External Review Draft

NCEA-S-1707
June 2004

TOXICOLOGICAL REVIEW

OF

NAPHTHALENE

(CAS No. 91-20-3)

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

August 1998
Revised June 2004
(Inhalation cancer assessment and other selected text, as indicated)

NOTICE

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U.S. Environmental Protection Agency
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June 2004:
Revision of the inhalation cancer assessment and other selected text, as indicated:
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Note to reviewers: Please review the highlighted sections.

CONTENTS—TOXICOLOGICAL REVIEW FOR NAPHTHALENE (CAS No. 91-20-3)

CONTENTS (continued)

CONTENTS (continued)

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 IRIS Hotline at 202-566-1676.

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

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

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). IRIS Summaries may include an oral reference dose (RfD), inhalation reference concentration (RfC) and a carcinogenicity assessment.

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

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

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

Council Handbook: *Risk Characterization* (U.S. EPA, 2000b), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c) and *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA 2000d).

The literature search strategy employed for this compound was based on the CASRN and at least one common name. At a minimum, the following data bases were searched: RTECS, HSDB, TSCATS, CCRIS, GENE-TOX, DART/ETIC, EMIC, TOXLINE, CANCERLIT, and MEDLINE. 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 as well as insect and animal 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 from contact of the diapers with mothballs or naphthalene flakes, although exposure concentration measurements were not taken and inhalation exposure cannot be excluded. Quantitative data in animals have been 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 has been demonstrated to change with time. At 24 hours after a single dose of 0.123 mg/kg, adipose was 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 were not located. In the case of 2-methylnaphthalene, which is similar in structure to naphthalene, 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 fat distribution in order of decreasing concentration.

3.3. METABOLISM

The metabolic pathways involved in the metabolism of naphthalene have been extensively studied (see ATSDR, 2003 for review; Buckpitt and Franklin, 1989; Buckpitt et al., 2002; Plopper et al., 1992a,b; Wells et al., 1989; Xu et al., 1992a, 1992b), and are diagrammed in Figure 1. In summary, naphthalene is initially metabolized by cytochrome P-450 (CYP)

enzymes, yielding naphthalene-1,2-oxide. The epoxide may be metabolized by glutathione S-transferase enzymes to a glutathione conjugate, which can then be further metabolized to mercapturic acids. Naphthalene-1,2-oxide may also spontaneously hydrate, restoring the aromatic ring and yielding 1-naphthol or 2-naphthol. 1-Naphthol may be metabolized by CYP enzymes to 1,4-naphthoquinone. Naphthalene-1,2-oxide can also be hydrated by epoxide hydrolase, resulting in 1,2-dihydroxy-1,2-dihydronaphthalene (naphthalene-1,2-dihydrodiol), which may then be oxidized by dihydrodiol dehydrogenase to 1,2-naphthoquinone. CYP enzymes may also oxidize naphthalene-1,2-dihydrodiol to yield the epoxide, 1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydronaphthalene, which can be further metabolized by a number of conjugation pathways.

The initial step in naphthalene metabolism involves epoxidation of the aromatic ring catalyzed by CYP enzymes resulting in the formation of naphthalene-1,2-oxide. Numerous studies have demonstrated that a wide variety of CYP isozymes can catalyze this reaction, including 1A1, 1A2, 1B1, 3A7, 3A5 (Juchau et al., 1998), 2B4 (Van Winkle et al., 1996), 2E1 (Wilson et al., 1996), and 2F (Buckpitt et al., 1995, 2002; Shultz et al., 1999). Three species-specific genes were identified for CYP2F; 2F1 in primates and human lung (Nhamburo et al., 1990), 2F2 in mice (Ritter et al., 1991), and 2F4 in rats (Buckpitt et al., 1995, 2002; Shultz et al., 1999). In some cases, stereoselectivity in the formation of the epoxide is known to occur; for example, CYP2B shows a preference for the formation of the (1*S*,2*R*)-naphthalene oxide (74%), whereas CYP1A1 and CYP2F2 preferentially generate (1*R*,2*S*)-naphthalene oxide (Shultz et al., 1999; Van Bladeren et al., 1984, 1985).

Baldwin et al. (2004) examined the respiratory tract of rodents to determine the relationship between levels of transcript (mRNA) and CYP2F protein with tissue susceptibility to injury. In addition, nasal tissues of rodents and primates were analyzed to determine CYP2F expression levels. In both mice and rats, the lung was found to contain higher levels of CYP2F transcript than liver while the levels in the kidney were undetectable. Mice expressed 4-fold greater CYP2F transcript in lung and 8-fold greater CYP2F transcript in liver tissue than rats. Quantitative immunoblot analysis of CYP2F in airway subcompartments revealed mice to have 6- to 30-fold higher levels of CYP2F protein than rats. Within the lungs of both rodent species, the highest CYP2F expression was found in the distal airways. The olfactory epithelium contained the greatest amount of CYP protein of all tissues studied in the rat, consistent with the observed pattern of *in vivo* injury. CYP2F expression levels in primate nasal tissues (ethmoturbinates, nasal and maxilloturbinates) demonstrate that only the nasal ethmoturbinates contained quantifiable amounts of CYP2F. The levels of CYP2F in primates were roughly 10- and 20-fold less than the corresponding tissues in rats and mice, respectively. Previous studies demonstrated that lung microsomal incubations of rhesus macaques metabolize naphthalene at a rate 100-fold less than mice and 10-fold less than rats (Buckpitt et al., 1992).

Spontaneous hydration of naphthalene-1,2-oxide results in either the formation of 1-naphthol or 2-naphthol. Either of these compounds may be enzymatically conjugated with glucuronic acid or sulfuric acid prior to elimination. Naphthalene-1,2-oxide may also be metabolized by glutathione S-transferase enzymes, resulting in conjugation with glutathione and

the formation of 1-hydroxy-2-glutathionyl-1,2-dihydronaphthalene (Warren et al., 1982; West et al., 2000). This compound may be further metabolized, resulting in mercapturic acids, which are generally eliminated in the urine. This is likely a detoxification mechanism, at least for acute effects, as evidenced by studies showing that glutathione depletion increased the degree of acute naphthalene-induced Clara cell injury in mice (Warren et al., 1982; West et al., 2000).

An additional pathway of naphthalene-1,2-oxide metabolism involves hydration by epoxide hydrolase, resulting in the formation of naphthalene-1,2-dihydrodiol (1,2-dihydroxy-1,2-dihydronaphthalene). Naphthalene-1,2-dihydrodiol can be transformed by dihydrodiol dehydrogenase (member of the aldo-keto reductase gene superfamily) to form the 1,2-naphthoquinone (see Bolton et al., 2000 for review; Smithgall et al., 1986, 1988). The initial product is an air sensitive catechol (Penning et al., 1999) which can undergo two 1 electron oxidations leading to a concurrent generation of reactive oxygen species. At least five members of the aldo-keto reductase superfamily have dihydrodiol dehydrogenase activity (AKR1C1-AKR1C4 and AKR1A1) (Burczynski et al., 1999). CYP enzymes may also further oxidize naphthalene-1,2-dihydrodiol, resulting in the epoxide, 1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydronaphthalene. Support for the *in vivo* formation of 1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydronaphthalene comes from the identification of several urinary metabolites, including a number of trihydroxytetrahydromethylthio derivatives (Horning et al., 1980) and trihydroxytetrahydromercapturic acid (Pakenham et al., 2002).

Zheng et al. (1997) incubated isolated Clara cells with naphthalene and demonstrated that a major metabolite, 1,2-naphthoquinone, was covalently bound to proteins. In rats given single oral doses of naphthalene ranging from 100 - 800 mg/kg levels of naphthalene adducts were found in decreasing order as follows: naphthalene-1,2-oxide, 1,2-naphthoquinone and 1,4-naphthoquinone (Troester et al., 2002). 1,2-Naphthoquinone is both inherently reactive (e.g., via Michael addition to nucleophiles) and capable of producing reactive oxygen species through redox cycling (Flowers et al., 1997). It is mutagenic in several strains of *Salmonella typhimurium* (Flowers-Geary et al., 1996). It has been proposed that 1-naphthol can also be further oxidized by CYP enzymes to form 1,4-naphthoquinone, but not 1,2-naphthoquinone, based on the finding that following incubation of liver microsomes with 1-naphthol and ethylene diamine, a compound that reacts readily with 1,2-naphthoquinone, no evidence of conjugate formation was found (D'Arcy Doherty et al., 1985).

In humans, limited information is available regarding the metabolism of naphthalene, most of which is based on data from human *in vitro* microsomal preparations. Human lung microsomal fractions demonstrated naphthalene metabolism to naphthalene dihydrodiol and other glutathione conjugates in the presence of glutathione (Buckpitt and Bahnson, 1986). In addition, human liver microsomes exposed to naphthalene demonstrated production of the metabolites 1-naphthol and naphthalene-1,2-dihydrodiol (Tingle et al., 1993). The metabolite 1,2-dihydrodiol predominated in human microsomes with the ratio of 1,2-dihydrodiol to 1-napthol at 8.6 and 0.4 in human and mouse microsomes, respectively. Naphthalene-1,2-dihydrodiol was formed following epoxidation and hydrolysis, however spontaneous rearrangement of the 1,2-epoxide led to the formation of 1-napthol. Microsomes from human

lymphoblastoid cells expressing recombinant human CYP2F1 were used to identify glutathione conjugates of naphthalene metabolism. Glutathione conjugates formed from the naphthalene-*1S,2R*-oxide enantiomer were found in quantities up to 4-fold greater than glutathione conjugates formed from naphthalene-*1R,2S*-oxide (Lanza et al., 1999). The preferential formation of the 1*S*,2*R*-naphthalene oxide enantiomer in human microsomes provides evidence that human transformation of naphthalene to reactive epoxides may be more similar to rats than mice with respect to stereoselectivity.

Polymorphisms in human metabolic enzymes including CYP could alter naphthalene metabolism. Yang et al. (1999) analyzed urinary napthol levels in individuals with CYP1A1, CYP2E1 or GSTMI genetic polymorphisms. Urinary 1- and 2-napthol levels were higher in individuals with the CYP2E1 and GSTM1 polymorphisms. The presence of naphthalene-metabolizing enzymes has been demonstrated in nasal respiratory epithelial and olfactory epithelial tissue from rodents and humans (Buckpitt et al., 1992, 2002; Plopper et al., 1992a; Thornton-Manning and Dahl, 1997), but information on the rates of naphthalene metabolism and the resulting metabolic products in human nasal tissue are not available.

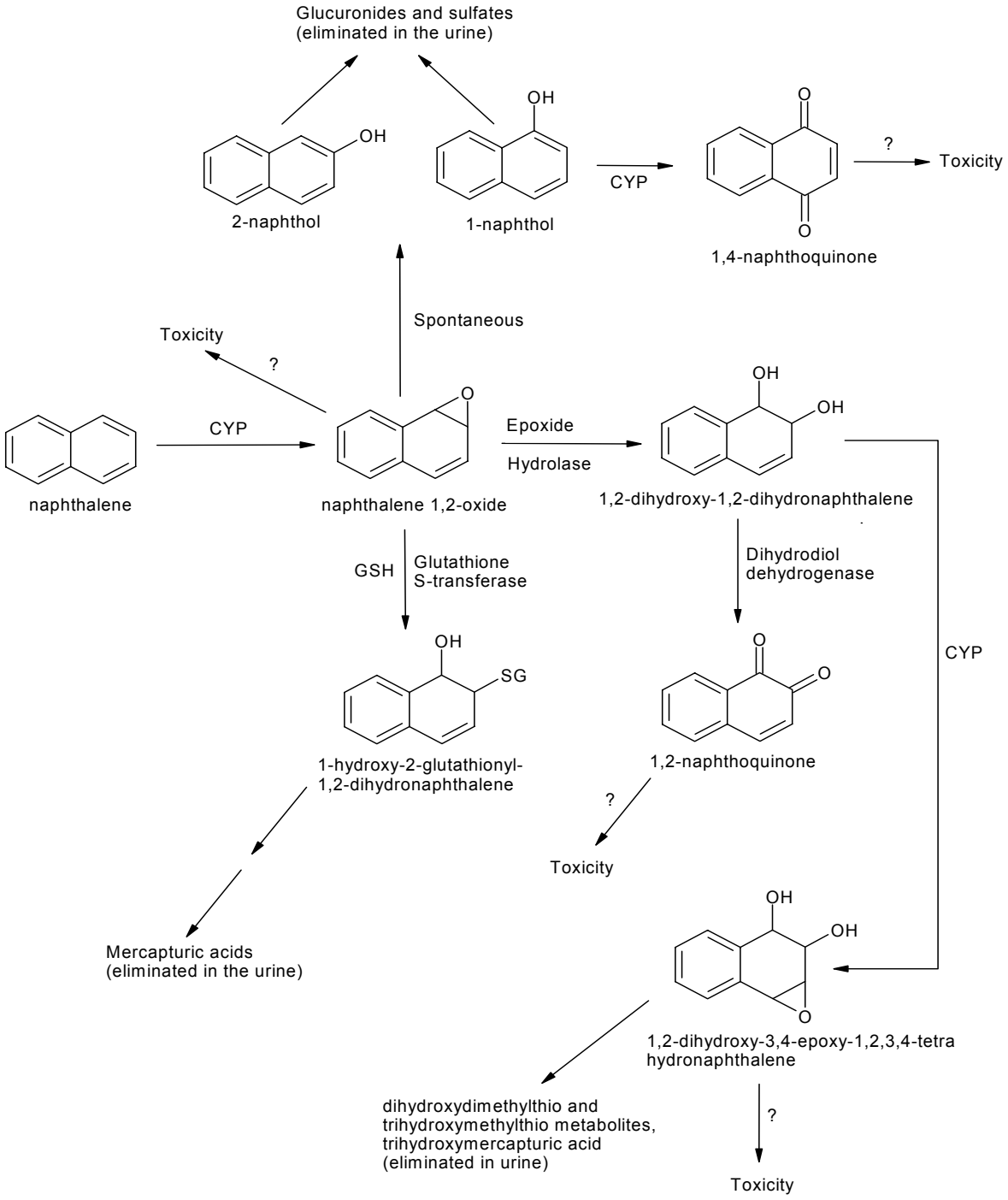

Figure 1. Scheme for naphthalene metabolism and formation of multiple reactive metabolites which may be involved in naphthalene toxicity (ATSDR, 2003; adapted from Buckpitt et al., 2002; Waidyanatha et al., 2002).

3.4. ELIMINATION AND EXCRETION

The majority of 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. Glutathione conjugates of naphthalene are catabolized to premercapturic and mercapturic acids before excretion in bile or urine.

Excretion of naphthalene in humans following inhalation exposure has been described by Bieniek (1994, 1997). Levels of urinary 1-naphthol, 2-naphthol and 1,4-naphthoquinone were quantitated in workers employed in tar and naphthalene oil distillation and correlated with levels of naphthalene measured in the workplace air (Bieniek, 1997). Peak levels of urinary 1-naphthol were found in workers employed in the same distillation plants 1 hour after the end of their shift. When compared to other workers at the plant or non-occupationally-exposed groups, naphthalene oil distribution plant workers had the highest urinary concentrations of 1-naphthol with excretion rates of 0.57% mg/hr (Bieniek, 1994). Following oral ingestion of naphthalene, urinary naphthol levels have been measured in patients up to 4 days post-ingestion (Zuelzer and Apt, 1949). The urine of an 18-month-old child was found to contain the metabolites1-naphthol, 2-naphthol, 1,2-naphthoquinone and 1,4-naphthoquinone up to 9 days following exposure (Mackell et al., 1951).

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) Urinary excretion of premercapturic acids and mercapturic acids represents a major excretory path in mice and rats (Chen and Dorough, 1979; Stillwell et al., 1978). Chen and Dorough (1979) reported that the glucuronide conjugate represented 12% of total radioactivity eliminated in urine in rodents after administration of radiolabeled naphthalene. In a recent study involving intraperitoneal administration of 1.6-200 mg/kg radiolabeled naphthalene to rats and mice, no significant differences were observed in the percentage of the administered dose excreted as mercapturic acids in the urine between mice (25-34%) and rats (24-35%) (Pakenham et al., 2002). In addition, no dose-response effect was found. High performance liquid chromatography (HPLC) elution profiles indicated that considerable amounts of the label were found in glucuronide conjugates (both species) and sulfate conjugates (in mice only) in the urine. N-acetylcysteine-trihydroxytetrahydronaphthalene was identified as a minor labeled urinary component in mice (Pakenham et al., 2002). 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 of the label recovered from ingested or dermally absorbed radioactive labeled naphthalene was 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-1,2-oxide formation (i.e., decreased CYP oxygenase activity)

in primates relative to rodents or increased rates of naphthalene-1,2-dihydrodiol formation (i.e., increased epoxide hydrolase activity).

3.5. PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS

A PBPK model for naphthalene in rats and mice has been developed and refined. The initial model description was presented by Sweeney et al. (1996). It consists of a parallel system of PBPK models that are bridged by the biotransformation of naphthalene to naphthalene-1,2-oxide in the lung and liver. The diffusion-limited model consists of arterial and venous blood compartments, as well as tissue compartments for the lung, liver, kidney, fat, and other tissues, which were assumed to be well-mixed and equilibrated with the existing blood. Inputs for the model included simulations of oral and intraperitoneal exposure routes. Naphthalene metabolized in the liver or lung enters the second system, which consists of an identical structure, reflecting the assumption that naphthalene-1,2-oxide can be formed in tissues and travel throughout the body. In both sections of the model, metabolism is assumed to occur only in the liver and the lung. For the naphthalene portion of the model, one metabolic pathway was used in the lung, while in the liver, two pathways were taken into account (one represented by Michaelis-Menten kinetics and the other by Hill kinetics). The model's physiological parameters (ventilation rate, cardiac output, tissue volumes, capillary volumes, and blood flow rates to tissues) were based on values obtained from the literature. Partition coefficients were calculated from the log octanol: water partition coefficient. Metabolic rates and permeability coefficients were estimated by optimizing the model to the available naphthalene time-course data.

Support for the concept of a parallel set of PBPK compartments for naphthalene-1,2-oxide, and the ability of naphthalene-1,2-oxide to travel in the bloodstream to target tissues, includes data indicating that toxic effects on the respiratory tract are due to naphthalene metabolites 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 epithelial cells in mice, rats and hamsters (Plopper et al., 1992a) occurs following intraperitoneal injection of naphthalene.

Subsequently, the model was updated (Quick and Shuler, 1999) and expanded to include the addition of model inputs for the inhalation and intravenous routes. Additionally, many of the metabolic parameters were adjusted, using data on naphthalene metabolism from studies with isolated Clara cells (for the lung) or hepatocytes (for the liver), as well as studies from isolated cell fractions. This model was evaluated both normally and with parameters designed to simulate the depletion of GSH with buthionine sulfoxamine; model predictions compared "reasonably well" with the experimental values.

The model was further refined with the use of a cell culture analog reactor experiment (Ghanem and Shuler, 2000). The cell culture analogue reactor (CCA) is a physical replica of the PBPK model; the device is a continuous, dynamic system composed of multiple cell types that interact through a common circulating cell culture medium. Where the PBPK model specifies an

organ or tissue compartment, the bioreactor contains compartments with a corresponding cell type. Two physically different CCA reactors were tested with naphthalene, yielding different results. In the prototype system using cells attached to glass dilution bottles, naphthalene dosing resulted in the generation of a circulating metabolite from the "liver" compartment (based on H4IIE cells from a rat hepatoma) that caused cell death in the "lung" compartment (L2 cells from a rat lung), as well as depletion of glutathione in the L2 cells. An improved CCA using packed bed reactors of microcarrier cultured cells did not show differences between naphthalene-dosed and non-dosed controls. To explain the different responses of the two CCA designs, PBPK models of the two reactors were tested with variations in physical and kinetic parameters, and toxic mechanism. When the toxic metabolite is assumed to be naphthalene-1,2-oxide, the PBPK model did not adequately predict the results from the CCA experiments. However, when the toxic metabolite was assumed to be a naphthoquinone, rather than naphthalene-1,2-oxide, the PBPK models adequately predicted the CCA results.

An additional refinement of the model was presented by Willems et al. (2001), who applied the data from the NTP bioassays in rats and mice (Abdo et al., 2001; NTP, 1992a, 2000) with the intent of exploring possible explanations for the induction of lung tumors in female mice, but not in rats following naphthalene inhalation exposure. Transfer of naphthalene from the capillary blood to the tissue compartments was no longer considered to be diffusion-limited, instead being characterized within the model. The following parameters were estimated: (1) the amount of naphthalene inhaled by rats and mice at the exposure concentrations used; (2) the amount of inhaled dose that was metabolized during 6 hour daily exposures and during the 18 hours post-exposure; (3) the steady-state concentrations of naphthalene in the liver and lung of rats and mice during exposure; and (4) the rate of naphthalene metabolism in the lung and liver of rats and mice at these steady-state concentrations. The inhalation data were used to select best-fitting models with the fewest assumptions possible and to optimize model parameters, while the intravenous data were used to examine the validity of the final models.

The results from the Willems et al. (2001) toxicokinetic model indicate that tissue dosimetry alone does not explain why naphthalene exposure induced tumor formation in the mouse lung but not the rat lung. Approximately 22 - 31% of inhaled naphthalene was metabolized by rats and 65 - 73% was metabolized by mice, such that the total amount of naphthalene metabolized was nearly equivalent for mice exposed to 10 ppm and rats exposed to 60 ppm. These differences are due to both the higher ventilation rate and greater metabolism rate of naphthalene in mice compared to rats. The models also indicate that the steady-state concentration of naphthalene in the lung of male rats is similar to that of mice exposed to equivalent concentrations. However, female rats exposed to 60 ppm naphthalene had a higher steady-state concentration in the lung than did female mice exposed to 30 ppm.

As stated in NTP (2000), the higher rates of naphthalene metabolism in the mouse lung may be a contributing factor to the species differences in response. The model does not include information on rates of detoxification of potential reactive intermediates. Thus, it is not possible to compare lung concentrations of naphthalene metabolites in the two species. For example, if naphthalene-1,2-oxide is the agent responsible for lung tumor development in mice, then the

species differences in response at this site may be due to a combination of higher rates of naphthalene oxide formation in the mouse lung and, possibly, a greater susceptibility of the mouse lung to this metabolite.

These models also do not account for nasal deposition, epithelial absorption, and nasal metabolism of naphthalene because these specific data are not available. Without such data, models similar to those developed for other nasal toxicants, such as acrylic acid (Frederick et al., 2001), cannot be developed for naphthalene. Validated PBPK models with metabolizing nasal compartments may also aid in determining why male and female rats develop nasal tumors with chronic inhalation exposure to naphthalene, but mice do not, even though both species develop high levels of
nonneoplastic lesions in the nasal tissues in which tumors developed in rats (Abdo et al., 2001; NTP, 1992a, 2000).

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. Reports of hemolytic anemia (or other effects on the blood) in humans following chronic or subchronic exposure are not available. 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; the mother had inhaled and ingested naphthalene from mothballs during pregnancy, particularly during the last trimester (Zinkham and Childs, 1958).

The only case report involving inhalation exposure where an attempt was made to quantitate exposure was that of 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 levels may have been much higher when fresh supplies of mothballs were introduced. When the use of mothballs was discontinued, the 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 as mothballs and subsequently developed hemolytic

anemia and other signs of naphthalene poisoning (estimated doses of 85.7 and 142.8 mg/kg, respectively, can be calculated, assuming a 70 kg body weight).

Neonates and persons deficient in glucose-6-phosphate dehydrogenase (G6PDH) or glutathione are believed to be particularly susceptible to naphthalene-induced hemolytic anemia (Valaes et al., 1963; U.S. EPA, 1987). 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. 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 mothballs (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 mothballs (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 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 (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 mothballs has also been reported (Schafer, 1951); no estimate 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) based on case studies reported at the time.

4.1.3. Cataracts

In humans, cataract formation has been associated with exposure to naphthalene. A pharmacist ingesting 5 g naphthalene developed blindness and bilateral cataracts (Lezenius, 1902). 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 otherwise. 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 human data on cancer incidence following naphthalene exposure are restricted to a few case series reports. No cohort mortality or morbidity studies or case-control studies examining possible associations between naphthalene exposure and increased risk of cancer are available, although 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).

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 lacked controls, utilized a limited number of subjects, and did not determine exposure levels. The subjects were exposed to complex mixtures (i.e., coal tar and tobacco smoke) containing other probable human carcinogens (e.g., polycyclic aromatic hydrocarbons such as benzo[a]pyrene).

In an abstract report of an East German study (Kup, 1978), 15 patients with cancer of the ear, nose, and/or throat were examined for an association with work-related causes. 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.

Twenty-three (23) cases of colorectal carcinoma admitted to a Nigerian hospital in a 2-year period were examined for possible associations with family history of polyposis and the ingestion of *Kafura*, a substance containing naphthalene (exact composition unknown), which was used for treating anorectal problems (Ajao et al., 1988). Eleven of the patients were 30 years or younger at diagnosis. No indication of a connection with familial history of polyposis was found among the cases. Half of the patients recalled a history of taking *Kafura*, and the remaining patients did not know if they had been given the substance during early childhood.

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-1,2- 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-1,2-dihydrodiol were similar and were similarly inhibited by ALϕ1537. Xu et al. (1992b) proposed that ALϕ1537 prevents cataract formation, possibly by inhibiting key enzymes involved in the metabolism of naphthalenein 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-1,2-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. *Other Effects*

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 were 250.6, 306.7, 333.4, 351.2, 353.4, and 348.9 g for males and 156.7, 190.5, 197.2, 203.5, 197.8, and 203.4 g for females for the 400, 200, 100, 50, 25, and 0 mg/kg 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. 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.

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.

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 than control males. 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. Over 50% of the males survived to week 92, indicating a sufficient number of controls were available. Thus, the study was considered adequate (NTP, 1992). In addition, because nasal hyperplasia and metaplasia were seen in virtually all exposed animals, but not in the controls, the doses chosen likely offered a sufficient challenge for the evaluation of carcinogenic potential. 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).

Lung inflammation in 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%). NTP stated that 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. The study concluded that the greater survival in the exposed group versus the control group was

likely related to the marginally increased tumor incidences in the control group. 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.

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.

In another 2-year inhalation bioassay, groups of 49 male and 49 female F344/N rats were exposed to naphthalene at concentrations of 0, 10, 30, or 60 ppm for 6 hours/day, 5 days/week for 105 weeks (Abdo et al. 2001; NTP, 2000). Rats were observed twice daily, and were weighed at the beginning of the study, at 4-week intervals beginning at week 4, and at 2-week intervals beginning at week 92. Complete necropsy and histopathology examinations were performed on all rats. Survival in all exposed groups was similar to control groups. Mean body weights of exposed male rats were lower than those of the control rats throughout the study, but a statistical analysis of the data was not conducted. Reported mean body weights for males during weeks 14-52 were 94%, 92%, and 89% of controls in the 10-, 30-, and 60-ppm groups, respectively; similar values were reported for weeks 52-104 (96%, 96%, and 91% of controls). Mean body weights of exposed female rats were similar to those of controls (101%, 100%, and 95% for the 10-60 ppm groups compared with controls).

Increased incidences of nonneoplastic and neoplastic lesions of the nose occurred in exposed rats of both sexes (see Table 1). Nonneoplastic nasal lesions included (1) hyperplasia, atrophy, chronic inflammation, and hyaline degeneration of the olfactory epithelium and (2) hyperplasia, metaplasia, or degeneration of the respiratory epithelium or glands. Mean severity scores on a 4-point scale from minimal (1) to marked (4) generally increased with increasing exposure level. Nasal lesions showing the greatest severity scores were olfactory epithelial atrophy (e.g., mean scores were 0, 2.1, 2.8, and 3.5 for control through 60-ppm males) and Bowman's gland hyperplasia (mean scores were 1.0, 2.2, 2.9, and 3.5 for males).

Neoplastic lesions associated with naphthalene exposure in rats were olfactory epithelial neuroblastomas and respiratory epithelial adenomas. No other tumors were observed. Olfactory neuroblastomas were masses originating in the ethmoid turbinates of the olfactory region of the nasal cavity. These aggressively invasive tumors extended rostrally involving the respiratory epithelium of the maxilloturbinates and nasal turbinates. The masses ranged from smaller lesions or early developing neuroblastomas, which involved thickening and replacement of olfactory epithelium with tumor cells, to larger masses that occluded the nasal passages and

distorted the nasal architecture. The neuroblastomas consisted of three morphologically distinct pattens as discussed in detail by Long et. al. (2003). Frequently, more than one pattern was seen in the same tumor. The first pattern was composed of "small island and nests of round to polygonal cells with prominent round nuclei; single, centrally located prominent nucleoli; marginated chromatin; poorly-defined cytoplasmic borders; and a large nuclear-to-cytoplasmic ratio". The second type of neuroblastoma consisted of irregular lobules with cells in the center which were usually necrotic. The lobules were separated by thin bands of fibrovascular connective tissue. The third morphologic type of neuroblastoma was less common and consisted of interlacing cords of tumor cells with areas of spindle cell differentiation. The cell of origin for the olfactory neuroblastoma is the olfactory basal cell, an undifferentiated cell capable of differentiating into sustentacular (supporting), sensory (neuronal) or ductal epithelial cells (Bowman's glands). The classification of these tumors as olfactory neuroblastomas is due to the fact that the tumors arise from the olfactory epithelium and contain cells morphologically similar to the differentiated olfactory basal cells. The growth pattern for the tumor cells consists of infiltration of the lamina propria along the mucosa and submucosal nerve fibers leading to gradual replacement of the normal architecture of the turbinates and nasal septum. Olfactory epithelial neuroblastomas are rare tumors in rats and in humans[1].

The respiratory epithelial adenomas developed in the naso- and maxillo-turbinates of the nasal cavity, which is lined by ciliated respiratory-type epithelium. The masses varied in size and the exophytic adenomas partially occluded and distorted the nasal passages. The well-differentiated cells of the neoplasms were composed into "variably-sized pseudoglandular structures surrounded by scant fibrovascular stroma with few focal solid areas of cells" (Long et al., 2003). Some adenomas displayed pseudostratified epithelium while others demonstrated squamous metaplasia. Less well-differentiated squamous cells made up a few adenomas with cells displaying eosinophilic cytoplasm and large nuclei containing one or two prominent nucleoli.

The first incidence of respiratory epithelial adenomas was 684, 685 and 552 days for the 10-, 30- and 60-ppm exposed male rats and 721 and 555 days for the 30- and 60-ppm exposed female rats, respectively. The first incidence of olfactory epithelial neuroblastomas was 433 and 399 days for the 30- and 60-ppm exposed male rats and 679, 480 and 429 days for the 10-, 30- and 60-ppm exposed female rats, respectively.

[1] Approximately 300 human cases of olfactory epithelial neuroblastomas were reported in the world research literature between 1924 and 1985 (McElroy et al., 1998). Olfactory epithelial neuroblastomas occurred in 0/299 control rats in NTP studies using the same type of feed as the NTP (2000) naphthalene bioassay in rats.

Table 1. Incidences of nonneoplastic‡ and neoplastic lesions of the nose in male and female F344/N rats exposed to naphthalene 6 hours/day, 5 days/week for up to 105 weeks (Abdo et al., 2001; NTP, 2000)

Lesion	Concentration (ppm)							
	0		10		30		60	
	M	F	M	F	M	F	M	F
NONCANCER LESIONS								
olfactory epithelium								
hyperplasia, atypical	0/49	0/49	48/49* (2.1)	48/49* (2.0)	45/48* (2.5)	48/49* (2.4)	46/48* (3.0)	43/49* (2.9)
atrophy	3/49	0/49	49/49* (2.1)	49/49* (1.9)	48/48* (2.8)	49/49* (2.7)	47/48* (3.5)	47/49* (3.2)
chronic inflammation	0/49	0/49	49/49* (2.0)	47/49* (1.9)	48/48* (2.2)	47/49* (2.6)	48/48* (3.0)	45/49* (3.4)
hyaline degeneration	3/49 (1.3)	13/49 (1.1)	46/49* (1.7)	46/49* (1.8)	40/48* (1.7)	49/49* (2.1)	38/48* (1.5)	45/49* (2.1)
respiratory epithelium								
hyperplasia	3/49 (1.0)	0/49	21/49* (2.2)	18/49* (1.6)	29/48* (2.0)	22/49* (1.9)	29/48* (2.2)	23/49* (1.7)
squamous metaplasia	0/49	0/49	15/49* (2.1)	21/49* (1.6)	23/48* (2.0)	17/49* (1.5)	18/48* (1.8)	15/49* (1.8)
hyaline degeneration	0/49	8/49 (1.0)	20/49* (1.2)	33/49* (1.2)	19/48* (1.4)	34/49* (1.4)	19/48* (1.2)	28/49* (1.2)
goblet cell hyperplasia	0/49	0/49	25/49* (1.3)	16/49* (1.0)	29/48* (1.2)	29/49* (1.2)	26/48* (1.2)	20/49* (1.0)
gland hyperplasia	1/49 (1.0)	0/49	49/49* (2.2)	48/49* (1.9)	48/48* (2.9)	48/49* (3.1)	48/48* (3.5)	42/49* (3.3)
gland squamous metaplasia	0/49	0/49	3/49 (3.0)	2/49 (2.0)	14/48* (2.1)	20/49* (2.5)	26/48* (2.5)	20/49* (2.8)
NEOPLASMS								
respiratory epithelial adenoma	0/49†	0/49	6/49*	0/49	8/48*	4/49	15/48*	2/49
olfactory epithelial neuroblastoma	0/49†	0/49†	0/49	2/49	4/48	3/49	3/48	12/49*

‡ Mean severity scores for rats with a specific lesion are noted in parentheses: 1=minimal; 2=mild; 3=moderate; 4=marked.
* Significantly ($p<0.05$) different from control value by the Poly-3 test, which adjusts for intercurrent mortality.
† Significant ($p<0.05$) trend by the Poly-3 test.

Increased incidences of neoplastic lesions were restricted to the nose in exposed rats compared with controls. Findings for naphthalene-induced nonneoplastic lesions at sites other than the nose were restricted to scattered observations for alveolar epithelial hyperplasia and chronic inflammation of the lung. Incidences of alveolar epithelial hyperplasia were statistically

significantly increased in 10- and 30-ppm female rats compared with controls, but not in 60-ppm female rats or in any of the exposed male rat groups. Incidences of alveolar epithelial hyperplasia in females in the control through 60-ppm groups were 4/49, 11/49, 11/49, and 9/49, respectively. Chronic pulmonary inflammation, described as minimal inflammatory foci often found in chamber control rats, was statistically significantly increased in male rats exposed to 10 or 60 ppm, but not in males exposed to 30 ppm (male incidences were 2/49, 13/49, 6/48, and 15/49), or in any of the exposed female groups.

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). 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. 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.

4.2.3. Other Routes of Exposure

Exposure to naphthalene by routes other than noninhalation 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 the same as when paraffin wax was used (2%-4%) and lower than with the implantation of cholesterol (12%).

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 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). 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 mmol to male and female newborn CD-1 mice on days 1, 8, and 15 of life (total dose = 1.75 mmol 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. Complete histological examinations were not conducted. No statistically significant increased incidence of liver tumors (adenomas or haematomas) 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. The observation period (52 weeks) was less than the lifetime for mice (approximately 2 years).

Male and female Swiss-Webster mice were given intraperitoneal injections of 0 or 200 mg/kg naphthalene in corn oil and lungs were removed at 1, 2, 3, 6, and 24 hours after treatment (n=3-5/sex/time period for exposed and n=1/sex/time period for vehicle controls) (Van Winkle et al., 2002). Acute lung injury was assessed by: (1) high-resolution microscopic assessment of differential permeability to fluorescent nuclear dyes in cells along the long axis of conducting airway trees of microdissected right middle lung lobes, and (2) high-resolution histopathology of sections of Karnovsky-fixed left lung lobes. The abundance of normal and cytotoxic (i.e., vacuolated) epithelial cells was determined by quantitative histopathology of the intrapulmonary lobar bronchus of groups of 5 male and 5 female mice, 24 hours following injection of 0 or 200 mg/kg naphthalene in corn oil. Clara cell injury occurred in the terminal bronchioles of both male and female mice. However, Clara cell injury in terminal bronchioles occurred earlier, affected cells farther up the airway tree, and showed a different temporal pattern of changes in Clara cells in female mice compared with male mice. Twenty-four hours after injection, Clara cell injury in the lobar bronchus of female mice was evidenced by numerous vacuolated cells, whereas normal bronchiolar epithelium containing Clara and ciliated cells was found in vehicle-control males and females, as well as in exposed male mice. The mean volume fraction of vacuolated epithelium in the lobar bronchus of exposed female mice (about 0.33) was statistically significantly increased compared with respective means for vehicle-treated female mice (0) or exposed male mice (about 0.05). *In vitro* CYP-mediated naphthalene metabolism was assessed in microdissected regions of airways from male and female mice by HPLC analysis. Two metabolites were detected in tissue from both sexes [naphthalene-1,2-dihydrodiol (dihydrodiol) and the glutathione conjugate of 1*R*,2*S*-naphthalene oxide], but the rate of formation of the dihydrodiol was statistically significantly greater in female tissue than in male tissue. For example, in terminal bronchioles the mean rates of formation of the dihydrodiol were about 0.48 and 0.30 nmol/minute/mg protein in females and males, respectively.

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, which was noted 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. NTP (1991) 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.

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. 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 another 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.

Naismith and Matthews (1986) performed a subsequent developmental toxicity study in New Zealand white rabbits with groups of 18 does utilizing gavage daily doses of 0, 40, 200, or 400 mg/kg naphthalene in 1% methylcellulose from gestation days 6-18. 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 dose 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

O'Brien et al. (1989) administered 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 naphthalene provided what was 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. 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 CYP monooxygenases in distal airways that are associated with the tolerance resulting from repeated exposure of mice to naphthalene. After repeated exposure to short-term cytotoxic doses of naphthalene, distal bronchioles of tolerant mice were lined by epithelium that resembled that of controls. Distal bronchioles had reduced levels of CYP activity towards naphthalene, but only at less than saturating concentrations, and had no decrease in covalent binding of reactive metabolites to proteins. Lastly, 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.

4.4.2. Studies With Methylnaphthalenes

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). No histopathological lesions were detected in any organ 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.

Statistically significant increases in the 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. 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 indicated 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. Incidences of 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, respectively. No other statistically significant exposure-related changes in incidence of nonneoplastic lesions were found in other organs or tissues.

Murata et al. (1997) exposed 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.

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 incidences 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, respectively. 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 also 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%, in females and 42.9% and 46.9% in males, respectively. Other nonneoplastic lesions were observed at 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; the 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. The results indicating 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.

The chronic lung effects observed in mice following ingestion of 1-methylnaphthalene or 2-methylnaphthalene are not likely to involve the same mode of action as the lung effects in mice following inhalation of naphthalene because of (1) the difference in the nature and tissue site of the lesions; (2) the difference in metabolism of naphthalene, which predominantly proceeds through an initial ring oxidation step, compared with methylnaphthalene metabolism, which proceeds predominantly through oxidation of the methyl side group; and (3) the difference in mouse gender susceptibility to naphthalene and methylnaphthalene carcinogenicity (with inhaled naphthalene, female mice, but not male mice, developed lung tumors; however, with ingested 1-methylnaphthalene, male mice but not female mice, showed increased incidence of lung tumors). Thus, the limited evidence for methylnaphthalene carcinogenicity is not considered to add supporting evidence for the carcinogenicity of naphthalene.

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, if any, were examined). No toxic effects were found with parenteral administration of naphthalene and 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

The available genotoxicity studies for naphthalene and its metabolites are presented in Table 2. Naphthalene has tested negative for reverse mutation in *S. typhimurium*, either with or without the use of an S9 fraction (McCann et al., 1975; Mortelmans et al., 1986; NTP, 1992a; Gatehouse, 1980; Bos et al., 1988; Florin et al., 1980; Sakai et al., 1985; Godek, 1985; Kaden et al., 1979; Narbonne et al., 1987; Connor et al., 1985) and for SOS response in *S. typhimurium* and *E. coli* (Nakamura et al., 1987; Mamber et al., 1984; Mersch-Sunderman et al., 1993), as well as in the Pol A^- or Rec assay in E. coli (Mamber et al., 1983). Naphthalene induced reverse mutations in genes controlling luminescence in the marine bacterium, Vibrio fischeri, in the presence of rat liver metabolic activation (Arfsten et al., 1994), but not if metabolic activation was not included.

In eukaryotic in vitro tests, naphthalene was negative for hprt mutations in MCL-5 human lymphoblastoid cells (Sasaki et al., 1997), sister-chromatid exchange in human mononuclear leukocytes (Tingle et al., 1993; Wilson et al., 1995), unscheduled DNA synthesis in primary rat hepatocytes (Barfknecht et al., 1985), and for induction of DNA-single-strand breaks, assessed by the alkaline elution assy, in rat hepatocytes (Sina et al., 1983). Naphthalene was found to induce chromosomal aberrations in Chinese hamster ovary cells in the presence of an S9 fraction (NTP, 1992a). A later evaluation of naphthalene's ability to induce chromosomal aberrations in whole mouse embryos was positive and was more pronounced by the addition of an S9 fraction (Gollahon et al., 1990). Naphthalene was negative for cell transformation in Fischer rat embryo cells (Freeman et al., 1973), a Syrian baby hamster kidney cell line (Purchase et al., 1978), BALB/c mouse whole mammary (Tonelli et al., 1979) and 3T3 cell cultures (Rundell et al., 1983), and in human diploid fibroblasts (Purchase et al., 1978).

While the majority of results from tests evaluating naphthalene's possible genotoxicity have been negative, there is some evidence of genotoxicity for the naphthalene metabolite 1,2-naphthoquinone. 1,2-Naphthoquinone induced reverse mutations (frameshift, point, and oxidative mutations) in several Salmonella typhimurium strains (TA97a, TA100 and TA104) without a metabolic activation system (Flowers-Geary et al., 1996) and sister-chromatid exchange in human mononuclear leukocytes (Wilson et al., 1996). The tester strain TA97a has a repetitive dinucleotide sequence (-GC-) near a base-pair deletion site and an added run of six cysteine molecules in the hisD gene that are postulated to be "hot spots" for frameshift mutations (Maron and Ames, 1983; Hartman et al., 1986). These frameshift mutations are reverted to wild-type by mutagens that stabilize the shifted base-pairing. The stabilization of the mispaired DNA occurs due to either stacking effects through intercalation alone or intercalation accompanied by covalent modification of the DNA (Maron and Ames, 1983; Hartman et al., 1986). Polycyclic aromatic hydrocarbons, including naphthalene, are generally planar molecules which could intercalate into DNA. In addition, benzo[a]pyrene-7,8-dione has been shown to form Michael adducts at C10 of the hydrocarbon and will form covalent adducts with 2'-deoxyguanosine with calf thymus DNA, plasmid DNA and oligonucleotides (Shou et al., 1993). These observations suggest that *ortho*-quinones, such as 1,2-naphthoquinone, may be frameshift mutagens due to intercalation followed by covalent modification of the tester strain. In addition, Yu et al. (2002) showed that 1,2-naphthoquinone was capable of inactivating the p53 tumor suppressor gene in a yeast reporter system in the presence of copper and a reducing agent. In summary, these data indicate that naphthalene itself may not be directly genotoxic, but that a metabolite, i.e., 1,2-naphthoquinone, is capable of producing genotoxic responses.

Table 2. Results of genotoxicity testing of naphthalene or metabolites*

Assay	Test system	Dose/Concentration	HID or LED	Result	Reference
Bacterial gene mutation assays					
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA98, TA100	100 µg/plate ± S9 activation	100	Negative	McCann et al., 1975
	S. typhimurium TA1535, TA1537, TA98, TA100	0.3-100 µg/plate ± S9 activation	100	Negative	Mortelmans et al., 1986
	S. typhimurium TA1535, TA1537, TA98, TA100	0.3-100 µg/plate ± S9 activation	100	Negative	NTP, 1992a
	S. typhimurium TA1537, TA1538	10-200 µg/plate ± S9 activation	100	Negative, toxic above 100 µg/plate	Gatehouse, 1980
	S. typhimurium TA98, TA100	10-50 µg/plate ± S9 activation	50	Negative	Bos et al., 1988
	S. typhimurium TA1535, TA1537, TA98, TA100	0.03-30 µmol/plate ± S9 activation	3	Negative, toxic above 3 µmol/plate	Florin et al., 1980
	S. typhimurium TA1535, TA1537, TA98, TA100	250 µg/plate ± S9 activation	250	Negative	Sakai et al., 1985
	S. typhimurium TA1535, TA1537, TA1538, TA98, TA100	3-300 µg/plate ± S9 activation	300	Negative, toxic above 300 µg/plate	Godek, 1985
	S. typhimurium TM677	1-2 mM ± S9 activation	2	Negative	Kaden et al., 1979
	S. typhimurium TA98, TA1535	5-1000 µg/plate ± S9 activation	1000	Negative	Narbonne et al., 1987

Table 2. Results of genotoxicity testing of naphthalene or metabolites*

Assay	Test system	Dose/Concentration	HID or LED	Result	Reference
	S. typhimurium UTH8413, UTH8414, TA98, TA100	100-2000 µg/plate ± S9 activation	2000	Negative	Connor et al., 1985
	S. typhimurium TA1535, TA1537, TA98, TA100	1000 µg/plate ± S9 activation	1000	Negative (1-naphthol)	McCann et al., 1975
	S. typhimurium TA98, TA1535	5-1000 µg/plate ± S9 activation	1000	Negative (1-naphthol)	Narbonne et al., 1987
	S. typhimurium TA1535, TA1537, TA98, TA100	250 µg/plate ± S9 activation	250	Negative (1,4-naphthoquinone)	Sakai et al., 1985
	S. typhimurium TA97a, TA98, TA100, TA104	0-100 nmol/plate ± S9 activation	17.5	Positive (1,2-naphthoquinone), 1.8- to 3.4-fold increase without S9; +S9 results similar to -S9 results for TA104 (only strain tested +S9)	Flowers-Geary et al., 1996
SOS response	*S. typhimurium* TA1535/p5K1002 (uMuC-lacZ)	83 µg/mL ± S9 activation	83	Negative	Nakamura et al., 1987
	E. coli K12 inductest (λ lysogen GY5027; uvrB$^-$, envA$^-$)	2000 µg/plate ± S9 activation	2000	Negative	Mamber et al., 1984
SOS chromotest	*E. coli* PQ37 (sfiA::lacZ fusion)	0.156-10.0 µg/assay ± S9 activation	10	Negative	Mersch-Sundermann et al., 1993
Pol A$^-$ or Rec assay	*E. coli* WP2/WP10 (uvrA$^-$, recA$^-$)	2000 µg/mL ± S9 activation	2000	Negative	Mamber et al., 1983
	E. coli WP2/WP67 (uvrA$^-$, pol A$^-$)	dose not specified ± S9 activation	NS	Negative	Mamber et al., 1983

Table 2. Results of genotoxicity testing of naphthalene or metabolites*

Assay	Test system	Dose/Concentration	HID or LED	Result	Reference
Pol A$^-$ or Rec assay	*E. coli* WP2/WP3478 (pol A$^-$)	dose not specified ± S9 activation	NS	Negative	Mamber et al., 1983
Mutatox (reversion to luminescence)	*V. fischeri* M169	Up to 5000 µg/tube ± S9 activation	0.203 0.625	Negative without S9 activation Positive with S9 activation	Arfsten et al., 1994
***In vitro* eukaryotic gene mutation, cytogenetic, or DNA damage assays**					
mutation at hprt and tk loci	Human B-lymphoblastoid cell line MCL-5	40 µg/mL	40	Negative	Sasaki et al., 1997
	Human B-lymphoblastoid cell line MCL-5	40 µg/mL	40	Negative (1,4-naphthoquinone)	Sasaki et al., 1997
Chromosomal aberrations	Chinese hamster ovary cells	15-75 µg/mL ± S9 activation	30 75	Positive, with S9 activation Negative without S9 activation	NTP, 1992a
Chromosomal aberrations	Preimplantation whole mouse embryos	0.16 mM ± S9 activation	0.16	Positive, more pronounced with S9 activation	Gollahon et al., 1990 [abstract only]
Sister chromatid exchange	Human mononuclear leukocytes	100 µM ± human liver microsomes	100	Negative	Tingle et al., 1993; Wilson et al., 1995
Sister chromatid exchange	Human mononuclear leukocytes	0-100 µM ± human liver microsomes	10	Positive (1,2- and 1,4-naphthoquinone) Negative (naphthalene-1,2-oxide)	Wilson et al., 1996
Sister chromatid exchange	Chinese hamster ovary cells	9-90 µg/mL ± S9 activation	27	Positive with S9 in the second of 2 trials and without S9 in both trials	NTP, 1992a

Table 2. Results of genotoxicity testing of naphthalene or metabolites*

Assay	Test system	Dose/Concentration	HID or LED	Result	Reference
***In vitro* eukaryotic gene mutation, cytogenetic, or DNA damage assays (continued)**					
Alkaline elution (*in vitro*)	Rat hepatocytes	3 mM, 3-hour exposure	3 mM	Negative for increased incidence of DNA single-strand breaks	Sina et al., 1983
Unscheduled DNA synthesis (*in vitro*)	Rat primary hepatocytes	0.16-5000 µg/mL	16	Negative, toxic above 16 µg/mL	Barfknecht et al., 1985
	Rat primary hepatocytes	0.5-1000 nM/mL	1000	Negative (1-naphthol, 2-naphthol)	Probst et al., 1981
Cell transformation	Fischer rat embryo cells (F1706P96)	0.1, 0.5 µg/mL	0.5	Negative	Freeman et al., 1973
	Syrian baby hamster kidney cells (BHK-21C13)	0.08-250 µg/mL + S9	250	Negative	Purchase et al., 1978
	Mouse (BALB/c) whole mammary gland cultures	0.001-1.0 µg/gland	0.1	Negative, cytotoxic above 0.1 µg/gland	Tonelli et al., 1979
	Mouse BALB/c 3T3 cell culture	15-150 µg/mL	150	Negative, toxic at highest dose	Rundell et al., 1983
	Human diploid fibroblasts (WI-38)	0.08-250 µg/mL + S9	250	Negative	Purchase et al., 1978
***In vivo* eukaryotic gene mutation, cytogenetic, or DNA damage assays**					
Somatic mutation, recombination	*D. melanogaster*	1, 5, 10 mM (feeding larvae)	5	Positive, loss of heterozygosity of 2 recessive wing genes (about 2-fold increase in # of wing spots)	Delgado-Rodriguez et al., 1995

Table 2. Results of genotoxicity testing of naphthalene or metabolites*

Assay	Test system	Dose/Concentration	HID or LED	Result	Reference
Micronuclei induction	Male ICR Swiss mice: bone marrow cells	50, 250, 500 mg/kg gavage	500	Negative	Harper et al., 1984
	Male and female CD-1 mice: bone marrow cells	250 mg/kg i.p.	250	Negative	Sorg, 1985
***In vivo* eukaryotic gene mutation, cytogenetic, or DNA damage assays (continued)**					
Micronuclei induction	Salamander larvae (*Pleurodeles waltl*): erythrocytes	0.125-0.5 ppm in the tank water	0.25	Positive at 0.5 ppm, weakly positive at 0.25 ppm	Djomo et al., 1995
Alkaline elution (*in vivo*)	DNA from hepatocytes of female rats given single oral doses	359 mg/kg oral	359	Negative for DNA single-strand breaks	Kitchin et al., 1992, 1994
Unscheduled DNA synthesis (*in vivo*)	Hepatocytes from rats given single oral doses	600, 1000, 1600 mg/kg gavage	1600	Negative	RTC, 1999
DNA fragmentation	DNA fragmentation in liver or brain tissue from mice given single doses	0, 3, 32, 158 mg/kg (0.01, 0.1, 0.5 of LD50=316 mg/kg)	32	Positive (1.0- to 1.5-fold &1.8- to 2.2-fold increase in DNA fragmentation at 32 and 158 mg/kg, respectively)	Bagchi et al., 2002
DNA fragmentation	DNA fragmentation in liver or brain tissue from rats given daily doses for up to 120 days	0, 110 mg/kg in corn oil	110	Positive (1.9- to 2.5-fold maximal increases in DNA fragmentation in brain and liver tissue)	Bagchi et al., 1998
DNA fragmentation	DNA fragmentation in liver or brain tissue from p53-deficient and standard mice given single oral doses	0, 3, 32, 158 mg/kg (0.01, 0.1, 0.5 of LD50=316 mg/kg)	158 (std) 3 (-p53)	Positive (1.8- to 3.9-fold increases in DNA fragmentation in brain and liver tissue; p53-deficient (tumor suppressor gene) strain was more sensitive	Bagchi et al., 2000

Table 2. Results of genotoxicity testing of naphthalene or metabolites*

Assay	Test system	Dose/Concentration	HID or LED	Result	Reference
Neoplastic transformation (*in vivo*)	F344 partially hepatectomized rats (sex not specified)	100 mg/kg gavage (in corn oil)	100	Negative for gamma-glutamyl transpeptidase foci	Tsuda et al., 1980

* Metabolites are noted in Result column; HID, highest ineffective dose for negative tests; LED, lowest effective dose for positive tests; NS, not specified; adapted from ATSDR, 2003

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

Human studies that establish an association between naphthalene exposure and health effects are restricted to reports of hemolytic anemia or cataracts following acute or occupational exposure to naphthalene, either by ingestion or inhalation of naphthalene vapors. These reports have not identified exposure levels associated with these effects (Dawson et al., 1958; Ghetti and Mariani, 1956; Gidron and Leurer, 1956; Gupta et al., 1979; Haggerty, 1956; Kurz, 1987; MacGregor, 1954; Mackell et al., 1951; Melzer-Lange and Walsh-Kelly, 1989; Ojwang et al., 1985; Santucci and Shah, 2000; Shannon and Buchanan, 1982).

Naphthalene metabolites may be involved in naphthalene-induced hemolytic anemia, but the molecular mechanisms are not clearly understood (U.S. EPA, 1987; ATSDR, 2003). 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-induced hemolysis which may be caused by the inability of G6PDH-deficient cells to replenish 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). Continued glutathione depletion could weaken cell membranes, caus hemoglobin to become unstable and ultimately lead to hemolysis of red blood cells. Other possible causes of hemolysis include inhibition of either glutathione peroxidase or glutathione reductase by a naphthalene metabolite (Rathbun et al., 1990; Tao et al., 1991b). Neonates are sensitive to naphthalene-induced hemolysis. Valaes et al. (1963) proposed that neonatal sensitivity may due to immaturity of the detoxication pathways responsible for the conjugation and excretion of naphthalene metabolites.

Wells et al. (1989) proposed that naphthalene-induced cataractogenesis in C57BL mice (from intraperitoneal injection) requires CYP-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 was decreased by pretreatment with either CYP 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 found 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. Several lines of evidence indicate 1,2-naphthoquinone may be involved in the formation of cataracts after acute, high-dose

exposure to naphthalene in rats and rabbits. Xu et al. (1992a) proposed that naphthalene-1,2-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. However, mice do not develop cataracts following naphthalene exposure, whereas both rats and rabbits do; it is not known if this difference is the result of a difference in enantiomeric epoxide production, a difference in enzymatic activities in the lens, or from some other cause. In the lens tissue, the formation of 1,2-naphthoquinone is likely catalyzed by aldose reductase (not shown in Figure 1, Section 3.3). Support for this hypothesis includes findings that: 1) aldose reductase inhibitors prevent cataract formation in naphthalene-fed rats (Tao et al., 1991a,b; Xu et al., 1992a); 2) dihydrodiol dehydrogenase is apparently absent in rat lens (Greene et al., 2000); and 3) aldose reductase appears to be the only enzyme in rat lens that can transform naphthalene-1,2-dihydrodiol to 1,2-naphthoquinone (Sugiyama et al., 1999).

Nasal and pulmonary effects have been identified as critical effects from inhalation exposure to naphthalene. Differences in sensitivity to naphthalene-induced respiratory effects have been found among animal species. Mice appear to be more sensitive to both acute and chronic naphthalene-induced pulmonary cytotoxicity than hamsters or rats (Abdo et al., 2001; Baldwin et al., 2004; Buckpitt and Franklin, 1989; Buckpitt et al., 1992, 1995; NTP, 1992a, 2000; Plopper et al., 1992a,b; West et al., 2001). Animal studies have linked naphthalene toxicity in the lung to CYP-mediated metabolism (Baldwin et al., 2004; Buckpitt et al., 1992, 1995; Kanekal et al., 1991; 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.

Kanekal et al. (1991) suggested that epoxides may be the primary toxicants based on the findings that in perfused mouse lung preparations racemic mixtures of naphthalene epoxides produced cytotoxicity at concentrations 10-fold lower than naphthalene. The epoxide is fairly short-lived with a half-life of approximately 2-3 minutes in pH 7.4 buffer which is extended by the presence of albumin to about 11 minutes (Buckpitt et al., 2002; Kanekal et al., 1991). These metabolic intermediates may be important in the generation of toxic effects of naphthalene on Clara cells (ciliated cells in the epithelium of proximal and distal airways of the lung) (Buckpitt et al., 2002; Zheng et al., 1997). Naphthalene-1,2-oxide has also been shown to be one of two naphthalene moieties covalently bound to proteins in isolated mouse Clara cells (Zheng et al., 1997). Due to its chemical reactivity, the epoxide can bind covalently to cellular macromolecules which could potentially cause cellular dysfunction. This characteristic and the knowledge that other aromatic hydrocarbon epoxides are cytotoxic, genotoxic, and/or carcinogenic has led to the hypothesis that naphthalene-1,2-oxide is, at least in part, responsible for the toxicity of naphthalene. 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 (1*S*,2*R*-naphthalene oxide) was different from that formed by mouse (1*R*,2*S*-naphthalene oxide). Rat and hamster lung microsomes exhibited results for naphthalene metabolic rates and stereoselectivity that were intermediate between those found with mice and monkey microsomes.

Buckpitt and Bahnson (1986) found earlier that human lung microsomes possessed metabolic activities similar to those measured with monkey lung microsomes (i.e., a slow rate and predominant formation of the 1*S*,2*R*-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 indicated 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, pulmonary region-specific (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) examined the correlation of Clara cell cytotoxicity with metabolism in different airway regions of rodents. The rate and stereoselectivity of naphthalene metabolism by microsomal preparations were found to correlate with pulmonary tissue and species differences in cytotoxicity. Obtained by microdissection, specific subcompartments of the pulmonary system were used to study the CYP-dependent metabolism of naphthalene and the epoxide hydrolase/glutathione transferase-dependent metabolism of naphthalene-1,2-oxide. 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 CYP2F2; little labeling was observed in any portion of rat and hamster lungs. Postmitochondrial supernatants prepared from mouse and hamster airways metabolized racemic naphthalene-1,2-oxide to dihydrodiol and glutathione adducts at substantially higher rates than did comparable preparations from rats. These data support the view that the rate and stereoselectivity of naphthalene metabolism to (1*R*,2*S*)-naphthalene oxide catalyzed by CYP2F2 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.

Species differences in susceptibility to toxicity of the olfactory and respiratory epithelia of the nose have not been correlated with differences in rates of transformation to epoxide derivatives in extracts of olfactory tissue (Buckpitt et al., 1992; Plopper et al., 1992a). Buckpitt et al. (1992) examined three segments of the nasal mucosa (lateral wall, septum, and olfactory epithelium) in mice, rats, and hamsters, evaluating these segments for differences in the rate of naphthalene metabolism. Metabolic rates (in nmol naphthalene converted to epoxide derivatives/min/mg protein) were highest in the olfactory epithelium in the following order: mouse (87.1) > rat (43.5) > hamster (3.9). However, rats were more susceptible to naphthalene-induced olfactory tissue cell injury than mice or hamsters. The lowest single intraperitoneal doses producing necrosis and exfoliation in olfactory epithelium were 200 mg/kg naphthalene in rats and 400 mg/kg in mice and hamsters. Thus, the rationale for species differences in susceptibility to naphthalene-induced nasal toxicity does not depend on the formation of naphthalene-1,2-oxide as it does for pulmonary toxicity.

CYP2F expression levels in primate nasal tissues (ethmoturbinates, nasal and maxilloturbinates) demonstrate that only the nasal ethmoturbinates contained quantifiable amounts of CYP2F. The levels of CYP2F in primates were roughly 10- and 20-fold less than the corresponding tissues in rats and mice, respectively. Previous studies demonstrated that lung microsomal incubations of rhesus macaques metabolize naphthalene at a rate 100-fold less than mice and 10-fold less than rats (Buckpitt et al., 1992). Based on the lower CYP2F expression levels in primate nasal tissues and the decreased level of metabolism of naphthalene in primates, rhesus macaques may be less susceptible to naphthalene-induced pulmonary injury than rats or mice. 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 evidence for toxicity caused by this pathway.

Another reactive metabolite that may be involved in naphthalene-induced toxicity is 1,2-naphthoquinone which can be formed from naphthalene-1,2-dihydrodiol by the actions of dihydrodiol dehydrogenase (member of the AKR superfamily). The initial product is an air sensitive catechol (Penning et al., 1999) which can undergo two 1 electron oxidations leading to a concurrent generation of reactive oxygen species. Thus, 1,2-naphthoquinone is both inherently reactive (e.g., via Michael addition to nucleophiles) and capable of producing reactive oxygen species through redox cycling (Flowers et al., 1997). 1,2-Naphthoquinone has been shown to be one of two naphthalene moieties covalently bound to proteins in isolated mouse Clara cells (Zheng et al., 1997). The remaining pathway of naphthalene metabolism that could result in reactive metabolites involves the oxidation of naphthalene-1,2-dihydrodiol by CYP enzymes resulting in the epoxide, 1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydronaphthalene. Support for the *in vivo* formation of 1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydronaphthalene comes from the identification of several urinary metabolites, including a number of trihydroxytetrahydromethylthio derivatives (Horning et al., 1980) and a trihydroxytetrahydromercapturic acid (Pakenham et al., 2002). While 1,2-dihydroxy-3,4-epoxy-,2,3,4-tetrahydronaphthalene would be expected to covalently modify cellular macromolecules, there is no direct evidence for the formation of this metabolite.

In humans, comparatively little information is available regarding the metabolism of naphthalene. It has 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). Microsomes from human lymphoblastoid cells expressing recombinant human CYP2F1 showed preferential formation of the 1*S*,2*R*-naphthalene oxide enantiomer, providing some evidence that human transformation of naphthalene to reactive epoxides may be more like rats than mice with respect to stereospecificity (Lanza et al., 1999). The presence of naphthalene-metabolizing enzymes has been demonstrated in nasal respiratory epithelial and olfactory epithelial tissue from rodents and humans (Buckpitt et al., 1992, 2002; Plopper et al., 1992a; Thornton-Manning and Dahl, 1997), but information on the rates of naphthalene metabolism and the resulting metabolic products in human nasal tissue are not available.

Studies following intraperitoneal injection of naphthalene indicate that toxic effects on the respiratory tract may be due to naphthalene metabolites that are formed either by the liver (or other tissues) or respiratory tract. Buckpitt and Franklin (1989) reported that pretreatment of mice with inhibitors of CYP (piperonal butoxide, SKF 525-A) protected against pulmonary damage from intraperitoneal injection of naphthalene. Furthermore, Clara cell necrosis in the bronchiolar epithelium of mice (Buckpitt et al., 1992; Rasmussen et al., 1986; O'Brien et al., 1985, 1989; Tong et al., 1981) and necrosis of olfactory epithelial cells in mice, rats and hamsters (Plopper et al., 1992a) was observed following intraperitoneal administration of naphthalene.

Available data regarding naphthalene-induced noncancer effects in orally exposed animals and associated dose levels are summarized in Table 3. A deficiency in the animal data base 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. The effects 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, 1990) 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 data indicating that pregnant animals are particularly sensitive to naphthalene via the oral route of exposure. 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 (≥ 50%) with doses ≥ 630 mg/kg (Naismith and Matthews, 1985, 1986). The basis for the apparent difference between the two rabbit gestational exposure studies is not known.

Table 3. 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
			NTP, 1992b
	Pregnant rabbits	120; 13 d	
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			BCL, 1980a
	Rats	200; 90 d	BCL, 1980b; Shopp et al., 1984
	Mice	100-133; 90 d	Naismith and Matthews, 1986
	Pregnant rabbits	40; 12 d	

Table 3. 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
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, 1990
	Pregnant rabbits	630; 12 d	Naismith and Matthews, 1985
No effect observed			
	Pregnant rabbits	400; 12 d	Naismith and Matthews, 1986 NTP, 1992b
	Pregnant rabbits	120; 13 d	NTP, 1991
	Pregnant rats	450; 10 d	

In general, results from the oral developmental studies in rats and rabbits are consistent with the conclusion that exposure to doses > 100 mg/kg during pregnancy produces no toxic effects in developing fetuses and no maternal effects that are unequivocally adverse. Gestational-exposure doses > 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 (> 150 mg/kg). Studies exposing pregnant mice to doses lower than 300 mg/kg were not available.

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

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION

4.6.1. Summary of Overall Weight of Evidence

Under the *Draft Revised Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1999), naphthalene is likely to be carcinogenic to humans by the inhalation route of exposure based on: (1) increased incidences of rare nasal tumors including olfactory epithelial neuroblastomas in female rats and respiratory epithelial adenomas in male rats exposed to naphthalene vapor concentrations of 10, 30, or 60 ppm for 2 years (Abdo et al., 2001; NTP, 2000); (2) increased incidences of alveolar/bronchiolar adenomas or carcinomas in female (but not male) B6C3F1 mice exposed to 30 ppm naphthalene for 2 years (NTP, 1992a); and (3) increased numbers of tumors in tumor-bearing A/J strain mice exposed to 10 or 30 ppm for 6 months (Adkins et al., 1986). Evidence from human studies is limited to a few case series reports of laryngeal cancer in naphthalene purification workers and colorectal cancer in several patients who reported taking an indigenous medication containing naphthalene.

The previous IRIS assessment classified naphthalene as a possible human carcinogen (Group C; inadequate human and limited animal data) via the oral and inhalation routes using criteria of the Guidelines for Carcinogenic Risk Assessment (U.S. EPA, 1986). In addition, using the Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996), it was stated that the human carcinogenic potential via the oral and inhalation routes cannot be determined based on human and animal data; however, there was suggestive evidence of potential human carcinogenicity based on increased lung tumor incidence in one animal species and one sex at the high dose only. Additional support included increased respiratory tumors associated with 1-methylnaphthalene. The current reassessment of inhalation carcinogenicity supersedes the previous assessment.

The International Agency for Research on Cancer has classified naphthalene as a 2B carcinogen (possibly carcinogenic to humans) based on inhalation data in animals (IARC, 2002). The National Toxicology Program is in the process of reevaluating the carcinogenicity of naphthalene for the upcoming 11th Report on Carcinogens (http://ntp-server.niehs.nih.gov/).

The mode of action by which naphthalene affects mouse lung epithelial tissue and mouse and rat nasal tissue may involve the metabolic intermediates, naphthalene-1,2-oxide, 1,2-naphthoquinone, and 1,4-naphthoquinone, which may damage tissue macromolecules either directly by their inherent electrophilicity or by the generation of reactive oxygen species (Buckpitt et al., 1992, 1995, 2002; D'Arcy Doherty et al., 1985; Flowers et al., 1997; Greene et al., 2000; Tao et al., 1991a, b; Xu et al., 1992; Zheng et al., 1997). However, identification of which metabolites are responsible for naphthalene toxicity and carcinogenicity is unknown. Tissue sites of nonneoplastic cellular damage (bronchoalveolar region in mice and nasal tissues in rats and mice) show some correlation with tissue sites of carcinogenicity (lung tumors in mice, but not rats, and nasal tumors in rats, but not mice), suggesting that naphthalene metabolites may act by nongenotoxic modes involving sustained cellular proliferation following cellular damage. This hypothesis is supported by negative results for naphthalene in numerous short-term

genotoxicity assays. However, an understanding of the mode of action is inadequate for determining why rats, but not mice, develop tumors originating in nasal tissues even though both species show nonneoplastic lesions in nasal epithelial tissues following inhalation exposure to naphthalene. Other events preceding tumor formation have not been identified. A possible genotoxic mode of carcinogenic action cannot be discounted as there are some data indicating genotoxicity of 1,2-naphthoquinone.

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

As discussed in Section 4.1.4, the only available studies of cancer in humans exposed to naphthalene are a few case series reports of cancer: one report of four laryngeal cancer cases (all of whom were smokers) among workers in a naphthalene purification plant in East Germany (Kup, 1978; Wolf, 1976, 1978), and a report of 23 patients with colorectal carcinoma admitted to a hospital in Nigeria, half of whom recalled taking sometime during their life a local medication, *Kafura*, which contains naphthalene (Ajao et al., 1988). No cohort mortality, morbidity studies or case-control studies examining possible associations between naphthalene exposure and increased risk of cancer (or other health effects) are available.

The NTP mouse (NTP, 1992a) and rat (Abdo, 2001; NTP, 2000) studies are adequately designed to examine the carcinogenicity of lifetime naphthalene exposure via inhalation. The studies involved exposure to 0, 10, or 30 ppm naphthalene for mice and 0, 10, 30, or 60 ppm for rats (6 hours/day, 5 days/week for 104-105 weeks). The studies provide evidence of naphthalene carcinogenicity in female mice (increased incidences of alveolar/bronchiolar adenomas and carcinomas) and in male and female rats (increased incidences of olfactory epithelial neuroblastomas and respiratory epithelial adenomas). Olfactory epithelial neuroblastomas and respiratory epithelial adenomas are considered rare tumors in the NTP tumor data base.

A statistically significant increased incidence of alveolar/bronchiolar adenomas and carcinoma was found in 30-ppm dosed female B6C3F1 mice, but not in 10-ppm dosed females or in males (females: 5/69, 2/65, 28/135, respectively; males: 7/70, 17/69, and 31/135, respectively) (NTP, 1992a). One female mouse exhibited an alveolar/bronchiolar carcinoma. 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 considered benign, and male mice did not show a statistically significant increased tumor incidence. Exposed mice of both sexes showed chronic inflammation of the lung (males: 0/70, 21/69, and 56/135 and females: 3/69, 13/65, and 52/135). Nearly all mice in both exposure groups (>95%) also showed nonneoplastic nasal lesions described as chronic inflammation, olfactory epithelial metaplasia, and respiratory epithelial hyperplasia.

In rats, incidences of nasal respiratory epithelial adenomas were statistically significantly elevated in males exposed to 0, 10, 30, and 60 ppm naphthalene (0/49, 6/49, 8/48, and 15/48, respectively), but not in females (0/49, 0/49, 4/49, 2/49, respectively) (Abdo, 2001; NTP, 2000). Incidences of olfactory epithelial neuroblastoma were 0/49, 0/49, 4/48, and 3/48, respectively, in male rats, and 0/49, 2/49, 4/48, and 12/49, respectively, in female rats. NTP (2000) concluded

that there was clear evidence of carcinogenic activity of naphthalene in male and female F344/N rats based on increased incidences of respiratory epithelial adenoma and olfactory epithelial neuroblastoma of the nose. As with mice, nearly all rats in all exposure groups showed nonneoplastic nasal lesions in both olfactory and respiratory epithelia, including atypical hyperplasia in olfactory epithelium, hyaline degeneration in olfactory and respiratory epithelia, and Bowman's gland hyperplasia. These results provide adequate evidence of carcinogenicity in two animal species exposed by inhalation to naphthalene vapors.

A 6-month inhalation study with female A/J strain mice exposed to 0, 10, or 30 ppm naphthalene concentrations found a statistically significant increased number of lung tumors (alveolar adenomas) per tumor-bearing lung, thus providing support to the weight of evidence that naphthalene induces lung tumors in mice (Adkins et al., 1986). 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.

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 (e.g., only one dose level was administered, the histopathological examination was not complete). 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, these studies has are inadequate for assessing the potential for lifetime naphthalene exposure to produce cancer.

As discussed in Section 4.4.4, results from genotoxicity testing for naphthalene are mostly negative. A genotoxic mode of action for carcinogenicity by the naphthalene metabolite, 1,2-naphthoquinone is plausible. Most results in bacterial mutation assays for naphthalene or its metabolites were negative, with the exception of two positive results. 1,2-Naphthoquinone induced reverse mutations in *S. typhimurium* without metabolic activation (Flowers-Geary et al., 1996), and naphthalene induced reverse mutations in *Vibrio fischeri* in the presence of metabolic activation from rat liver enzymes (Arfsten et al., 1994). *In vitro* genotoxicity assays with eukaryotic cells are limited and show positive or negative results in different assays (see Section 4.4.4). These *in vitro* tests include observations that naphthalene metabolites generated from rat liver microsomes induced chromosomal aberrations or sister chromatid exchanges in Chinese hamster ovary cells (NTP, 1992a), and that 1,2- and 1,4-naphthoquinone induced sister chromatid exchanges in human leukocytes (Wilson et al., 1996). The potential genotoxicity of 1,2-naphthoquinone is supported by *in vitro* findings that 1,2-naphthoquinone formed N7 adducts with deoxyguanosine (McCoull et al., 1999) and caused DNA strand scission in the presence of NADPH and copper via reactive oxygen species (Flowers et al., 1997). Yu et al. (2002) showed that 1,2-naphthoquinone was capable of inactivating the p53 tumor suppressor gene in a yeast reporter system in the presence of copper and a reducing agent. Although copper is not present in high concentrations in the cytoplasm of cells, it is present on nucleoprotein

complexes and is associated with DNA bases (Bryan et al., 1981; Gierestanger et al., 1991). Limited *in vivo* genotoxicity assays with naphthalene provide both negative or positive results for naphthalene genotoxicity (including a report that naphthalene induced wing spot mutations in *Drosophila melanogaster*).

Information on the carcinogenicity of 1- and 2-methylnaphthalene is included for informational purposes due to the similarity in chemical structure. 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. As discussed in Section 4.4.2, the limited evidence for methylnaphthalene carcinogenicity in mice is not considered to add supporting evidence for the carcinogenicity of naphthalene, because of likely differences in the mode of action by which methylnaphthalene and naphthalene may affect the lung.

4.6.3. Mode of Action Information

Naphthalene exposure is know to result in cellular toxicity as demonstrated by intraperitoneal administration of naphthalene resulting in cellular swelling, and vacuolization and necrosis of Clara cells in mice but not rats (Plopper, 1992). The same study (Plopper et al., 1992) demonstrated that naphthalene was cytotoxic to the olfactory epithelium of both rats and mice but the cytotoxicity was seen at higher doses in mice, suggesting increased sensitivity of the rat olfactory epithelium. As discussed in Section 3.3. and a recent review by Buckpitt et al. (2002), there is considerable evidence to indicate that the cytotoxic action of naphthalene in mouse lung epithelial tissue and mouse and rat nasal epithelial tissue involves the inherent electrophilicity of reactive metabolic intermediates (e.g., naphthalene1-2-oxide, 1,2-naphthoquinone, or 1,4-naphthoquinone) or their propensity to generate reactive oxygen species (e.g., from 1,2-naphthoquinone), but the relationship of naphthalene cytotoxicity to the mode of tumor formation is uncertain.

In nasal epithelial tissue of chronically exposed male and female F344/N rats, a number of nonneoplastic lesions were observed in the same tissues in which elevated incidences of nasal tumors were observed (adenomas in the nasal respiratory epithelium and neuroblastomas in the olfactory epithelium) (Abdo et al., 2001; NTP, 2000). The nonneoplastic lesions included atypical (basal-cell) hyperplasia, atrophy, chronic inflammation and hyaline degeneration of the olfactory epithelium; hyperplasia, squamous metaplasia, hyaline degeneration and goblet-cell hyperplasia of the respiratory epithelium; and glandular hyperplasia and squamous metaplasia of the glandular epithelium. With the exception of the atypical hyperplasia, these nonneoplastic lesions have been observed in previous inhalation studies of chemicals that are nasal irritants and appear to be adaptive responses that were not associated with the development of tumors (NTP, 2000). In contrast, atypical olfactory epithelial hyperplasia was the principal nonneoplastic lesion observed in naphthalene-exposed F344/N rats. These lesions were morphologically similar to the neoplastic lesions and appeared to form a morphologic continuum with the

neuroblastomas. This suggests that the atypical hyperplasia may represent a precursor event for nasal olfactory carcinogenesis (Long et al., 2003). Neuroblastomas of the nasal olfactory epithelium are rare neoplasms in rodents (Pino et al., 1999) and humans (McElroy et al., 1998). The induction of these neoplasms has been shown to occur in a limited number of chemical exposure studies. In some of these studies, the induction of nasal neoplasms occurred in conjunction with olfactory epithelial nonneoplastic lesions (squamous and respiratory metaplasia, basal-cell hyperplasia, and glandular hyperplasia). However, the association between these lesions and the development of neuroblastomas is not clear because in other studies similar lesions occurred without the development of neuroblastomas (Miller et al., 1985). Some rats displayed localized proliferation of the respiratory epithelium that was considered morphologically similar to the respiratory epithelial adenomas but were included in the respiratory epithelial hyperplasia classification (Long et al., 2003). Likewise, B6C3F1 mice chronically exposed to naphthalene did not develop elevated incidences of respiratory epithelial adenomas or olfactory epithelial neuroblastomas, but nearly all exposed mice showed olfactory epithelial metaplasia and respiratory epithelial hyperplasia (NTP, 1992a).

Current information is inadequate to identify which metabolites may be responsible for nonneoplastic nasal lesions that develop in rodents following chronic inhalation exposure, or to explain the tendency for rats to develop nasal tumors while mice develop lung tumors following exposure to naphthalene. These distinct species differences could be due to site-specific metabolism in rats versus mice or anatomic differences in the nasal passages of the two species (Long et al., 2003). Species differences in tumor development could also be due to different rates of production and elimination of carcinogenic metabolites by the nasal epithelia and lungs. Rodents and humans are known to display distinct differences in nasal anatomy and respiratory physiology that may cause different deposited doses, and subsequently different responses, in human nasal tissue relative to rats or mice. However, the anatomical and physiological differences alone may be insufficient to rule out the relevance of naphthalene-induced nasal lesions in rats. There is evidence that both rodents and humans have enzymes in nasal tissues that are capable of transforming naphthalene to reactive metabolites (Buckpitt et al., 1992, 2002; Plopper, 1992; Thornton-Manning and Dahl, 1997). Gervasi et al. (1991) described activities of xenobiotic metabolizing enzymes (CYPs, epoxide hydrolase and several phase II enzymes) in nasal epithelium taken from 10 human subjects and found a wide variation. CYP enzymes have been identified in all regions of the nasal mucosa of rodents, dogs and monkeys, with the olfactory tissue having the highest level (Dahl et al., 1982). Baldwin et al. (2004) have shown 10- and 20-fold differences in CYP2F expression in primate nasal tissues versus tissues in rats and mice, indicating primates may be less sensitive to naphthalene-induced toxicity than rodents.

In contrast to the lack of mechanistic understanding of why naphthalene-exposed rats develop nasal tumors and mice do not, the relative susceptibility of mice, compared with rats, to acute Clara cell cytotoxicity (Buckpitt et al., 1992; West et al., 2001) and chronic lung inflammation and lung tumor development (NTP, 1992a; 2000) has been correlated with higher rates of formation of specific enantiomeric epoxide derivatives of naphthalene in mice compared with rats (Buckpitt et al., 1992, 1995). Although there is evidence that formation of naphthalene epoxides by human lymphoblastoid cell microsomes (Lanza et al., 1999) shows stereoselective

preference for formation of the 1*S*-2*R*-oxide, as opposed to the 1*R*-2*S*-oxide formed by mouse lung microsomes (Buckpitt et al., 1987), the possibility of species differences in downstream metabolic capabilities precludes using this information to conclude that humans are more like rats than mice in susceptibility to the development of lung tumors. For example, human liver microsomes have been reported to convert naphthalene to the dihydrodiol intermediate at faster rates than mouse and rat liver microsomes (Kitteringham et al., 1996). A possible consequence of this species difference is that human tissue may form relatively greater amounts of 1,2-naphthoquinone from the dihydrodiol via dihydrodiol dehydrogenase. The observation that 1,2-naphthoquinone was the predominant naphthalene metabolite covalently bound to proteins from Clara cells incubated *in vitro* with naphthalene (Zheng et al., 1997) provides some evidence that naphthalene lung cytotoxicity may not only involve epoxide metabolites. The available mechanistic data are currently inadequate to determine which metabolites are responsible for the development of naphthalene-induced lung tumors or to preclude the relevance of the mouse lung tumors to humans.

4.7. SUSCEPTIBLE POPULATIONS

4.7.1. Possible Childhood Susceptibility

Children, especially neonates, may be more susceptible to acute naphthalene poisoning based on the number of reports of lethal cases in children and infants (Gerarde, 1960; Santucci and Shah, 2000; U.S. EPA, 1987). Valaes et al. (1963) proposed that the possible susceptibility of children to naphthalene-induced hemolytic anemia may be due to immaturity of the detoxication pathways responsible for the conjugation and excretion of naphthalene metabolites. The role of metabolites in hemolytic anemia has not been ascertained with respect to childhood susceptibility.

A recent study of possible associations between maternal indoor exposure to 28 volatile organic chemicals (including naphthalene) and putative immune status at birth assessed by cord-blood levels of cytokine-producing T cells, [interleukin-4 (IL-4), interleukin-2 (IL-2), interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α)], reported an association between increased exposure to naphthalene and increased maternal cord-blood levels of the T cell cytokine, IL-4 (Lehmann et al., 2002). Levels of 28 volatile organic chemicals in air samples collected during a 4-week postnatal period in bedrooms of 85 newborn children were measured as a surrogate index of maternal indoor exposure to volatile organic chemicals. A logistic regression analysis which adjusted for possible confounding factors of family allergic (i.e., atopic) history and maternal smoking during pregnancy found an elevated odds ratio (OR=2.9; 95% CI 1.0-8.2) for elevated naphthalene air concentrations (>75th percentile) and elevated percentage of IL-4-producing T cells in cord blood. The significance of the observed variations in cord blood T cell levels to the immune status of the newborn children is unknown. The findings from this study are inadequate to determine if maternal exposure to naphthalene may influence the immune status of newborn children.

Comparison of naphthalene-induced lung injury in neonatal mice (7 and 14 days after birth) and adult mice indicate that neonatal mice may be more susceptible to single intraperitoneal doses of 0, 25, 50, or 100 mg/kg naphthalene (Fanucchi et al., 1997a). Epithelial damage in terminal bronchioles (principally in the Clara cells) was observed at the lowest dose tested in 7-day old mice, but was absent in adult mice at the same dose level. In adult mice exposed to 50 mg/kg, injury was mild and variable (from mouse to mouse) and only became consistent with exposure to 100 mg/kg. Epithelial damage in 14-day-old mice was less severe than the damage in 7-day-old mice. Activities of CYP-mediated (CYP2F2) naphthalene metabolism in bronchiolar tissues were 2.5 times lower in neonatal mice than in adult mice (Fanucchi et al., 1997b), suggesting that the difference in susceptibility is not explained by differences in ability to form reactive metabolites alone (e.g., naphthalene-1,2-oxide). Differences between neonates and adults in the balance between formation of reactive naphthalene metabolites and downstream metabolic transformations could potentially explain the difference in susceptibility to naphthalene toxicity, but the possibilities for specific, age-related differences in downstream enzyme activities for naphthalene (e.g., epoxide hydrolase, dihydrodiol dehydrogenase) have not been studied to date. Alternatively, toxicodynamic differences may exist between neonatal and adult mice exposed to naphthalene (e.g., different target macromolecules). Based on findings that *in utero* exposure to other chemicals which are bioactivated by CYP enzymes caused Clara cell tumors in adult offspring, Fanucchi et al. (1997a) postulated that naphthalene exposure during the neonatal period, when increased susceptibility to naphthalene-induced cytotoxicity occurs, may lead to loss of regulatory mechanisms resulting in Clara cell proliferation and tumor formation in adult animals. Direct evidence in support of this hypothesis is not available (e.g., demonstration that *in utero* or neonatal naphthalene exposure will cause an increased incidence of lung tumors in adult mice). Green et al. (2000) reported a 600-fold less capacity of the human lung to metabolize trichloroethylene than that in the mouse and indicated the human lung differs markedly from the mouse lung in the number and morphology of its Clara cells. Thus, species differences in the number and morphology of lung Clara cells may play a role in any potential differential toxicity.

The initial step in naphthalene metabolism involves several of the CYP enzymes including 1A1, 1B1, 3A7, 3A5, 2E1 and 2F1. Levels of these enzymes change during development and may not reach adult levels until later in development. CYP1A2, 3A4, 2E1 and 2D6 tend to be at low levels at birth and increase slowly within the first year following birth, usually reaching levels closer to adult values by one year of age (Cresteil, 1998). However, CYP3A7 and CYP4A are found at high levels in fetal liver and peak at 1-7 days following birth, slowly declining during development. CYP2F may play an important role in the metabolism of naphthalene in animals and humans, however, there is currently no data on developmental levels of this isozyme. Overall, varying CYP levels may alter the metabolism of substrates, thereby altering the toxicity seen in children following exposure to certain chemicals.

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 may be involved in the conjugation of naphthalene metabolites. These deficits could 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 from naphthalene exposure have not been found in animal studies involving repeated inhalation or oral exposure to naphthalene. Males and females displayed equivalent incidences of nonneoplastic nasal lesions in mice and rats and nonneoplastic lung lesions in mice exposed by inhalation to naphthalene for 2 years (Abdo et al., 2001; NTP, 1992a, 2000). A statistically significant increased incidence of alveolar/bronchiolar adenomas and carcinomas compared with controls was found in female mice exposed to 30 ppm naphthalene by inhalation (NTP, 1992a). Incidences of adenomas were also elevated in male mice in the two exposure groups (10 and 30 ppm), but not to a statistically significant degree. Female incidences were 5/69, 2/65, 29/135 for the 0-, 10-, and 30-ppm groups; and male incidences were 7/70, 17/69, and 31/135, respectively (NTP, 1992a). In rats, both males and females exposed to naphthalene for 2 years showed statistically significant increased incidences of naphthalene-induced nasal tumors, although the pattern of the sites of tumors was different in the two sexes. For example, incidences for respiratory epithelial adenomas for male rats were 0/49, 6/49, 8/48, and 15/48 for control through 60-ppm groups and 0/49, 0/49, 4/49, and 2/49 for females, respectively (Abdo et al., 2001; NTP, 2000). Showing a different pattern, incidences for olfactory epithelial neuroblastomas were 0/49, 0/49, 4/48, and 3/48 for male rats and 0/49, 2/49, 3/49, and 12/49 for female rats, respectively. In a 13-week gavage exposure toxicity study in rats, both male and female rats showed exposure-related decreases in body weight, the only toxic effect found in the study (NTP, 1980a). In contrast, Van Winkle et al. (2002) reported results indicating that female mice are more susceptible than male mice to lung injury from acute parenteral exposure to naphthalene.

4.7.3. Other

A relationship may exist between an inherited deficiency in the enzyme, glucose 6-phosphate dehydrogenase (G6PD), and susceptibility to naphthalene-induced hemolysis (Dawson et al., 1958; Owa, 1989; Owa et al., 1993; Shannon and Buchanan, 1982; Santucci and Shah, 2000; Valaes et al., 1963).

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. Thus, in the absence of 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 3 (Section 4.5) identify body weight decreases as the most appropriate critical effect for the purpose of RfD derivation. 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 data base 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. Alternatives for Deriving an 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 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 data base 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, 1992a), 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 4). 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 < ½ of mice exposed at 10 and 30 ppm, respectively (Table 4). 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.

Table 4. 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

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 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 4). 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 data base 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 Deriving an RfC

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 data base 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

5.3.1. Choice of Study/Data - with Rationale and Justification

As discussed in Section 4.6, naphthalene is likely to be carcinogenic to humans by the inhalation route of exposure based on increased incidences of rare nasal tumors (olfactory epithelial neuroblastomas and respiratory epithelial adenomas) in rats exposed to naphthalene vapor concentrations of 10, 30, or 60 ppm for 2 years (Abdo, 2001; NTP, 2000), increased incidences of alveolar/bronchiolar adenomas or carcinomas in female mice exposed to 30 ppm for 2 years (NTP, 1992a), and increased numbers of tumors in tumor-bearing A/J strain mice exposed to 10 or 30 ppm for 6 months (Adkins et al., 1986). Evidence from human studies is inadequate to determine naphthalene carcinogenicity. There are no human data and inadequate laboratory animal data to determine the carcinogenicity of naphthalene by oral or dermal routes of exposure.

All tumors from the NTP (1992a, 2000) studies were considered relevant for the determination of the inhalation unit risk. Tumor incidences are indicated in Table 5 below. According to the Draft Revised Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999), benign tumors that are not observed to progress to malignancy should be considered on a case-by-case basis. The respiratory epithelial adenomas observed following inhalation exposure were dependent on the concentration of naphthalene. They are rare tumors that were not observed in the study controls or the NTP historical control database. A number of nonneoplastic lesions were observed in rats that developed tumors, including hyperplasia, squamous metaplasia, hyaline degeneration and goblet-cell hyperplasia of the respiratory epithelium. These lesions have been observed in previous inhalation studies of chemicals that are nasal irritants and may be adaptive responses not associated with tumor development (NTP, 2000). Note, however, that similar nonneoplastic lesions were found in B6C3F1 mice which did not develop nasal tumors. Thus, the mechanism of adenoma formation in rats is unknown. For these reasons, the adenomas in rats were considered relevant to humans and were included in the quantitative analyses.

Tumor responses for olfactory epithelial neuroblastomas in male rats and respiratory epithelial adenomas in female rats were not statistically significant. The responses in these animals were, however, modeled and included in the estimation of risk since: (1) the occurrence of neuroblastomas in female rats and adenomas in male rats was statistically significant; and (2) these tumors types are rare and not seen in historical NTP controls.

Table 5. Incidences of neoplastic lesions in the lung of male and female B6C3F1 mice (NTP, 1992a) and the nose of male and female F344/N rats (Abdo et al., 2001; NTP, 2000) exposed to naphthalene 6 hours/day, 5 days/week for up to 105 weeks

	Concentration (ppm)							
	Males				Females			
Lesion	0	10	30	60	0	10	30	60
alveolar/bronchiolar adenomas and carcinomas in mice	7/70	17/69	31/135	----	5/69	2/65	29/135*	----
respiratory epithelial adenoma in rats	0/49†	6/49*	8/48*	15/48*	0/49	0/49	4/49	2/49
olfactory epithelial neuroblastoma in rats	0/49†	0/49	4/48	3/48	0/49†	2/49	3/49	12/49*

* Significantly ($p<0.05$) different from control value by the Poly-3 test, which adjusts for intercurrent mortality
† Significant ($p<0.05$) trend by the Poly-3 test

5.3.2. Calculation of Inhalation Unit Risk

Note: EPA recognizes that the derivation of the human equivalent concentration differs in the proposed inhalation cancer assessment and the existing RfC assessment. EPA may revisit the RfC assessment in the future.

5.3.2.1. Quantal Analyses

Multistage models (i.e., polynomial exponential models) were fit to the tumor incidence data shown in Table 5 using the Benchmark Dose Modeling Software (version 1.3.1) (U.S. EPA, 2003). The maximum likelihood estimates of the benchmark doses (BMCs) and 95% lower bounds (BMCLs) for 10% extra risk for each tumor are provided, consistent with the BMDS Technical Guidance (U.S.EPA, 2000c) and the Draft Revised Guidelines for Carcinogen Risk Assessment (U.S.EPA, 1999). $BMCL_{10}$s from the best-fitting models for each data set were considered as potential points of departure for an inhalation cancer unit risk estimate for inhaled naphthalene in humans (Table 6). For each data set, a one-degree polynomial provided an adequate ($p>0.1$) fit as assessed with a chi-square goodness of fit test. Higher degree models (up to 3-degree polynomial models) did not show improved fit to the data. The incidence rate for

adenoma formation in female mice is less than that for respiratory epithelial adenomas in male rats and olfactory epithelial neuroblastomas in female rats. In addition, data from female mice (alveolar/bronchiolar adenomas and carcinomas) yielded a $BMCL_{10}$ that was higher than that observed for respiratory epithelial adenomas in male rats and was not considered further.

Olfactory epithelial neuroblastomas and respiratory epithelial adenomas are considered rare tumors in the NTP tumor data base. Neither tumor type occurred in the chamber controls nor have they been observed in the NTP historical control data bases (NTP, 2000). The olfactory neuroblastomas are considered malignant neoplasms. The neuroblastomas tended to occur earlier than the adenomas. In addition, the neuroblastomas originate from a different cell type than the adenomas. The summation of risks from multiple tumor sites when tumor formation occurs independently in different organs or cell types is considered superior to the calculation of risk from individual tumor sites alone. Consequently, to further characterize the tumor incidence data from male and female rats, the distribution of unit risks ("q1s") for respiratory epithelial adenomas and the distribution of unit risks for olfactory neuroblastomas as indicated in Table 6 were added to obtain a single distribution of unit risks for developing either respiratory epithelial adenomas or olfactory epithelial neuroblastomas, for both male and female rats. More complete descriptions of modeling results are found below.

The distribution of unit risks ("q1s") for respiratory epithelial adenomas and the distribution of unit risks for olfactory neuroblastomas were added to obtain a single distribution of unit risks for developing either respiratory epithelial adenomas or olfactory epithelial neuroblastomas. Based on the assumption that each MLE unit risk estimate (i.e., q1 in Table 6 above) is the mean value of a normal distribution of unit risks with a standard deviation of σ, values of σ for distributions of unit risks for adenomas alone or neuroblastomas alone were calculated with the following equation :

Equation 1: 95% upper confidence limit on q1= q1*= q1 + 1.645 σ.

Under the rules for adding normal distributions, the parameters of the distribution of unit risks for developing adenomas or neuroblastomas ($q1_{combined}$ and $\sigma_{combined}$) were calculated as follows:

Equation 2: $q1_{combined} = q1_{REA}+q1_{OEN}$

$q1_{combined, male} = 8.45 \times 10^{-3}$ per ppm

$q1_{combined, female} = 5.06 \times 10^{-3}$ per ppm

Equation 3: $\sigma_{combined} = [\sigma_{REA}^2+\sigma_{OEN}^2]^{0.5}$

$\sigma_{combined, male} = 1.59 \times 10^{-3}$

$\sigma_{combined, female} = 1.23 \times 10^{-3}$.

Table 6. Results from modeling of incidence data for lung tumors (alveolar/bronchiolar adenomas and carcinomas, ABAC) in female mice and nasal tumors (respiratory epithelial adenomas, REA; olfactory epithelial neuroblastomas, OEN) in rats exposed by inhalation to naphthalene for 105 weeks (Abdo et al., 2001; NTP, 1992a, 2000).

Tumor type	Model degree†	chi-square p-value	Model parameters				Model-predicted exposure levels (and lower 95% confidence limits) associated with 10% extra risk .	
			MLE q0	MLE q1 (risk/ppm)	95%UCL q1 (i.e., q1*) (risk/ppm)	SD (σ) of q1	BMC_{10} (ppm)	$BMCL_{10}$ (ppm)
Female mice ABAC	2	0.1472	0.053	0	NA	NA	22.30	16.80
Male rat REA	1	0.2575	0	$6.95x10^{-3}$	$9.30x10^{-3}$	$1.43x10^{-3}$	15.16	11.32
Female rat REA	1	0.1287	0	$1.26x10^{-3}$	$2.30x10^{-3}$	$6.32x10^{-4}$	83.92	45.86
Male rat OEN	1	0.2417	0	$1.50x10^{-3}$	$2.64x10^{-3}$	$6.93x10^{-4}$	70.11	39.91
Female rat OEN	1	0.4433	0	$3.80x10^{-3}$	$5.53x10^{-3}$	$1.05x10^{-3}$	37.63	20.90

† The degree polynomial which gave the best fit as assessed by p-value for the chi-square goodness-of-fit test (p>0.1 was taken as an adequate fit). Model form (where d = exposure level in ppm): $P(d)= 1-exp(-q0-q1d-q2d^2.....)$.
NA = not applicable

The 95th percentile of the combined distribution of unit risks for developing respiratory epithelial adenomas or olfactory neuroblastomas was calculated. The 95th percentile values of the unit risk for developing adenomas or neuroblastomas in male rats ($q1^*_{combined, male}$= 11.1x10^{-3} per ppm) and female rats ($q1^*_{combined, female}$=7.1x10^{-3} per ppm) were calculated using Equation 1. In the absence of more specific biological reasoning for selection of a particular risk, the highest risk, 11.1x10^{-3} per ppm for the sum of nasal tumor risks in male rats, was selected as the point of departure for deriving an inhalation cancer unit risk estimate for naphthalene using a quantal analysis.

Naphthalene-related effects on the nasal epithelium are expected to result following direct exposure and subsequent metabolism in nasal tissues and indirect exposure due to absorption and metabolism in other tissues (e.g., liver). As discussed in Sections 3.3 and 4.6.3, respiratory effects are likely due to reactive naphthalene metabolites formed either within the affected tissue or as a result of systemic metabolism. Currently available PBPK models for naphthalene do not include nasal compartments that metabolize naphthalene, because specific data are not available on nasal deposition and epithelial absorption of naphthalene (NTP, 2000; Willems et al., 2001). Without such data, reliable models for nasal deposition, tissue dosimetry, and nasal-tissue metabolism cannot be developed for naphthalene. In the absence of appropriate naphthalene-specific PBPK models for humans and rats, default dosimetric equations (U.S. EPA, 1994b) for both category 1 and category 3 gases were initially considered for converting rat exposure levels to human equivalent concentrations.

If the nasal effects from inhalation exposure to naphthalene are considered to be extrarespiratory effects (category 3 gas), as defined in the U.S. EPA guidance for deriving RfCs (U.S. EPA, 1994b), risks would be adjusted to a mg/m^3 basis, adjusted to a continuous exposure basis, and converted to human equivalent risks by multiplying by the ratio of mouse:human blood/gas partition coefficients. Because the blood/gas coefficients for naphthalene are not available, the default ratio of 1 would be used. Using the category 3 gas equation, the sum of nasal tumor risks in male rats was converted to a human equivalent risk:

Category 3 unit risk = (11.1 x10^{-3} per ppm) x 7/5 (days) x 24/6 (hours) / 5.2 [(mg/m^3)/ppm]
= 1.2 x 10^{-2} per mg/m^3.

If the nasal effects are considered to be extrathoracic respiratory effects (category 1 gas), U.S. EPA (1994b) guidance specifies that the experimental exposure concentrations would be adjusted to a mg/m^3 basis, adjusted to a continuous exposure basis, and converted to human equivalent risks by multiplying by the ratio of regional deposited doses in the extrathoracic (ET) region for the experimental animal and humans as follows:

Category 1 unit risk = (11.1 x10^{-3} per ppm) x 7/5 (days) x 24/6 (hours) x 1/0.132 RGDR
= (11.1 x10^{-3} per ppm) / 0.1236
= 9 x 10^{-2} per mg/m^3,

where $RGDR_{ET}$ = regional gas dose ratio in the extrathoracic (ET) region

$= (DoseET)_A/(DoseET)_H$
$= [\text{minute volume/ETsurface area}]_A \div [\text{minute volume/ETsurface area}]_H$;
Reference minute volumes (L/min): 13.8 human, 0.137 rat;
Reference ET surface area (cm^2): 200 human, 15 rat;
$RGDR_{ET}$(Rat to Human) = [0.137/15]÷[13.8/200] = 0.132.

The corresponding maximum likelihood estimate of risk for a category 1 gas is 8.45×10^{-3} per ppm/ 0.1236, or 7×10^{-2} per mg/m^3.

The category 1 gas dosimetric equation was selected as the appropriate method for dose conversion because naphthalene is reactive in the nasal cavity. Lee et al. (2004) demonstrated that a limited region of the olfactory mucosa was injured following inhalation exposure to naphthalene and this area of injury was correlated with nasal airflow patterns as well as *in situ* metabolism of naphthalene. In addition, enzymes capable of transforming naphthalene to reactive metabolites exist in these tissues in rodents and humans (Buckpitt et al., 1992, 2000; Plopper et al., 1992a; Thornton-Manning and Dahl, 1997). Thus, the recommended risk estimates resulting from the quantal dose-response modeling are 9×10^{-2} per mg/m^3 (unit risk) and 7×10^{-2} per mg/m^3 (central tendency), for extra risk of nasal tumors in male rats.

5.3.2.2. Time-to-Tumor Modeling

In addition to a quantal analysis as described above, the characteristics of the dose-response relationships for the male and female rat respiratory epithelial adenomas and male and female nasal olfactory neuroblastomas were assessed through time-to-tumor analyses, in order to adjust for competing mortality and differing time courses of tumor incidence with increasing dose. The times of observation of these tumors are presented in Appendix C. Note that the neuroblastomas tended to occur earlier than the adenomas, suggesting that the adenomas are not precursors to the neuroblastomas, and mostly occur independently of the neuroblastomas; only one high dose female and mid-dose male were observed with both tumors.

The general model used for the time-to-tumor (or time-to-response) analyses is the multistage-Weibull model, which has the form

$$P(d,t) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)*(t - t_0)^z]$$

where P(d,t) represents the probability of a tumor (or other response) by age t (in bioassay weeks) for dose d (i.e., human equivalent exposure), and parameters $z \geq 1$, $t_0 \geq 0$, and $q_i \geq 0$ for i = 0, 1, ..., k, where k = the number of dose groups - 1. The parameter t_0 represents the time between when a potentially fatal tumor becomes observable and when it causes death (see below). The analyses were conducted using the computer software TOX_RISK version 5.2 (K.S. Crump Group, 2000-2001), which is based on Weibull models drawn from Krewski et al. (1983). Parameters are estimated using the method of maximum likelihood. Note that it was not necessary to adjust the administered dose for lifetime exposure prior to modeling, because the

software program characterizes the tumor incidence over time from which it provides an extrapolation to lifetime exposure.

The tumor types were categorized by tumor context as either fatal or incidental tumors, in order to adjust appropriately for competing risks. Incidental tumors are those tumors thought not to have caused the death of an animal, while fatal tumors are thought to have resulted in animal death. The neuroblastomas were treated as fatal tumors unless observed at the terminal sacrifice, in which case they were considered incidental. The parameter t_0 was set equal to 0, as there were insufficient data to reliably estimate t_0. The work of Portier et al. (1986) in analyzing tumor types in NTP historical controls lends support to these tumor context assumptions.

Specific n-stage Weibull models were selected for the individual tumor types for each sex based on the values of the log-likelihoods according to the strategy used by EPA (U.S. EPA, 2002). If twice the difference in log-likelihoods was less than a chi-square with degrees of freedom equal to the difference in the number of stages included in the models being compared, the models were considered comparable and the most parsimonious model (i.e., the lowest-stage model) was selected.

The results of applying the multistage-Weibull data to the time-to-tumor data are summarized in Table 7, and the model output and dose-response curves are provided in Appendix C. The maximum likelihood estimates of the benchmark doses (BMCs) and 95% lower bounds (BMCLs) for 10% extra risk for each tumor are provided, consistent with the BMDS Technical Guidance (U.S.EPA, 2000) and Guidelines for Carcinogen Risk Assessment (U.S.EPA, 1999). In general, site- and sex-specific $BMCL_{10}$s using the time-to-tumor approach were approximately 20% higher than those calculated using the quantal approach.

Unit risks using the time-to-tumor approach were calculated by dividing the response rate of 0.10 (10%) by the $BMCL_{10}$. The highest unit risk for individual tumors was 0.10/15.2 ppm = 6.6×10^{-3} $(ppm)^{-1}$, corresponding to neuroblastomas in female rats. In terms of human equivalent continuous exposure, the corresponding unit risk is

$$6.6 \times 10^{-3} \text{ (ppm)}^{-1} \times 7/5 \text{ (days)} \times 24/6 \text{ (hours)} \times 1/0.132 \text{ RGDR} = 0.28 \text{ per ppm},$$

where the RGDR was derived in the previous section (category 1 gas). Also as derived in the previous section, one ppm of naphthalene is equivalent to 5.2 mg/m^3. Hence, this inhalation unit risk can be expressed as

$$\text{Unit risk} = 0.28 \text{ (ppm)}^{-1} / 5.2 \text{ [(mg/m}^3\text{)/ppm]} = 5.4 \times 10^{-2} \text{ per mg/m}^3.$$

This unit risk should not be used with continuous human exposures greater than 2.5 ppm (the exposure corresponding to 10% extra risk of neuroblastomas, adjusted to a human equivalent exposure assuming category 1 properties), or 13 mg/m^3. Above this level, the dose-response is not linear, and the modeled dose-response relationship should be used to estimate risk.

Reliance on single tumor sites may underestimate the carcinogenic potential of naphthalene by the inhalation route (see Section 5.3.2.1). Thus, time-to-tumor modeling was applied to calculate the summed risk for multiple tumor types in rats. Accordingly, a statistically appropriate upper bound risk was estimated using the following steps. Summing the cancer risks in this manner, after the dose-response for each tumor site has been evaluated, is superior to EPA's previous practice of carrying out one dose-response analysis of tumor-bearing animals. The primary reason is that the biological relevance of the multistage model is maximized, by allowing different multistage models to be fit to qualitatively different tumor types which might not be expected to develop through exactly the same modes of action. The time courses of the tumor types evaluated here did vary, for example.

First, the central tendency, or maximum likelihood estimates (MLE) of unit potency for each tumor type were summed for male and for female rats. Then an estimate of the 95% upper bound on the summed unit risk was calculated by assuming a normal distribution for the individual risk estimates, and deriving the variance of the risk estimate for each tumor site from its 95% upper confidence limit (UCL) according to the formula

$$95\%\ \text{UCL} = \text{MLE} + 1.645 \cdot \text{s.d.},$$

where 1.645 is the t-statistic corresponding to a one-sided 95% confidence interval and >120 degrees of freedom, and the standard deviation (s.d.) is the square root of the variance of the MLE. The variances were summed across tumor sites to obtain the variance of the sum of the MLE. The 95% UCL on the sum of the individual MLEs was calculated from the variance of the sum using the same formula.

For the male rats, the summed unit risk was 1.5×10^{-2} per ppm, and the summed central tendency risk was 1.1×10^{-2} per ppm. In terms of human equivalent exposures in mg/m^3, the summed risks were 12.1×10^{-2} per mg/m^3 (upper bound) and 8.9×10^{-2} per mg/m^3 (central tendency). Both estimates were approximately 35% higher than the summed risks resulting from the quantal dose-response modeling. Results for female rats were proportionately similar, and are not presented.

Table 7. Estimation of benchmark concentration (BMC) associated with an extra risk of 10%, lower 95% confidence limits (BMCL), and estimated model parameters; using multistage-Weibull time-to-tumor modeling, for animals exposed by inhalation to naphthalene (NTP, 2000).

Sex, Species	Tumor type	Parameter estimates[a]	Point of Departure		Unit Risk[b]	Human Equivalent Unit Risk[c]
			BMC_{10} (ppm)	$BMCL_{10}$, (ppm)	$(ppm)^{-1}$	$(ppm)^{-1}$
Male Rats	Olfactory epithelial neuroblastomas	$q_1 = 4.2 \times 10^{-18}$ $z = 7.3$	57.3	34.2	2.9×10^{-3}	3.3×10^{-3}
	Respiratory epithelial adenomas	$q_1 = 8.5 \times 10^{-13}$ $z = 5.0$	10.9	8.0	1.3×10^{-2}	1.5×10^{-2}
Female Rats	Olfactory epithelial neuroblastomas	$q_1 = 7.5 \times 10^{-15}$ $z = 5.7$	22.1	15.2	6.6×10^{-3}	7.6×10^{-3}
	Respiratory epithelial adenomas	$q_1 = 1.2 \times 10^{-10}$ $z = 3.5$	67.1	35.0	2.9×10^{-3}	3.3×10^{-3}

[a] All tumor incidence data were fit using the multistage-Weibull model: $P(d) = 1 - \exp[(-q_0 - q_1 d - \ldots - q_6 d^6)t^z]$. Parameters not specified were estimated to be zero.
[b] Unit risks are reported in terms of the administered bioassay exposures, and have not been adjusted to human equivalent continuous exposure levels.
[c] Adjusted for continuous exposure by dividing by 5/7 (days) x 6/24 (hours) and for human equivalent exposure by dividing by RGDR = 0.132.

5.3.3. Rationale for Extrapolation Method

According to the Draft Revised Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999), a linear mode of extrapolation to low doses was selected (i.e., linear extrapolation to the origin from the point of departure) because mode of action data are incomplete and inadequate to invoke a nonlinear low-dose extrapolation. Naphthalene has been negative in most tests of genotoxicity. Tissue sites of nonneoplastic cellular damage (bronchoalveolar region in mice) show some correlation with tissue sites of carcinogenicity (lung tumors in mice, but not rats), suggesting that naphthalene metabolites may act by a nongenotoxic mode of action involving sustained cellular proliferation following cellular damage. However, an understanding of the mode of action is inadequate for determining why rats, but not mice, develop tumors originating in nasal epithelial tissue, even though both species show nonneoplastic lesions in nasal epithelial tissue following inhalation exposure to naphthalene. In addition, currently available chronic inhalation data do not provide indications of the shapes of dose-response relationships for naphthalene-induced nonneoplastic nasal lesions in rats or mice. At the lowest exposure level in both chronic inhalation studies, 10 ppm, nearly all mice and rats showed nonneoplastic lesions in nasal epithelial tissue (Abdo et al., 2001; NTP, 1992a; 2000). Other intermediate precursor events have not been identified.

Support for a possible genotoxic mode of carcinogenic action includes evidence for genotoxicity of the naphthalene metabolite, 1,2-naphthoquinone (Flowers et al., 1997; Flowers-

Geary et al., 1996; McCoull et al., 1999; Wilson et al., 1996), several positive genotoxicity results for naphthalene in the presence of metabolic activation (Arfsten et al., 1994; Delgado-Rodriquez et al., 1995; NTP, 1992a), and evidence that nasal epithelial tissue contains enzymes capable of transforming naphthalene to 1,2-naphthoquinone (Buckpitt et al., 1992, 2000; Plopper et al., 1992a; Thornton-Manning and Dahl, 1997). Available evidence in support of a nongenotoxic mode of action is inadequate to preclude a mode of action involving direct covalent modification of DNA by 1,2-naphthoquinone or indirect DNA damage via reactive oxygen species from redox cycling involving the *ortho*-quinone group of 1,2-naphthoquinone. Other nongenotoxic modes of action, e.g., protein modification and resultant alterations in cell signaling, are possible but likewise lack experimental evidence.

5.3.4. Oral Slope Factor and Inhalation Unit Risk

An inhalation unit risk of 0.1 per mg/m^3, based on time-to-tumor modeling and a summed risk for respiratory epithelial adenomas and olfactory epithelial neuroblastomas in male rats, was chosen as the best estimate of human risk. This value is approximately 35% higher than the summed unit risk resulting from the quantal dose-response modeling of the same data.

There are no human data and inadequate laboratory animal data to determine the carcinogenicity of naphthalene by oral or dermal routes of exposure. Thus, no oral slope factor was derived.

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. It is used in manufacturing phthalate plasticizers, resins, dyes, and insect repellents. Naphthalene has also been used as a moth repellent in the form of balls or flakes and as a bathroom deodorant.

Naphthalene may be absorbed via the gastrointestinal tract, the respiratory tract, and the skin (U.S. EPA, 1987; ATSDR, 1995; see NTP, 2000 for a comparison of absorption via inhalation in rats and mice). The metabolism of naphthalene, as indicated by studies in rodents and humans, results in the formation of several reactive metabolic intermediates (naphthalene-1,2-oxide, 1,2-naphthoquinone, and 1,4-naphthoquinone), which are thought to be involved in the expression of naphthalene toxicity and carcinogenicity. Metabolism of naphthalene has been demonstrated in several tissues, including the liver, lung, nasal epithelium, and ocular tissue. Naphthalene may be eliminated from the body in the urine as metabolites conjugated to glutathione, glucuronic acid, or sulfate.

Health hazards of concern from exposure to naphthalene include hemolytic anemia, cataracts, and respiratory toxicity (both noncancer and cancer effects). Naphthalene metabolites may 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 may experience hemolysis after naphthalene exposure by the inhalation, oral, and dermal routes. 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 may cause cells, particularly erythrocytes, to be vulnerable to reactive naphthalene metabolites. 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 detoxication pathways responsible for the conjugation and excretion of naphthalene metabolites. There are no reports of naphthalene-induced hemolysis in either rats or mice; however, hemolysis has been observed in dogs.

1,2-Naphthoquinone formed in lens tissue is thought to be involved in naphthalene-induced cataracts in rats and rabbits. The enzyme involved in the transformation of the 1,2-dihydrodiol to 1,2-naphthoquinone in lens tissue is thought to be aldose reductase. Support for this hypothesis includes findings that aldose reductase inhibitors prevent cataract formation in

naphthalene-fed rats (Tao et al., 1991a,b; Xu et al., 1992a), dihydrodiol dehydrogenase is apparently absent in rat lens (Greene et al., 2000), and aldose reductase appears to be the only enzyme in rat lens that can transform naphthalene-1,2-dihydrodiol to 1,2-naphthoquinone (Sugiyama et al., 1999).

Toxicity and carcinogenicity studies in rats and mice exposed by inhalation to naphthalene vapors for 2 years have identified nonneoplastic and neoplastic lesions in the respiratory tract as likely health hazards from chronic-duration inhalation exposure (Abdo et al., 2001; NTP 1992a, 2000). With chronic inhalation exposure to concentrations as low as 10 ppm naphthalene, rats and mice developed nonneoplastic lesions in the nose including hyperplasia of respiratory epithelium and metaplasia of olfactory epithelium. In addition, increased incidences of pulmonary inflammation were found in mice of both sexes. Neoplastic lesions in naphthalene-exposed animals included lung tumors in mice and nasal tumors in rats.

Naphthalene is likely to be carcinogenic to humans by the inhalation route of exposure based on: (1) increased incidences of olfactory epithelial neuroblastomas and respiratory epithelial adenomas in F344/N rats of both sexes exposed to naphthalene for 2 years; (2) increased incidences of alveolar/bronchiolar adenomas or carcinomas in female B6C3F1 mice exposed to 30 ppm for 2 years; and (3) increased numbers of tumors in tumor-bearing A/J strain mice exposed to 10 or 30 ppm for 6 months. Evidence from human studies, which consists of a few case series reports of laryngeal cancer in naphthalene purification workers and colorectal cancer in several patients who reported taking an indigenous medication containing naphthalene, is inadequate by itself to determine naphthalene carcinogenicity in humans. The carcinogenic response in rats and mice assumed to be relevant to humans; current mechanistic understanding is inadequate. There are no human data and inadequate laboratory animal data to determine the carcinogenicity of naphthalene by oral or dermal routes of exposure.

The mode of action by which naphthalene affects mouse lung epithelial tissue and mouse and rat nasal epithelial tissue may involve the metabolic intermediates naphthalene-1,2-oxide, 1,2-naphthoquinone or 1,4-naphthoquinone, which may damage tissue macromolecules either directly by their inherent electrophilicity or by the generation of reactive oxygen species. Tissue sites of nonneoplastic cellular damage show some correlation with tissue sites of carcinogenicity suggesting that naphthalene metabolites may act by nongenotoxic modes involving sustained cellular proliferation following cellular damage. This hypothesis is supported by negative results for naphthalene in numerous short-term genotoxicity assays. A possible genotoxic mode of carcinogenic action cannot be discounted and is supported by evidence for genotoxic actions by 1,2-naphthoquinone and several positive genotoxicity results for naphthalene in the presence of metabolic activation.

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 data base 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 data base, including the lack of chronic oral data and the lack of dose-response data for hemolytic anemia in a sensitive animal model.

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 data base 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 data base.

6.2.3. Cancer/Oral and Inhalation

Naphthalene is considered likely to be carcinogenic to humans by the inhalation route of exposure predominantly due to the findings of increased incidences of lung tumors in mice (NTP, 1992a) and nasal tumors in rats (Abdo et al., 2001; NTP, 2000) following 2-year exposures.

Multistage (quantal) models were fit to the incidence data for lung adenomas in female mice and nasal tumors found in the respiratory epithelium or olfactory epithelium of male and female rats exposed to naphthalene for up to 2 years. Concentrations associated with a 10% extra risk for tumors (BMC_{10}s) and their lower 95% confidence limits ($BMCL_{10}$s) were calculated.

BMC_{10}s from the best-fitting models for each data set were considered as potential points of departure for a cancer unit risk estimate for inhaled naphthalene in humans. The summation of risks from multiple tumor sites when tumor formation occurs independently in different organs or cell types is considered superior to the calculation of risk from individual tumor sites alone. Consequently, to further characterize the tumor incidence data from male and female rats, the distribution of unit risks ("q1s") for respiratory epithelial adenomas and the distribution of unit risks for olfactory neuroblastomas were added to obtain a single distribution of unit risks for developing either respiratory epithelial adenomas or olfactory epithelial neuroblastomas, for both male and female rats. Time-to-tumor modeling using the individual animal tumor data was also performed to calculate the risk for individual tumors and summed (or total) nasal tumors. A linear mode of extrapolation to low doses was selected, because available data are inadequate to ascertain a plausible mode of action and there is evidence of a possible genotoxic mode of carcinogenic action involving reactive metabolites.

An inhalation unit risk of 0.1 per mg/m^3, based on time-to-tumor modeling and a summed risk for respiratory epithelial adenomas and olfactory epithelial neuroblastomas in male rats, was chosen as the best estimate of human risk. This value is approximately 35% higher than the summed unit risk resulting from the quantal dose-response modeling of the same data. Results for female rats were proportionately similar.

There are no human data and inadequate laboratory animal data to determine the carcinogenicity of naphthalene by oral or dermal routes of exposure. Thus, no oral slope factor was derived.

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APPENDIX A. EXTERNAL PEER REVIEW OF THE 1998 TOXICOLOGICAL REVIEW OF NAPHTHALENE AND IRIS SUMMARY —SUMMARY OF COMMENTS AND DISPOSITION

The 1998 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 data base 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 data base 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., 1997a). 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 mothballs (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 proposed 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 data base (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 data base? 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 data base (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 C. CANCER DOSE-RESPONSE MODELING RESULTS

TIME-TO-TUMOR MODELING

Table C-1: Incidence by week of nasal tumors in female rats exposed to naphthalene by inhalation (NTP, 2000)

		Neuroblastomas		Adenomas	
Dose Group	Week	Death without tumor	Death with tumor	Death without tumor	Death with tumor
Control	73	1	0	1	0
	75	1	0	1	0
	82	1	0	1	0
	86	1	0	1	0
	87	1	0	1	0
	90	1	0	1	0
	94	2	0	2	0
	96	3	0	3	0
	97	3	0	3	0
	98	1	0	1	0
	99	1	0	1	0
	101	2	0	2	0
	102	1	0	1	0
	104	2	0	2	0
	105	28	0	28	0
10 ppm	63	1	0	1	0
	68	1	0	1	0
	72	1	0	1	0
	73	1	0	1	0
	74	1	0	1	0
	82	1	0	1	0
	83	3	0	3	0
	84	1	0	1	0
	86	1	0	1	0
	87	1	0	1	0
	89	1	0	1	0
	90	1	0	1	0
	91	1	0	1	0
	93	1	0	1	0
	95	2	0	2	0
	97	5	0	5	0
	100	0	1	1	0
	102	1	0	1	0
	103	1	0	1	0
	104	1	1	2	0
	105	21	0	21	0

Table C-1: Incidence by week of nasal tumors in female rats exposed to naphthalene by inhalation (NTP, 2000)

Dose Group	Week	Neuroblastomas		Adenomas	
		Death without tumor	Death with tumor	Death without tumor	Death with tumor
30	63	2	0	2	0
	69	0	1	1	0
	74	1	0	1	0
	83	1	0	1	0
	84	4	0	4	0
	88	1	0	1	0
	90	1	0	1	0
	90	0	1	1	0
	94	2	0	2	0
	95	1	0	1	0
	96	1	0	1	0
	98	1	0	1	0
	100	1	0	1	0
	101	0	1	1	0
	102	1	0	1	0
	103	1	0	0	1
	105	28	0	25	3
60	16	1	0	1	0
	63	0	1	1	0
	64	0	1	1	0
	69	2	0	2	0
	72	1	0	1	0
	77	0	1	1	0
	79	0	1	1	0
	80	0	1	0	1
	83	1	0	1	0
	86	1	0	1	0
	89	0	1	1	0
	90	2	0	2	0
	90	0	1	1	0
	92	2	0	2	0
	94	1	0	1	0
	96	0	1	1	0
	97	1	0	1	0
	98	2	0	2	0
	98	0	1	1	0
	103	1	0	1	0
	104	1	0	1	0
	105	21	3	23	1

Table C-2: Incidence by week of nasal tumors in male rats exposed to naphthalene by inhalation (NTP, 2000)

Dose Group	Week	Neuroblastomas death without tumor	Neuroblastomas death with tumor	Adenomas death without tumor	Adenomas death with tumor
Control	66	2	0	2	0
	68	1	0	1	0
	72	1	0	1	0
	74	1	0	1	0
	82	1	0	1	0
	88	2	0	2	0
	90	1	0	1	0
	91	1	0	1	0
	92	1	0	1	0
	94	1	0	1	0
	95	3	0	3	0
	97	2	0	2	0
	99	1	0	1	0
	100	1	0	1	0
	101	1	0	1	0
	102	3	0	3	0
	104	2	0	2	0
	105	24	0	24	0
10 ppm	56	1	0	1	0
	63	1	0	1	0
	72	1	0	1	0
	73	1	0	1	0
	75	1	0	1	0
	76	1	0	1	0
	79	1	0	1	0
	82	1	0	1	0
	85	1	0	1	0
	86	1	0	1	0
	88	1	0	1	0
	92	3	0	3	0
	93	1	0	1	0
	95	1	0	1	0
	97	2	0	2	0
	98	5	0	4	1
	100	2	0	2	0
	102	1	0	1	0
	104	1	0	1	0
	105	22	0	17	5
30	62	0	1	1	0
	70	1	0	1	0
	75	1	0	1	0
	79	1	0	1	0
	80	1	0	1	0
	81	1	0	1	0

Table C-2: Incidence by week of nasal tumors in male rats exposed to naphthalene by inhalation (NTP, 2000)

Dose Group	Week	Neuroblastomas death without tumor	Neuroblastomas death with tumor	Adenomas death without tumor	Adenomas death with tumor
	82	1	0	1	0
	84	1	0	1	0
	86	1	0	1	0
	88	3	0	3	0
	90	1	0	1	0
	92	0	2	2	0
	93	1	0	1	0
	95	1	0	1	0
	97	0	1	1	0
	98	4	0	3	1
	101	1	0	1	0
	102	1	0	1	0
	104	1	0	1	0
	105	23	0	16	7
60	57	0	1	1	0
	60	1	0	1	0
	67	0	1	1	0
	71	1	0	1	0
	76	2	0	2	0
	77	0	1	1	0
	79	1	0	0	1
	80	1	0	1	0
	81	1	0	0	1
	82	1	0	1	0
	83	1	0	0	1
	86	1	0	1	0
	87	1	0	1	0
	90	1	0	0	1
	91	1	0	1	0
	92	1	0	0	1
	94	1	0	1	0
	95	2	0	2	0
	96	1	0	1	0
	98	2	0	0	2
	101	2	0	2	0
	102	1	0	1	0
	104	1	0	0	1
	105	21	0	14	7

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                                                  Generating Model Fit Table ---
                                        TITLE: Naphthalene, NTP 2000, female rat adenomas

Model: One Stage Weib         Dataset: D:\Program Files\TOX_RISK\naph_aden_f.ttd
   Functional form: 1 - EXP[( -Q0 - Q1 * D ) * (T - T0)^Z]
                                      Maximum Log-Likelihood =  -2.377199e+001

                                                     Parameter Estimates :
                                                        Q 0 = 0.000000E+000
                                                        Q 1 = 1.219963E-010
                                                      Z   = 3.524734E+000
                                                T0  = 0.000000E+000   Set by User


              Avg. Doses         -------------------- Number --------------------
                (ppm)              of animals       with fatal    with incidental
                                                        tumors          tumors
                         0                  49               0               0
                        10                  49               0               0
                        30                  49               0               4
                        60                  49               0               2



                                                  Generating Model Fit Table ---
                                        TITLE: Naphthalene, NTP 2000, female rat adenomas

Model: Two Stage Weib         Dataset: D:\Program Files\TOX_RISK\naph_aden_f.ttd
   Functional form: 1 - EXP[( -Q0 - Q1 * D - Q2 * D^2) * (T - T0)^Z]
                                      Maximum Log-Likelihood =  -2.377199e+001

                                                     Parameter Estimates :
                                                        Q 0 = 0.000000E+000
                                                        Q 1 = 1.219963E-010
                                                        Q 2 = 0.000000E+000
                                                      Z   = 3.524734E+000
                                                T0  = 0.000000E+000   Set by User


              Avg. Doses         -------------------- Number --------------------
                (ppm)              of animals       with fatal    with incidental
                                                        tumors          tumors
                         0                  49               0               0
                        10                  49               0               0
                        30                  49               0               4
                        60                  49               0               2



                                             Generating Extrapolated Doses Table ---
                                        TITLE: Naphthalene, NTP 2000, female rat adenomas

                                     Dataset: D:\Program Files\TOX_RISK\naph_aden_f.ttd
                                                                    Exposure Pattern
                          Model: One Stage Weib     Age Begins: 0     Age Ends: 70
                    Target Species: Human           Weeks/Year: 52   Days/Week:  7
                          Route: Air                                 Hours/Day : 24
                Animal to human conversion method: PPM IN AIR

                          Unit Potency [ per mg/kg/day ] (computed for Risk of 1.0E-6)
            Lower Bound = Not Reqstd   MLE = 1.0306E-003   Upper Bound(q1*) = 1.9745E-003

                                          Induction Time (T0) Set by User to 0


                                                                    Dose Estimates (ppb)
                                                95.00 %                             95.00 %
          Incid Extra Risk   Time     Lower Bound          MLE          Upper Bound
              1.0000E-006     70.00   3.3250E-001     6.3701E-001     Not Reqstd
              1.0000E-005     70.00   3.3250E+000     6.3702E+000     Not Reqstd
              0.0001          70.00   3.3251E+001     6.3704E+001     Not Reqstd
              0.01            70.00   3.3417E+003     6.4022E+003     Not Reqstd
              0.05            70.00   1.7055E+004     3.2674E+004     Not Reqstd
              0.10            70.00   3.5032E+004     6.7116E+004     Not Reqstd
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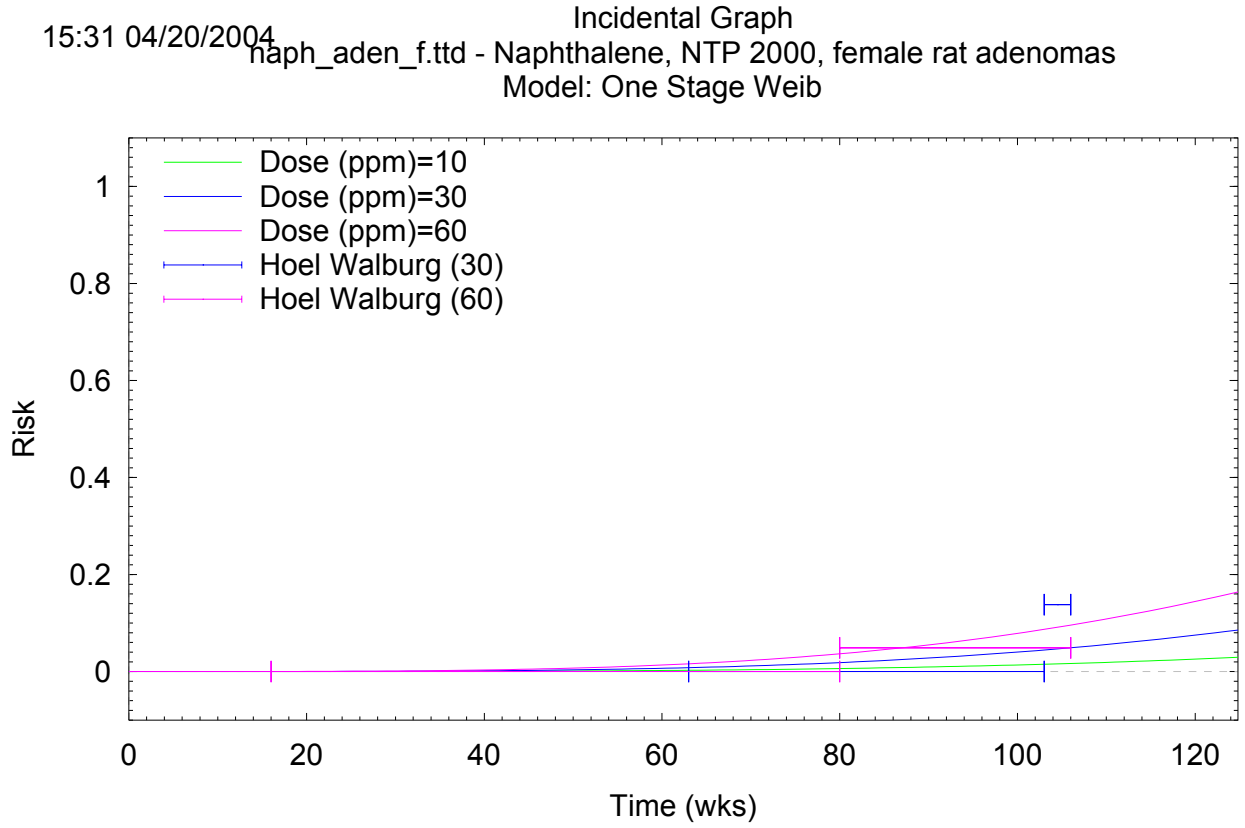
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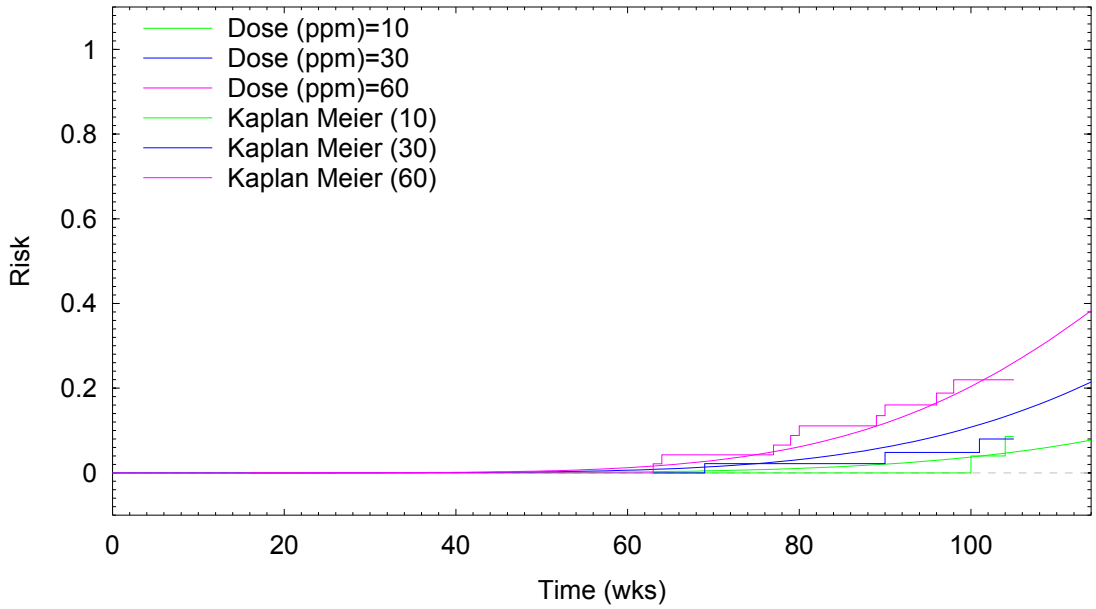
naph_aden_f.ttd - Naphthalene, NTP 2000, female rat adenomas
Model: One Stage Weib
Dose (ppm)=10
Dose (ppm)=30
Dose (ppm)=60
Hoel Walburg (30)
Hoel Walburg (60)
Risk
1
0.8
0.6
0.4
0.2
0
0
20
40
60
80
100
120
Time (wks)

Fatal Graph
15:53 04/20/2004
naph_blast_f.ttd - Naphthalene, NTP 2000, female rat neuroblastomas
Model: One Stage Weib



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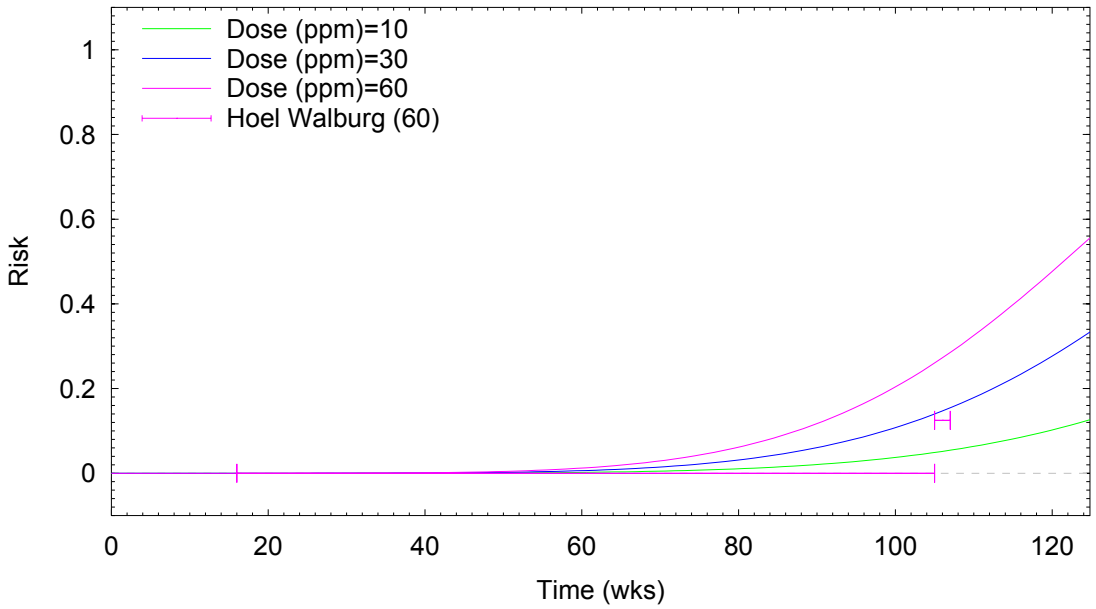
Model: One Stage Weib



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                          TITLE: Naphthalene, NTP 2000, female rat neuroblastomas

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                                    Maximum Log-Likelihood =  -9.806274e+001

                                                  Parameter Estimates :
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                                                     Q 1 = 1.348641E-014
                                                   Z   = 5.724964E+000
                                          T0  = 0.000000E+000   Set by User


              Avg. Doses         -------------------- Number --------------------
                (ppm)              of animals       with fatal    with incidental
                                                        tumors          tumors
                       0                  49               0              0
                      10                  49               2              0
                      30                  49               3              0
                      60                  49               9              3


                                        Generating Model Fit Table ---
                          TITLE: Naphthalene, NTP 2000, female rat neuroblastomas

Model: Two Stage Weib         Dataset: D:\Program Files\TOX_RISK\naph_blast_f.ttd
    Functional form: 1 - EXP[( -Q0 - Q1 * D - Q2 * D^2) * (T - T0)^Z]
                                    Maximum Log-Likelihood =  -9.775256e+001

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                                                     Q 1 = 7.481838E-015
                                                     Q 2 = 1.194329E-016
                                                   Z   = 5.735127E+000
                                          T0  = 0.000000E+000   Set by User


              Avg. Doses         -------------------- Number --------------------
                (ppm)              of animals       with fatal    with incidental
                                                        tumors          tumors
                       0                  49               0              0
                      10                  49               2              0
                      30                  49               3              0
                      60                  49               9              3




                                     Generating Extrapolated Doses Table ---
                          TITLE: Naphthalene, NTP 2000, female rat neuroblastomas

                              Dataset: D:\Program Files\TOX_RISK\naph_blast_f.ttd
                                                             Exposure Pattern
                         Model: One Stage Weib     Age Begins: 0     Age Ends: 70
              Target Species: Human                Weeks/Year: 52   Days/Week:  7
                         Route: Air                                 Hours/Day : 24
                 Animal to human conversion method: PPM IN AIR

                        Unit Potency [ per mg/kg/day ] (computed for Risk of 1.0E-6)
           Lower Bound = Not Reqstd   MLE = 3.1231E-003   Upper Bound(q1*) = 4.5604E-003

                                          Induction Time (T0) Set by User to 0


                                                                 Dose Estimates (ppb)
                                                95.00 %                          95.00 %
          Incid Extra Risk   Time     Lower Bound          MLE          Upper Bound
              1.0000E-006     70.00   1.4396E-001      2.1021E-001     Not Reqstd
              1.0000E-005     70.00   1.4396E+000      2.1021E+000     Not Reqstd
              0.0001          70.00   1.4397E+001      2.1022E+001     Not Reqstd
              0.01            70.00   1.4468E+003      2.1127E+003     Not Reqstd
              0.05            70.00   7.3841E+003      1.0783E+004     Not Reqstd
              0.10            70.00   1.5168E+004      2.2148E+004     Not Reqstd
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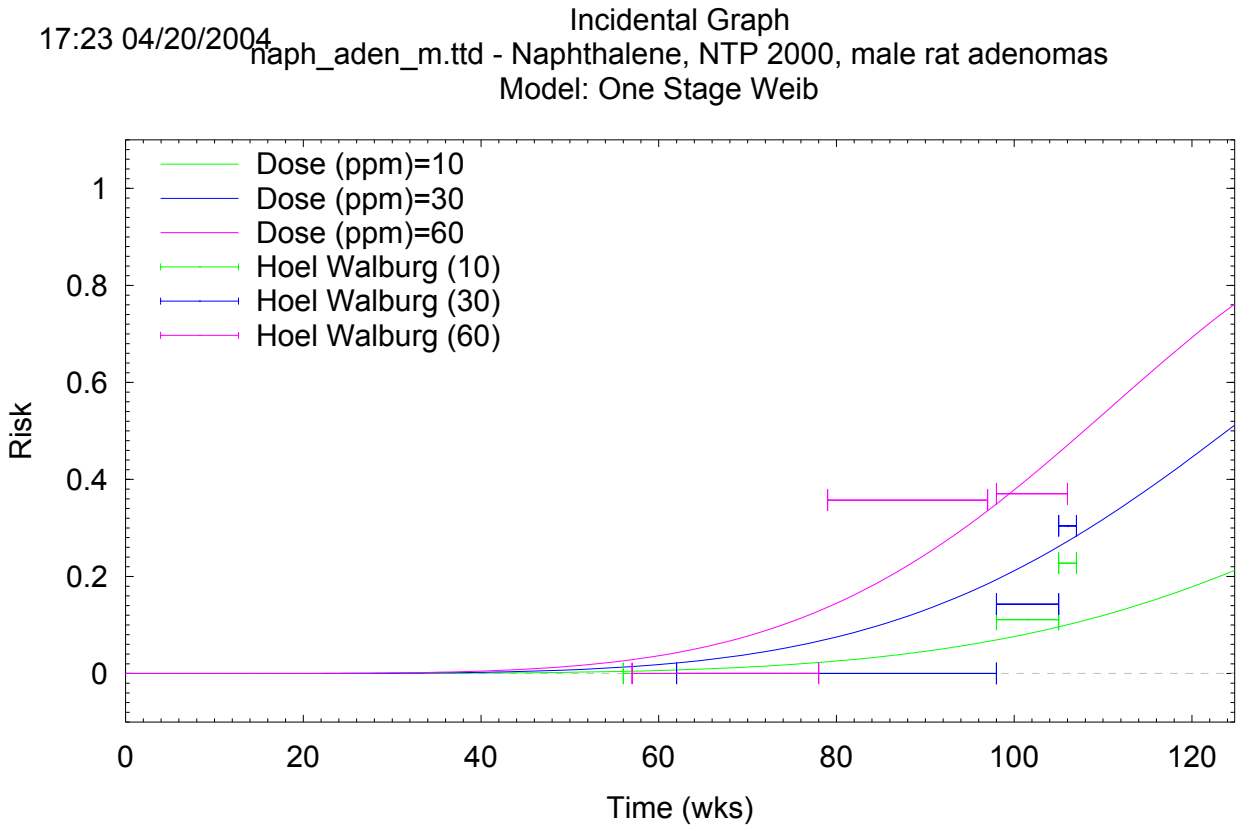


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                    Time to tumor modeling for male rat adenomas



                                                  Generating Model Fit Table ---


                                          TITLE: Naphthalene, NTP 2000, male rat adenomas

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                                        Maximum Log-Likelihood =  -6.671755e+001

                                                        Parameter Estimates :
                                                           Q 0 = 0.000000E+000
                                                           Q 1 = 8.506490E-013
                                                           Q 2 = 0.000000E+000
                                                         Z   = 4.984588E+000
                                                T0  = 0.000000E+000   Set by User


                    Avg. Doses          -------------------- Number --------------------
                      (ppm)               of animals       with fatal    with incidental
                                                                 tumors          tumors
                               0                  49                0             0
```

10	49	0	6
30	48	0	8
60	48	0	15

Generating Model Fit Table ---
TITLE: Naphthalene, NTP 2000, male rat adenomas

Model: One Stage Weib Dataset: D:\Program Files\TOX_RISK\naph_aden_m.ttd
Functional form: 1 - EXP[(-Q0 - Q1 * D) * (T - T0)^Z]
Maximum Log-Likelihood = -6.671755e+001

Parameter Estimates :
Q 0 = 0.000000E+000
Q 1 = 8.506490E-013
Z = 4.984588E+000
T0 = 0.000000E+000 Set by User

Avg. Doses (ppm)	Number of animals	with fatal tumors	with incidental tumors
0	49	0	0
10	49	0	6
30	48	0	8
60	48	0	15

Generating Extrapolated Doses Table ---
TITLE: Naphthalene, NTP 2000, male rat adenomas

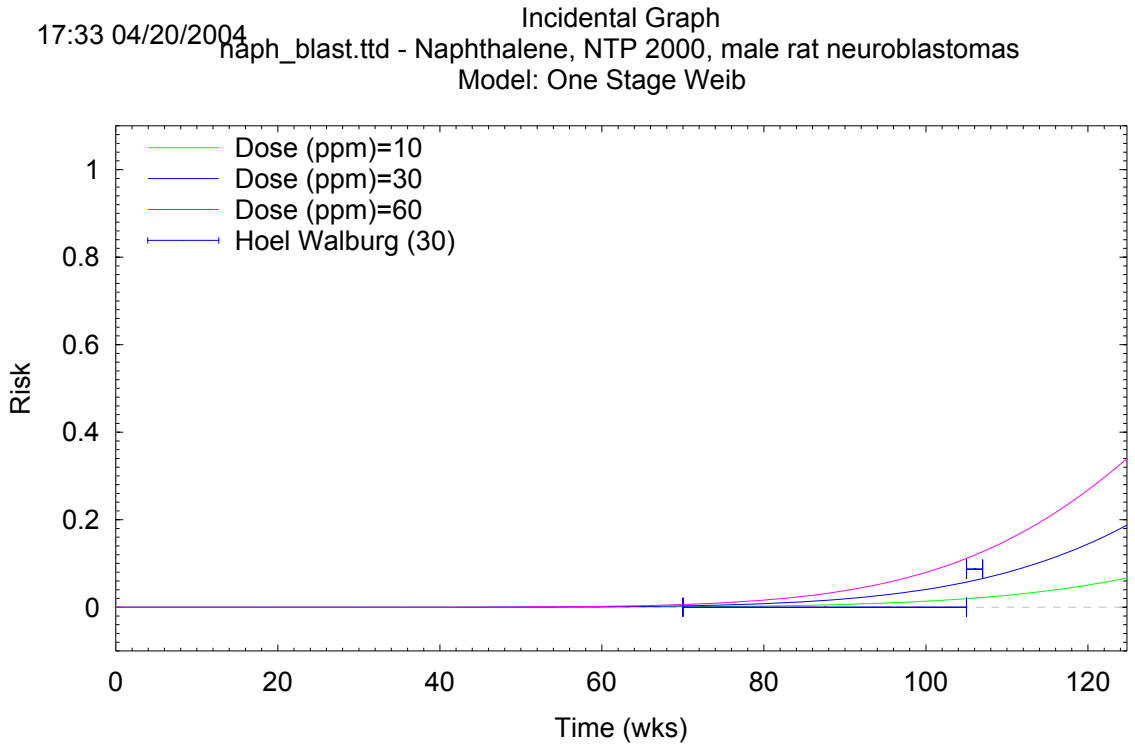
Dataset: D:\Program Files\TOX_RISK\naph_aden_m.ttd
Exposure Pattern
Model: One Stage Weib Age Begins: 0 Age Ends: 70
Target Species: Human Weeks/Year: 52 Days/Week: 7
Route: Air Hours/Day : 24
Animal to human conversion method: PPM IN AIR

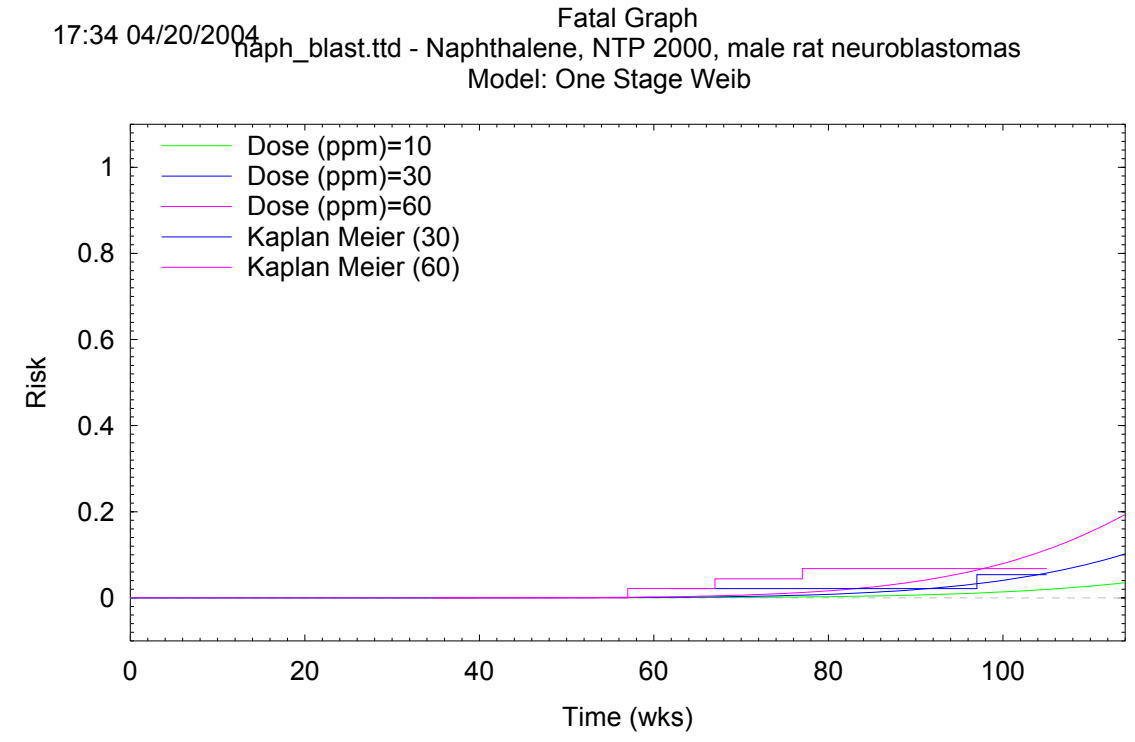
Unit Potency [per mg/kg/day] (computed for Risk of 1.0E-6)
Lower Bound = Not Reqstd MLE = 6.3252E-003 Upper Bound(q1*) = 8.6902E-003

Induction Time (T0) Set by User to 0

Dose Estimates (ppb)

Incid Extra Risk	Time	95.00 % Lower Bound	MLE	95.00 % Upper Bound
1.0000E-006	70.00	7.5546E-002	1.0379E-001	Not Reqstd
1.0000E-005	70.00	7.5546E-001	1.0379E+000	Not Reqstd
0.0001	70.00	7.5550E+000	1.0380E+001	Not Reqstd
0.01	70.00	7.5926E+002	1.0432E+003	Not Reqstd
0.05	70.00	3.8750E+003	5.3239E+003	Not Reqstd
0.10	70.00	7.9596E+003	1.0936E+004	Not Reqstd


Incidental Graph
17:33 04/20/2004
naph_blast.ttd - Naphthalene, NTP 2000, male rat neuroblastomas
Model: One Stage Weib
Dose (ppm)=10
Dose (ppm)=30
Dose (ppm)=60
Hoel Walburg (30)
Risk
0
0.2
0.4
0.6
0.8
1
0
20
40
60
80
100
120
Time (wks)


Fatal Graph
17:34 04/20/2004
naph_blast.ttd - Naphthalene, NTP 2000, male rat neuroblastomas
Model: One Stage Weib
Dose (ppm)=10
Dose (ppm)=30
Dose (ppm)=60
Kaplan Meier (30)
Kaplan Meier (60)
Risk
0
0.2
0.4
0.6
0.8
1
0
20
40
60
80
100
Time (wks)

```
                                        Generating Model Fit Table ---
                          TITLE: Naphthalene, NTP 2000, male rat neuroblastomas

Model: One Stage Weib         Dataset: D:\Program Files\TOX_RISK\naph_blast.ttd
  Functional form: 1 - EXP[( -Q0 - Q1 * D ) * (T - T0)^Z]
                                    Maximum Log-Likelihood =  -5.955715e+001

                                                  Parameter Estimates :
                                                     Q 0 = 0.000000E+000
                                                     Q 1 = 4.141071E-018
                                                    Z   = 7.262002E+000
                                          T0  = 0.000000E+000   Set by User


            Avg. Doses         -------------------- Number --------------------
              (ppm)              of animals       with fatal    with incidental
                                                      tumors          tumors
                       0                  49               0              0
                      10                  49               0              0
                      30                  48               2              2
                      60                  48               3              0



                                        Generating Model Fit Table ---
                          TITLE: Naphthalene, NTP 2000, male rat neuroblastomas

Model: Two Stage Weib         Dataset: D:\Program Files\TOX_RISK\naph_blast.ttd
  Functional form: 1 - EXP[( -Q0 - Q1 * D - Q2 * D^2) * (T - T0)^Z]
                                    Maximum Log-Likelihood =  -5.955715e+001

                                                  Parameter Estimates :
                                                     Q 0 = 0.000000E+000
                                                     Q 1 = 4.141071E-018
                                                     Q 2 = 0.000000E+000
                                                    Z   = 7.262002E+000
                                          T0  = 0.000000E+000   Set by User


            Avg. Doses         -------------------- Number --------------------
              (ppm)              of animals       with fatal    with incidental
                                                      tumors          tumors
                       0                  49               0              0
                      10                  49               0              0
                      30                  48               2              2
                      60                  48               3              0



                                     Generating Extrapolated Doses Table ---
                          TITLE: Naphthalene, NTP 2000, male rat neuroblastomas

                              Dataset: D:\Program Files\TOX_RISK\naph_blast.ttd
                                                             Exposure Pattern
                        Model: One Stage Weib     Age Begins: 0     Age Ends: 70
                 Target Species: Human            Weeks/Year: 52   Days/Week:  7
                        Route: Air                                 Hours/Day : 24
          Animal to human conversion method: PPM IN AIR

                       Unit Potency [ per mg/kg/day ] (computed for Risk of 1.0E-6)
   Lower Bound = Not Reqstd   MLE = 1.2080E-003   Upper Bound(q1*) = 2.0209E-003

                                                          Dose Estimates (ppb)
                                          95.00 %                        95.00 %
      Incid Extra Risk   Time     Lower Bound          MLE          Upper Bound
          1.0000E-006     70.00   3.2487E-001      5.4349E-001     Not Reqstd
          1.0000E-005     70.00   3.2487E+000      5.4349E+000     Not Reqstd
          0.0001          70.00   3.2488E+001      5.4351E+001     Not Reqstd
          0.01            70.00   3.2650E+003      5.4622E+003     Not Reqstd
          0.05            70.00   1.6664E+004      2.7877E+004     Not Reqstd
          0.10            70.00   3.4228E+004      5.7262E+004     Not Reqstd
```