% at the low concentration and compared to females at 4% in the control group and 20% at the low concentration. These response rates are much lower than the 97%-100% response level for nasal inflammation (including all severity levels) at the same exposure level, and the BMC value for lung inflammation is correspondingly higher. Thus, although the lowest NOAEL does not necessarily correspond to the lowest BMC, judicious consideration of the available data can limit the amount of modeling that needs to be done. However, it is important to note that with doses that are close together, the impact on the predicted dose-response relationship (and hence the BMD) of differences in response rates may not always be obvious.

G.2. Data Gaps

All studies with endpoints appropriate for modeling presented the data in a form appropriate for benchmark modeling. However, nasal inflammation in female mice (when all severity levels were included) could not be modeled because the response level was 100% at the low concentration, so no information on the concentration-response between 0 and the low concentration was available. The primary toxicological data gaps are related to the applicability to humans of the endpoints observed in animals. For inhalation exposure, it is unclear whether and at what levels the nasal effects observed in mice would occur in humans. As discussed further in the RfC documentation (Clement International Corporation, 1996c), naphthalene cytotoxicity of the respiratory tract is highly correlated with the production of a specific enantiomer of the naphthalene metabolite naphthalene-1,2-epoxide (Buckpitt et al., 1987). The amount of the

metabolite produced is highly species dependent; in vitro data with lung microsomes indicate that mice produce much more of the implicated metabolite than do humans (Buckpitt and Bahnson, 1986) or Rhesus monkeys (Buckpitt et al., 1992). Experiments have not been conducted to determine whether naphthalene inhalation results in respiratory tract histopathology in primates.

An additional issue relates to the fact that the most sensitive endpoints observed in humans (hemolytic anemia and cataracts) are not the most sensitive endpoints in animal studies. For anemia, this difference is largely related to the fact that this effect occurs in specific sensitive subpopulations; naphthalene toxicity has not been adequately tested in animal models for these subpopulations. Although animal models have been identified for naphthalene-induced cataracts (e.g., Wells et al., 1989), cataracts appear to occur at levels higher than those causing the body weight effects reported by BCL (1980a) and NTP (1991). However, most studies that investigated cataractogenesis were conducted at doses high enough to cause cataracts in all animals, and data are not readily available to determine whether the animal strains used in the studies described in this document are among those that are genetically responsive to naphthalene-induced cataracts.

G.3. Definition of BMR for Body Weight Endpoints

The most appropriate definition of the BMR for decreased body weight relative to controls has not yet been determined. For this task, we used several different definitions of the BMR. The definition of the BMR most consistent with current toxicological practice appears to be a 10% decrease in mean body weight, consistent with the commonly accepted practice of considering a 10% change in mean body weight to represent an adverse effect. However, the nature of the modeling makes it difficult to incorporate such definitions of a threshold "adverse effect" into the definition of the BMR, because this threshold is, by definition, a LOAEL, while a BMR should correspond to a no-effect level. This difficulty is related to the fact that the NOAEL/LOAEL method uses discreet, actual dose levels, while the BMD can be any value in the dose range of interest. Thus, while the NOAEL is simply the dose below the one that causes a 10% decrease in body weight, it is unclear how much below this threshold one should go when using the BMD approach. To some degree this is addressed by the use of the lower bound on the 10% change (i.e., the BMD, rather than the MLE). However, it appears that use of the lower bound still introduces an overall upward bias to the definition of BMDs based on decreased body weight. This is because, for a standard study design, this definition of the BMR will result in a fairly consistent percentage decrease in body weight slightly lower than 10%, maybe a 7%-8% decrease. Using the traditional approach, some NOAELs would correspond to a decrease of this magnitude, but the overall range of decrease observed at the NOAEL would be 0%- ~ 9%, or an average of about 5%. Thus, while the calculated BMDs would be acceptable as NOAELs, the overall average of BMDs would be expected to be higher than the overall average of NOAELs.

We note that this issue of translating a threshold or study design sensitivity limit into a BMR definition will be relevant to any attempt to provide a rational endpoint-specific definition of the BMR. Because such efforts are aimed at determining the level at which an effect is adverse, or the level at which an effect can be detected statistically, the most immediate result will be more

similar to a LOAEL than to a NOAEL. As discussed for the body weight endpoint, it will be necessary to devise some method of translating this threshold/detection limit into something more like a NOAEL for regulatory purposes.

Note that the considerations discussed above apply to body weight per se, rather than body weight changes. If one were considering body weight change as the endpoint, a 10% difference in change would be predicted to occur at quite different doses. As an example, if the starting average weight for a control group were 250 g and that group exhibited an increase in weight to 300 g on average (a change of 50 g), then a 10% decrement in body weight change would occur at a dose that produced a 45 g change in body weight, corresponding to a final body weight of 295 g. By contrast, a 10% change in final body weight would correspond to a final body weight of 270 g. For this reason, BMDs that are defined as corresponding to 10% decreases in body weight change will tend to be less than those corresponding to 10% difference in body weight per se and less than the common toxicological practice of considering a 10% difference in average body weight to be adverse.

When the BMD for body weight was calculated using a BMR defined as a probability of response, the BMDs calculated for decreased body weight in the subchronic study (BCL, 1980a) were consistently smaller than the corresponding NOAELs, for all choices of p_0, x_0, and BMR, apparently introducing a consistent downward bias for these data sets. While the rule-of-thumb that a 10% decrease in body weight compared with controls is considered adverse has been developed over many years of experience, it must be noted that this rule-of-thumb applies to comparisons of mean values across dose groups.[1] In other words, the LOAEL is defined as the dose at which at least 50% of the animals are adversely affected (i.e., have a 10% decrease in *individual* body weight). Thus, as much as 49% of the animals theoretically could be adversely affected at a NOAEL (assuming a symmetrical distribution). By contrast, only 10% of the animals are adversely affected at the BMD. In light of this difference in the definitions of the NOAEL and BMD, it is not surprising that the BMDs tend to be lower than the NOAELs.

This point is highlighted by the male body weight data for the BCL (1980a) study. The BMDs calculated for fixed x_0 (57-77 mg/kg-day for 10% BMR) might appear on first analysis to be unreasonably low, since mean male body weight at 50 mg/kg-day is actually slightly *higher* than the control value, and only a moderate decrease is apparent at 100 mg/kg-day. However, analysis of the individual data (Appendix E of the study) indicates that one rat (10%) at 50 mg/kg-day had a body weight (315 g) very close to the cut-point (314 g). In addition, at 100 mg/kg-day, there was one rat below the cut-point and one rat at 315 g.

The BMDs estimated for female body weight with x_0 fixed are also consistent with the experimental data. For 10% BMR, the values range from 101 to 117 mg/kg-day. Although the NOAEL is identified as 200 mg/kg-day in Table B-4, the mean body weight at 200 mg/kg-day (190.5 g) is clearly reduced compared with the controls, although it does not reach the cut-point

[1]Note that it is important to differentiate between a 10% decrease in the mean body weight and a 10% increase in the *probability* of an adverse effect (i.e., an increased proportion of animals considered to be adversely low in weight.)

(183 g). Mean body weight is also reduced at 100 mg/kg-day, although the mean is the same as at the low dose. Therefore, it is not surprising that the BMD predicts that 10% of the rats would have adversely low body weights at doses slightly above 100 mg/kg-day.

G.4. Applications

The BMC of 0.93 mg/m^3 calculated based on metaplasia of the olfactory epithelium of severity grade 3 or higher in female mice exposed to naphthalene via inhalation has been used as the basis for a proposed RfC (Clement International Corporation, 1996c). As discussed above, several different modeling approaches were attempted in order to address the plateauing at less than 100% response. Because the BMC calculated for this endpoint when the lower bound on the power was allowed to be less than 1 was 0, the BMC proposed here was calculated with the high exposure level dropped. A limitation to using this method to improve the fit is that only one positive exposure level was modeled. An uncertainty factor of 10 was proposed for protection of sensitive human subjects. Because of the extensive database (Buckpitt et al., 1987, 1992; Buckpitt and Bahnson, 1986) showing that mice are more sensitive than humans and other primates, as well as more sensitive than rats, to the effects of naphthalene on the respiratory tract, an uncertainty factor of 1 was used for extrapolation from mice to humans. Although no two-generation reproductive study has been conducted, data from oral developmental toxicity studies indicated that naphthalene causes developmental toxicity only at doses that cause frank maternal toxic effects (Hardin et al., 1987; Mesa Corporation, 1983; NTP, 1991, 1992b; Plasterer et al., 1985).

The severity grades for metaplasia may be less reliable than those for the other nasal lesions, since it appears that the criteria for the severity grades reported by Peckham (1991) did not differentiate well among the different severity grades. For this lesion, the only reported difference among the different severity grades was the percentage of the olfactory epithelium that was replaced. Unlike the other lesions, no qualitative differences among severity categories were reported. However, there was no clear severity-response for the severity categories. The range (and average) degree of replacement was as follows: minimal, 50%-100% (average 71.1%); mild, 40%-100% (average 70.0%); moderate, 60%-95% (average 79.4%); and marked, 90%-100% (95%). Thus, it is not clear if the apparent higher response for severity categories greater than or equal to 3 for olfactory metaplasia (compared with the other nasal lesions) was due, at least partially, to the similarity in severity categories. This consideration may suggest that respiratory hyperplasia in the nose of male mice is a more appropriate endpoint for the derivation of the RfC. However, the BMC estimated for the hyperplasia endpoint (0.98 mg/m^3) is very close to that for the metaplasia endpoint (0.93 mg/m^3), indicating that this consideration would not have a significant impact on the RfC.

The major alternatives for RfD determination appear to be decreased body weight (with males more sensitive) in a subchronic study (BCL, 1980a) and decreased maternal body weight gain in the developmental study (NTP, 1991). As discussed above in the context of choice of endpoints to model, although a BMD was calculated for the developmental endpoint of increased number of litters with malformed fetuses, the increase in malformations can be attributed to one malformation (enlarged lateral ventricles of the brain). Due to wide variations in response in

control group replicates, it is unclear whether this increase is naphthalene related; the biological significance of this endpoint is also unclear. The change in maternal body weight in the developmental study is not recommended as a basis for an RfD, because the pregnant animals were exposed for such a small percentage of their lives. Furthermore, as discussed above, the appropriate definition of a BMD is unclear when only body weight gain (rather than mean body weight) is reported. Thus, the most appropriate critical effect for an RfD would be decreased male (92.8 mg/kg-day; duration adjusted) body weight in the subchronic study (BCL, 1980a). The calculated BMD of 130 mg/kg-day (using a BMR of 10% decrease in mean body weight; duration adjusted 93 mg/kg-day) is close to, but slightly higher than, the NOAEL of 100 (duration adjusted 71.4 mg/kg-day) for this endpoint.

H. CONCLUSIONS

Benchmark modeling was conducted for chronic inhalation toxicity and for systemic effects in a subchronic oral study and maternal effects in an oral developmental study. When lesions of severity greater than or equal to 3 were modeled, the most sensitive endpoint in the inhalation study was hyperplasia of the respiratory epithelium of the nose in male mice. Because the response plateaued at less than 100%, it was difficult to obtain a good fit using standard modeling methods. We attempted to improve the fit by allowing the lower bound on the power to be less than 1. Although this resulted in a markedly improved fit for all endpoints, the resulting BMC for hyperplasia of the respiratory epithelium in male mice was very conservative (0.008 mg/m^3); BMCs calculated for the other endpoints tended to be more similar to the BMCs calculated with the lower bound on power restricted. Because the most sensitive BMC using this method appeared to be overly conservative, we also attempted to resolve the fit issue by dropping the high exposure level. The resulting BMC of 0.98 mg/m^3 for hyperplasia of the respiratory epithelium in the nose of male mice was initially proposed as the basis for an inhalation RfC. As described in Section 5.2 of this Toxicological Review, however, a NOAEL/LOAEL approach was ultimately used for the derivation of the RfC.

Using a BMR of a 10% decrease in the mean body weight, the BMDs for male and female body weight in the subchronic oral study (BCL, 1980a) were slightly higher than the corresponding NOAELs, but below the corresponding LOAELs. Although this definition of the BMR is expected to introduce a slight bias compared with NOAELs (when the NOAEL is defined as the dose below that at which a 10% decrease in the mean body weight is observed) as discussed above, the BMD calculated here does appear to be sufficiently reliable for use in the derivation of an RfD.

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