Long-term Health Effects of Exposure to Naphthalene

Background and status of Naphthalene as a Toxic Air Contaminant and Potential Carcinogen

Naphthalene (CAS Registry Number: 91-20-3) is a natural constituent of coal tar, comprising approximately 11% of that material by weight (HSDB, 2003). It is present in gasoline and diesel fuels. All pesticide registrations of naphthalene, including use as a moth repellent, were cancelled in California in 1991 due to data gap inadequacies. However, naphthalene is included on a list of "inert" or "other ingredients" found in registered pesticide products (U.S. EPA, 2004). It is used in the manufacture of a wide variety of industrial products. A profile dated 1987 showed proportionate naphthalene use for various products as: Phthalic anhydride, 60%; 1-naphthyl methyl carbamate insecticide and related products (tetralin and 1-naphthol), 10%; dispersant chemicals, 10%; moth repellent, 6%; synthetic tanning agents, 5%; miscellaneous uses, 5%; exports, 4% (HSDB, 2003). Other products listed as derived from naphthalene include phthalic and anthranilic acids, naphthols, naphthylamines, naphthalene sulfonates, synthetic resins, celluloid, lampblack, smokeless powder, anthraquinone, indigo, perylene, and hydronaphthalenes (NTP, 1992; HSDB, 2003).

Naphthalene enters the atmosphere both from emissions from industrial facilities and other localized sources, and from mobile sources. Vehicle exhaust contains naphthalene both due to its presence in fuel oil and gasoline, and its formation as a combustion by-product. In addition, there are discharges of naphthalene on land and into water from spills during the storage, transport and disposal of fuel oil, coal tar, etc. Naphthalene is emitted when wood is burned (IARC, 2002). Naphthalene is a component of environmental tobacco smoke and a number of consumer products, resulting in its presence as a contaminant of indoor air. The statewide annual emissions from facilities reporting under the Air Toxics Hot Spots Act in California, were estimated to be 164,459 pounds of naphthalene (CARB, 1999). U.S. EPA's Toxics Release Inventory most recent database (U.S. EPA, 2001) listed 2,603,377 pounds total on- and off-site releases of naphthalene in the United States, of which 2,002,901 pounds were air emissions and the rest divided among land, surface water and underground emissions.

In polluted urban areas, the primary route of atmospheric transformation for naphthalene is daytime reaction with the OH radical. For a 12-hr average OH radical concentration of 2.0×10^6 molecule cm^{-3}, the calculated lifetime of naphthalene is 6 hr (Atkinson and Arey, 1994; Arey and Atkinson, 2003). Nighttime reaction of naphthalene with NO_3 radicals can be important in the formation of nitronaphthalenes (Atkinson and Arey, 1994; Arey and Atkinson, 2003). Observed products of naphthalene reaction with the OH and/or NO_3 radicals include 2-formylcinnamaldehyde, 1-naphthol, 2-naphthol, 1-nitronaphthalene, 2-nitronaphthalene, 1-hydroxy-2-nitronaphthalene, and 1,4-naphthoquinone (Sasaki *et al.*, 1997).

In addition to emissions from facilities and other localized sources, mobile sources also contribute to the ambient levels of naphthalene in California air. Atkinson (1995) measured 12-hour average ambient concentrations of naphthalene in Redlands, California in October 1994. The levels observed ranged from 348 to 715 ng/m^3.

Naphthalene is individually identified under the section 112(b)(1) of the U.S. Clean Air Act amendment of 1990 as a Hazardous Air Pollutant (HAP). This followed the U.S. EPA's determination that it is known to have, or may have, adverse effects on human health or the environment. On April 8, 1993, the California Air Resources Board (ARB) identified, by regulation, all 189 of the then listed HAPs as Toxic Air Contaminants (TACs). This was in response to the requirement of Health and Safety Code Section 39657(b). In addition, naphthalene meets the U.S. Clean Air Act definition of Polycyclic Organic Matter (POM; "Includes organic compounds with more than one benzene ring, and which have a boiling point greater than or equal to 100ºC"), so its identification as a California TAC could be regarded as arising either from its individual listing as a HAP, or from the listing of POM. The health effects summary (OEHHA, 1993) prepared for benzo[a]pyrene included cancer risk estimates for a number of other polycyclic aromatic hydrocarbons and related derivatives which fall under the general classification of POM. However, estimates for naphthalene were not included in that document, since at the time unequivocal evidence for the carcinogenicity of naphthalene was not available.

Non-cancer health effects of naphthalene have been recognized for some time, and these were the basis for a Chronic Inhalation Reference Exposure Level (cREL) developed by OEHHA (2000) for use in the Air Toxics Hot Spots (AB2588) program. The cREL adopted was 9 $\mu g/m^3$ (2 ppb), based on respiratory effects (nasal inflammation, olfactory epithelial metaplasia, respiratory epithelial hyperplasia) in mice.

Summary of Health Effects of Naphthalene

Non-cancer Health Effects

Hemolysis has been reported in infants exposed to high doses of naphthalene (Siegel and Wason, 1986; U.S. EPA, 1998). The effect appears to be caused by the metabolites (1- and 2- naphthol and naphthoquinones), which produce methemoglobinemia. Infants appear to be more sensitive than adults, due to their lower capacity for methemoglobin reduction. However, even in infants the doses at which this effect occurs are high. A few cases related to high (but unquantified) inhalation exposure have been reported, but the incidents generally involved absorption by the dermal route (contact with substantial amounts of solid naphthalene), or ingestion of several grams of naphthalene as a solid or dissolved in oils. Individuals with glucose-6-phosphate dehydrogenase deficiency are especially susceptible to this effect.

Several studies in animals report damage to the respiratory tract as a result of exposure to naphthalene. In a study by the National Toxicology Program (NTP, 1992), male and female $B6C3F_1$ mice were exposed to naphthalene (>99% pure) vapor for 6 hours per day, 5 days per week over 104 weeks. Concentrations used were 0 (150 mice), 10 (150 mice), or 30 ppm (300 mice) naphthalene. Lesions were observed in the nose and lungs of exposed mice, including increased incidences of chronic nasal inflammation, olfactory epithelial metaplasia, and respiratory epithelial hyperplasia.

Naphthalene causes damage to both ciliated and Clara cells of the bronchiolar epithelium in mice (Van Winkle *et al*., 1995; Plopper *et al*., 1992a,b). Neonatal mice were more sensitive to this damage than adult mice (Fanucchi *et al.*, 1997). Swiss Webster Mice at post-natal day (PND) 7

or 14, or adults, received 25, 50 or 100 mg/kg naphthalene by intraperitoneal injection, and the lungs were prepared for histological examination. Both observational and morphometric evaluation showed dose-dependent damage to the bronchiolar epithelium. There was loss of both ciliated and non-ciliated (Clara) cells, as indicated by changes in total epithelial thickness and in volume fractions of the various cell types, and appearance of vacuolated (injured) cells. Effects were similar in adults and young mice, but whereas the adult mice showed a LOAEL of 100 mg/kg for most effects, the 7 and 14-day old mice showed LOAELs of 25-50 mg/kg. Although the doses in this experiment were given intraperitoneally, the effects appear to depend on metabolism of naphthalene in the target tissues and are therefore anticipated to occur regardless of the dose route. Mice, which have high cytochrome P-450 activity in the bronchiolar epithelium, show more severe effects of naphthalene in the bronchiolar region of the respiratory system than rats or hamsters where local cytochrome P-450 activity is lower (Plopper *et al.,* 1992b).

There are a number of case reports of human exposures to naphthalene, generally involving acute poisonings or other situations with high exposures. Toxic effects seen generally include respiratory or hematological effects similar to those described above, nausea, vomiting, and ocular effects such as cataracts and retinal damage. These reports, and additional studies in animals, are further described in the chronic toxicity summary in support of the cREL derivation (OEHHA, 2000).

Carcinogenicity

Early carcinogenicity studies (by various routes) of naphthalene had mostly equivocal or non-positive results, although those studies were of low power (Adkins *et al*., 1986; Kennaway, 1930; Schmahl, 1955). Genetic toxicology results were mixed: *Salmonella* reverse mutation assays were generally negative, but some test results with eukaryotic systems *in vivo* or *in vitro* were positive (NTP, 2000). Inhalation studies in mice by the National Toxicology Program (NTP, 1992) were generally considered at the time to provide only equivocal evidence of carcinogenic activity, when considered in conjunction with the earlier studies. However, the observation of possible tumor responses in the mice prompted the National Toxicology Program to undertake

inhalation studies in rats, the results of which became available more recently (NTP, 2000). These studies found clear evidence of carcinogenic activity in rats, based on increased incidences of respiratory epithelial adenoma and olfactory epithelial neuroblastoma of the nose in both sexes. These additional findings prompted IARC to re-evaluate naphthalene, which was re-classified as Group 2B: possibly carcinogenic to humans (IARC, 2002). The State of California's Proposition 65 program listed naphthalene as a substance known to the State to cause cancer on April 19, 2002. In view of these new data and conclusions, it is appropriate to provide a cancer risk estimate for naphthalene for use in the Toxic Air Contaminants program, in addition to the Reference Exposure Level already available for the chronic non-cancer effects. The following summary (to be included as an addendum to the *Air Toxics Hot Spots Program Risk Assessment Guidelines: Part II, Technical Support Document for describing available Cancer Potency Factors)* provides an analysis of the carcinogenicity data for naphthalene, and derives a cancer potency factor and unit risk factor for use in risk assessment of inhalation exposures to naphthalene.

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NAPHTHALENE

CAS No: 91-20-3

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 2003 except as noted)

Molecular weight	128.2
Boiling point	218ºC
Melting point	80.5 ºC
Vapor pressure	0.078 Torr @ 25ºC (Sonnenfeld *et al*., 1983); 0.10 Torr @ 27ºC (CRC, 1994)
Air concentration conversion	1 ppm = 5.24 mg/m^3 (NIOSH, 2004)

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 3.4 E-5 $(\mu g/m^3)^{-1}$
Slope Factor: 1.2 E-1 $(mg/kg\text{-}day)^{-1}$

[Male rat nasal respiratory epithelial adenoma and nasal olfactory epithelial neuroblastoma incidence data (NTP, 2000), linearized multistage procedure (OEHHA, 2002).]

III. CARCINOGENIC EFFECTS

Animal Studies

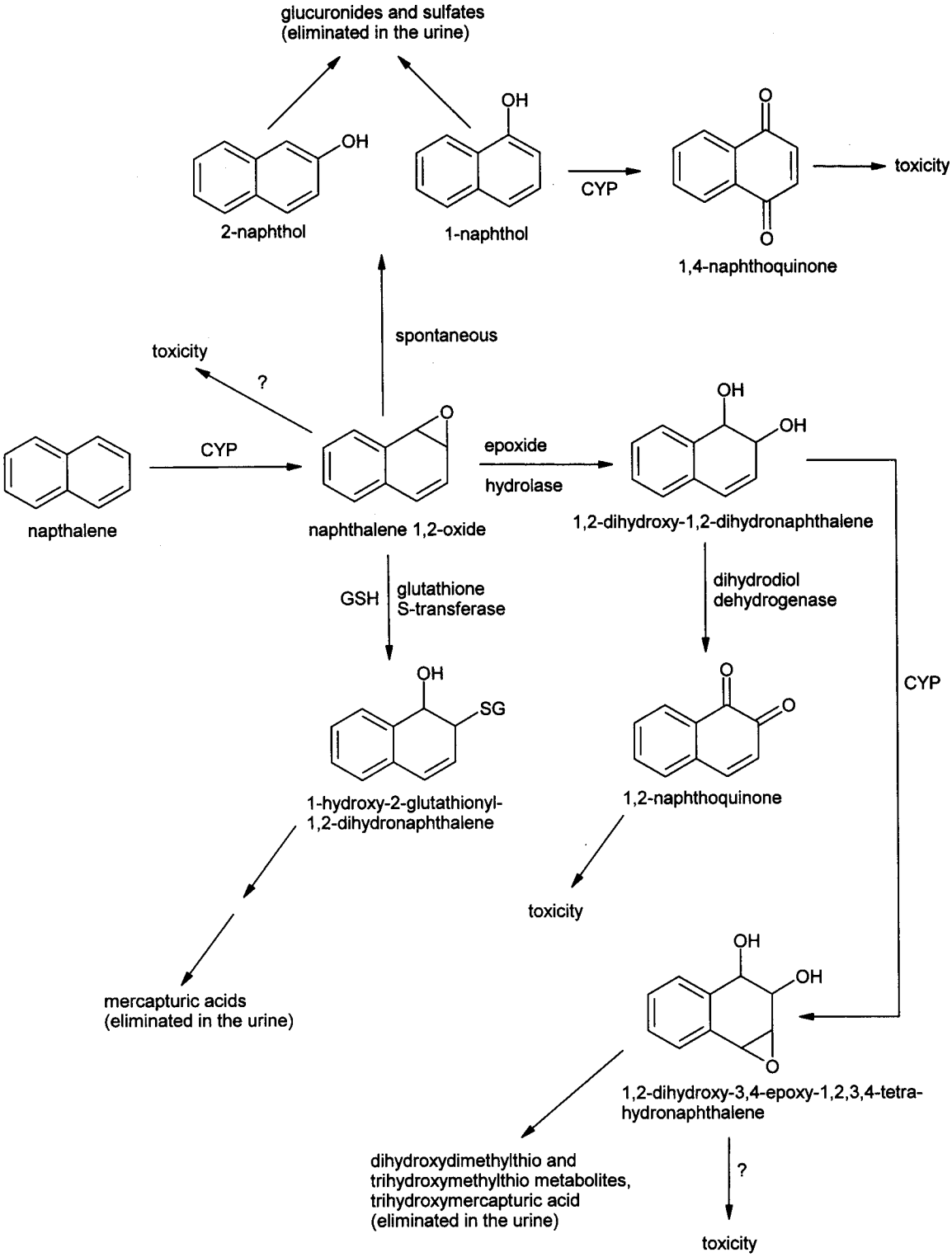
Metabolism

The metabolism of naphthalene is similar to that of many other aromatic hydrocarbons (reviews: Buckpitt and Franklin, 1989; Buckpitt *et al*., 2002). Initially, naphthalene is oxidized by the cytochrome P450 monooxygenase system to naphthalene oxide enantiomers. The primary site of metabolism is the liver, but oxidation also occurs in the lung and kidneys. Naphthalene oxides may be converted to dihydrodiols by epoxide hydrolase enzymes. Naphthalene oxides also undergo nonenzymatic conversion to 1-naphthol, which may be subsequently conjugated or may be further oxidized to 1,4-naphthoquinone. The naphthalene dihydrodiols may also be oxidized to 1,2-naphthoquinone (Figure 1). Toxicity is apparently related to protein binding by quinone metabolites and/or their participation in redox cycles leading to oxidative stress including DNA damage (O'Brien, 1991).

Naphthalene-1,2-epoxide

Naphthalene is oxidized to two reactive enantiomers: (1*R*, 2*S*)-naphthalene oxide and (1*S*, 2*R*)-naphthalene oxide. A purified cytochrome P450 monooxygenase (CYP2F2) from mouse liver that metabolized naphthalene rapidly with high stereoselectivity was used to clone and sequence the cDNA coding for a 50-kDa protein present at high levels in mouse lung and liver. The protein had 82% sequence homology to a cDNA cloned earlier from human lung (Ritter *et al*., 1991). The human CYP2F1 and mouse CYP2F2 were expressed in HEPG2 cells and yeast,

Figure 1. Metabolic Scheme for Naphthalene (adapted from ATSDR, 2003)



CYP = cytochrome P450 enzyme(s); GSH = reduced glutathione; SG = glutathione

respectively. In human lymphoblastoid cells the CYP2F1 demonstrated a naphthalene turnover of about 0.035 nmol/min/nmol P450 or about 0.1% the rate observed with the mouse CYP2F2 (Shultz *et al*., 1999). The human CYP2F1 showed slight stereopreference in the generation of the (1S, 2R) naphthalene oxide enantiomer (7.7:1). The recombinant mouse CYP2F2 exhibited a high degree of stereoselectivity for the (1*R*, 2*S*) enantiomer (66:1), a very high V_{max} (107 nmol/min/nmol P450), and a low K_m (3 μM). This high level of activity is consistent with the metabolism and toxicity of naphthalene in the mouse lung. CYP2F2 also oxidizes a number of other substrates including the lung toxicant 1-nitronaphthalene.

Characterization of naphthalene oxidizing P450 enzymes in the rat lung has been more difficult. A CYP2F4 has been cloned from the rat lung that has 93 percent identity with the mouse and 83 percent with the human (Buckpitt *et al*., 2002). While further characterization of the rat enzyme is necessary the results overall indicate that there are likely major interspecies catalytic differences in these CYP2F enzymes between mouse, rat and human. It is unknown whether CYP2F is responsible for the activation of naphthalene in the nasal epithelium. The rate of metabolism appears to be substantial in olfactory postmitochondrial supernatants from mice and rats, 87 and 43.5 nmol/min/mg protein, respectively (Buckpitt *et al*., 1992). However, there are several P450 isoforms in the nasal mucosa including CYP1A2, 2A, 2B, 2C, 2G1, 2J, and 3A and catalytic activities with naphthalene have not been established.

In contrast to the results of *in vitro* enzymatic studies, Willems *et al*. (2001) estimated overall kinetic parameters for intact mice and rats exposed to concentrations of naphthalene that were used in the NTP cancer bioassays in conjunction with a physiologically based pharmacokinetic model. The V_{max} (nmol/min/mg microsomal protein) and Km (μM) for the male and female rats were 16.5, 6.0; and 24.6, 3.2, respectively. For male and female mice the V_{max} and K_m values were 38.7, 1.5; and 54.8, 5.8, respectively. While some differences in metabolic saturation capacity are apparent the values overall are similar. The overall metabolic capacities based on these figures are higher than indicated by in vitro derived kinetic parameters of Quick and Shuler (1999). The values above do not distinguish between lung and liver.

1-Naphthol

Naphthalene-1,2-epoxide undergoes spontaneous rearrangement to1-naphthol. Like naphthalene, 1-naphthol is subject to oxidation by cytochrome P450 enzymes. Wilson *et al*. (1996) studied the metabolism and cytotoxicity of naphthalene and 1-naphthol in vitro with human hepatic microsomes and mononuclear leukocytes (MNL). 1-Naphthol was observed to be more toxic than naphthalene (49.8 ± 13.9% vs. 19.0 ± 10.0% cell death; $p < 0.01$). CYP2E1-induced rat liver microsomes increased the metabolism of naphthalene giving increased yields of both naphthalene dihydrodiol and 1-naphthol. The cytotoxicity of naphthalene but not 1-naphthol was increased by CYP2E1 induction. The metabolites of 1-naphthol; 1,2-naphthoquinone and 1,4-naphthoquinone were directly toxic to MNL and depleted glutathione to one percent of control level. Both quinone metabolites of 1-naphthol were also genotoxic to human lymphocytes. Buckpitt *et al*. (1986) found that in vivo, reactive metabolites from [^{14}C]-1-naphthol became covalently bound to proteins in lung, liver, and kidney, but that the amount of binding was similar to that seen after administration of naphthalene. Zheng *et al*. (1997) incubated murine Clara cells with naphthalene and found adducts generated from the 1,2-quinone metabolite vs. the 1,2-epoxide had a ratio of 32:1. They found no evidence for formation of the 1,4-

naphthoquinone. Conversely, Doherty *et al*. (1985) incubated microsomal preparations and reported that the reactive metabolite generated from 1-naphthol was not trapped by ethylene diamine, which reacts rapidly with 1,2-naphthoquinone, and hence was likely 1,4-naphthoquinone. Current thinking is that 1-naphthol is oxidized to 1,4-naphthoquinone, which binds to proteins and plays a role in toxicity of naphthalene, particularly in the lung (Buckpitt *et al*., 2002).

1,2-Dihydroxy-1,2-dihydronaphthalene

Naphthalene-1,2-oxides are hydrolyzed to naphthalene dihydrodiols via epoxide hydrolase (EH). While generally less toxic than their parent epoxides dihydrodiols may serve as precursors to diol epoxides that represent ultimate carcinogens for many PAHs. The formation of diol epoxides and/or diepoxides from naphthalene is supported by the observation of trihydroxytetrahydro-urinary metabolite derivatives (Buckpitt *et al*., 2002). In addition to this microsomal oxidation of naphthalene dihydrodiol, a competing biotransformation by cytosolic dihydrodiol dehydrogenase (DD) generates the catechol, 1,2-dihydroxynaphthalene, which is readily autoxidized to 1,2-naphthoquinone. During oxidation via single electron steps reactive oxygen species (ROS) are generated together with an *o*-semiquinone anion radical intermediate. 1,2-Naphthoquinone can be reduced by NAD(P)H back to the catechol creating a futile redox cycle, which may lead to oxidative stress including DNA damage (Penning *et al*. 1999). As noted above, studies in isolated Clara cells indicated that the 1,2-naphthoquinone was the major metabolite, which bound covalently to proteins (Zheng *et al*., 1997). 1,2-Naphthoquinone is mutagenic in the Salmonella microsome assay (Bolton *et al*., 2000) and forms *N*-7 adducts with deoxyguanosine *in vitro* (McCoull *et al*., 1999). 1,2-Naphthoquinone is a key metabolite in naphthalene toxicity and may be more important in humans than rodents due to the higher activities of both EH and DD in human tissues compared to rodent tissues (Penning *et al*., 1999; Kitteringham *et al*., 1996)

Conjugates of Naphthalene Metabolites

Naphthalene-1,2-oxides are converted via the action of glutathione *S*-transferase to 1-hydroxy-2-glutathionyl-1,2-dihydronaphthalenes. These are subsequently converted to mercapturic acids (*S*-conjugates of *N*-acetyl-L-cysteine) and excreted in the urine (25 to 35 percent of dose in mice and rats). In mice the ratio of diastereomeric mercapturates derived from (1*R*, 2*S*):(1*S*, 2*R*) epoxide ranged from 1:1 to 3:1 (1-200 mg/kg i.p.) whereas in rats the ratio was always less than 1:1 at all doses. For inhalation exposures the ratios were higher in mice 6:1 to 3:1 (15-100 ppm) but unchanged in rats (Pakenham *et al*., 2002). Three mercapturic acids have been identified in urine derived from the following conjugates: 1) 1*S*-hydroxy-2*S* –glutathionyl; 2) 1*R*-hydroxy-2*R*-glutathionyl; and 3) 1*R*-glutathionyl-2*R*-hydroxy. There are no substantial species differences in the percentage of dose eliminated as diastereomeric mercapturates. The ratios of the diastereomers do vary with species, administration route and dose level. Overall these urinary mercapturates appear to provide a useful biomarker of internal naphthalene dose.

Alternative biomarkers are the albumin and hemoglobin cysteinyl adducts of naphthalene-1,2-oxide, 1,2- and 1,4-naphthoquinones formed after administration of naphthalene to rats (Waidyanatha *et al*., 2002). In human coke oven workers only albumin adducts of 1,2-naphthoquinone (1,2-NQ-Alb) were found to significantly exceed background levels seen in control steel industry workers (Waidyanatha *et al*., 2004). This study also observed that 1,2-NQ-

Alb levels were significantly correlated with urinary levels of naphthalene, 1-naphthol, 2-naphthol, and 1-pyrenol but negatively correlated with age, suggesting a diminished cytochrome P450 metabolism of about three percent/year.

The conjugation of dihydroxy dihydronaphthalene metabolites and their subsequent urinary elimination as mercapturates is generally considered a detoxication process. However, reaction of these metabolites with glutathione may give rise to naphthoquinone thioethers that possess a variety of toxic properties (Monks and Lau, 1992,1998; Monks *et al*., 1992). ESR studies have shown the formation of GSH-conjugated semiquinone free radicals of 3-(glutathion-*S*-yl)-1,4-naphthoquinone and 2,3 (di-glutathion-*S*-yl)-1,4-naphthoquinone in rat hepatocytes (Takahashi *et al*., 1987; Rao *et al*., 1988). Conjugation of naphthoquinones with glutathione or *N*-acetylcysteine may exacerbate redox cycling by reducing the redox potential of the conjugate vs. the parent quinone. Quinone thioethers participate in protein crosslinking; serve as substrates for DT-diaphorase, or as inhibitors of important enzymes such as NADP-linked 15-hydroxyprostglandin dehydrogenase and glutathione sulfotransferase (GST). Some quinone thioethers also exhibit nephrotoxicity as indicated by menadione 3-thioethers (e.g., 2-methyl-3-(*N*-acetylcysteine-*S*-yl)-1,4-naphthoquinone) induction of renal proximal tubular necrosis in rats (Lau *et al*., 1990).

In addition to the GST mediated conjugation of naphthalene dihydrodiols and naphthoquinones, 1-naphthol and 2-naphthol arising from the rearrangement of the naphthalene-1,2-oxides are also conjugated and eliminated in the urine as sulfates and glucuronides.

Genotoxicity

Naphthalene

Naphthalene has been tested for genotoxicity in a variety of *in vitro* and *in vivo* genotoxicity assays. Those studies have recently been reviewed by ATSDR (2003). Naphthalene has not demonstrated genotoxicity in *Salmonella* reverse mutation assays. Those studies are listed in Table 1. All studies were performed in the presence and absence of metabolic activation (rat liver S9), and were negative.

Table 1. Naphthalene *Salmonella* reverse mutation studies

Test strains	Reference
TA98, TA100, TA1535, TA1537	McCann *et al*., 1975
TM677	Kaden *et al*., 1979
TA98, TA100, TA1535, TA1537	Florin *et al*., 1980
TA1537, TA1538	Gatehouse, 1980
TA98, TA100, UTH8413, UTH8414	Connor *et al*., 1985
TA98, TA100, TA1535, TA1537, TA1538	Godek, 1985
TA98, TA100, TA1535, TA1537	Mortelmans *et al*., 1986
TA98, TA1535	Narbonne *et al*., 1987
TA98, TA100	Bos *et al*., 1988
TA98, TA100, TA1535, TA1537	NTP, 1992a
TA98, TA100, TA1535, TA1537	Sakai *et al*., 1985

Naphthalene has also been tested in other bacterial mutation assay systems. These studies are listed in Table 2. All studies were performed in the presence and absence of metabolic activation (rat liver S9), and were negative except for the study by Arfsten *et al*. (1994). This study used Vibrio fischeri (strain M169) in a Mutatox assay (reversion to luminescence). Naphthalene was negative in the absence of S9, but positive in the presence of S9.

Table 2. Other naphthalene bacterial mutation studies

Assay system	Species/strain	Reference
SOS response	*E. coli* K12 inductest (λ lysogen GY5027; *uvr*B-, *env*A-)	Mamber *et al*., 1984
	S. typhimurium TA1535/p5K1002 (uMuC-IacZ)	Nakamura *et al*., 1987
SOS chromotest assay	*E. coli* PQ37 (*sfi*A::*lac*Z fusion)	Mersch-Sundermann *et al*., 1993
Pol A- or Rec	*E. coli* WP2/WP10 (*uvr*A-, *rec*A-)	Mamber *et al*., 1983
	E. coli WP2/WP67 (*uvr*A-, *pol* A-)	
	E. coli WP2/WP3478 (*pol* A-)	
Mutatox (reversion to luminescence)	*Vibrio fischeri* M169	Arfsten *et al*. 1994

Naphthalene has not demonstrated genotoxicity activity in mammalian *in vitro* DNA damage and gene mutation assays. The relevant studies are listed in Table 3.

Table 3. Naphthalene mammalian *in vitro* DNA damage and gene mutation studies

Assay	Cell type	Reference
Alkaline elution (single strand DNA breaks)	Rat primary hepatocytes	Sina *et al*., 1983
Unscheduled DNA synthesis	Rat primary hepatocytes	Barfknecht *et al*., 1985
hprt and *tk* loci mutations	Human MCL-5 B-lymphoblastoid cells	Sasaki *et al*., 1997

Naphthalene has been demonstrated to induce chromosomal damage in mammalian cells *in vitro*. Wilson *et al*. (1995) found that naphthalene did not induce sister chromatid exchanges (SCEs) in human lymphocytes in the presence or absence of human liver microsomes. However, NTP (1992) found that naphthalene induced SCEs in Chinese hamster ovary (CHO) cells in the presence of rat liver S9 in the second of two trials and in the absence of S9 in both trials. Naphthalene also induced chromosomal aberration in CHO cells in the presence but not absence of rat liver S9. Additionally, naphthalene caused an increase in the frequency of $CREST^{-}$ micronuclei (indicative of chromosomal breakage) in human MCL-5 B-lymphoblastoid cells (Sasaki *et al*., 1997). These cells express microsomal epoxide hydrolase (EH) and CYP1A2, CYP2A3, CYP3A4, and CYP2E1 P450 isoforms.

Data on the induction by naphthalene of DNA damage *in vivo* are mixed. Single strand DNA breaks (measured by alkaline elution) were not induced in hepatocytes from female rats exposed to a single oral dose of naphthalene (Kitchin *et al*., 1992, 1994). In contrast, naphthalene caused DNA fragmentation in liver and brain tissue from rats given daily oral doses for up to 120 days (Bagchi *et al*., 1998), mice given single oral doses (Bagchi *et al*., 2000, 2002), and *p*53-deficient mice given single oral doses (Bagchi *et al*., 2002).

Naphthalene did not cause micronucleus induction (indicative of chromosomal damage) in the bone marrow cells of mice exposed to naphthalene either by gavage (Harper *et al*., 1984) or by intraperitoneal injection (i.p.) (Sorg, 1985). However, naphthalene was reported to induce chromosomal aberrations in preimplantation mouse embryos in an abstract by Gollahon *et al*. (1990).

Naphthalene has been demonstrated to have genotoxic effects in nonmammalian assay systems. Delgado-Rodriguez *et al*. (1995) reported positive results for naphthalene in the *Drosophila melanogaster* wing spot assay. This assay detects both somatic mutations and mitotic recombination in cells of the wing imaginal discs, based on the induced loss of heterozygosity for two recessive wing cell markers. Micronuclei induction was reported in the erythrocytes of salamander larvae (*Pleurodeles waltl*) exposed to naphthalene in their tank water (Djomo *et al*., 1995).

Naphthalene metabolites

1-Napthol, 2-Napthol

1-Naphthol was reported to be negative in the *Salmonella* reverse mutation assay by McCann *et al*. (1975) (test strains TA98, TA100, TA1535 and TA1537) and Norbonne *et al*. (1987) (test strains TA98 and TA1535). 1-Naphthol and 2-napthol also did not induce UDS in primary rat hepatocytes (Probst *et al*., 1981).

1,2-Napthoquinone

Flowers-Geary *et al*. (1996) tested 1,2-napthoquinone in the *Salmonella* reverse mutation assay (test strains TA97a, TA98, TA100 and TA104) in the presence and absence of rat liver S9. 1,2-Napthoquinone caused a 2.5-fold increase in revertants in strain TA104, a strain that is sensitive to oxidative DNA damage, compared to controls. 1,2-Napthoquinone also caused SCEs in human lymphocytes in the absence of metabolic activation (Wilson *et al*., 1996).

1,4-Napthoquinone

1,4-Napthoquinone was negative in the *Salmonella* reverse mutation assay (test strains TA98, TA100, TA1535 and TA1537) (Sakai *et al*., 1985). 1,4-Napthoquinone also did not induce mutations at either the *hprt* or *tk* loci in the human B-lymphoblastoid MCL-5 cell line (Sasaki *et al*., 1997). However, 1,4-napthoquinone did cause SCEs in human lymphocytes in the absence of metabolic activation (Wilson *et al*., 1996), and caused a significant increase in the frequency

of both $CREST^+$ (indicative of chromosomal loss) and total micronuclei in the human B-lymphoblastoid MCL-5 cell line (Sasaki *et al*., 1997).

Naphthalene 1,2-epoxide

Naphthalene 1,2-epoxide did not cause SCEs in human lymphocytes in the absence of metabolic activation (Wilson *et al*., 1996).

Naphthalene Atmospheric Reaction Products

Naphthalene is one of the more abundant PAH air pollutants in California. Atmospheric naphthalene occurs partially in the vapor phase and enters into rapid gas-phase reactions with hydroxyl radical (HO, daytime) and nitrate radicals (NO_3, nighttime). Reaction products include 1-nitronaphthalene (1NN), 2-nitronaphthalene (2NN), 1-hydroxy-2-nitronaphthalene (1H2NN), and 2-hydroxy-2-nitronaphthalene (2H2NN). Sasaki *et al*. (1997) evaluated the genotoxicity of these reaction products in the human B-lymphoblastoid MCL-5 cell line. 2-Nitronaphthalene caused a significant increase both in the mutation frequency at the thymidine kinase (*tk*) locus, and in the total micronucleus number (indicative of chromosomal damage).

The above data indicate that naphthalene generally has not been shown to cause gene mutations, but has been demonstrated to cause chromosomal damage and may cause DNA damage. The naphthalene metabolite 1,4-napthoquinone also causes chromosomal damage, and 1,2-napthoquinone causes both gene mutations and chromosomal damage, as does the atmospheric reaction product 2-nitronaphthalene.

Cancer Bioassays

The National Toxicology Program (NTP) conducted inhalation cancer studies of naphthalene using male and female $B6C3F_1$ mice (NTP, 1992). Animals were exposed to 0 (70 males, 69 females), 10 (69 males, 65 females) or 30 ppm naphthalene (135 males, 135 females) for 6 hours/day, 5 days/week for 104 weeks.

The survival rates of exposed female mice were similar to that of controls (86%, 88% and 76% for controls, 10 and 30 ppm exposure groups, respectively). However, survival of male control mice was significantly less than that of exposed male mice (37%, 75% and 89% for controls, 10 and 30 ppm exposure groups, respectively). NTP stated that the reduced control survival was due to wound trauma and secondary infections due to fighting among the group-housed mice.

Almost all of the male and female mice in the NTP 1992 mouse inhalation studies demonstrated an increased incidence of nasal respiratory epithelium hyperplasia and olfactory epithelium metaplasia (Table 4).

Table 4. Incidence of nonneoplastic nasal lesions in male and female $B6C3F_1$ exposed to naphthalene by inhalation for 104 weeks (NTP, 1992).

Lesion type	Sex	Naphthalene concentration		
		0 ppm	10 ppm	30 ppm
respiratory epithelium hyperplasia	male			
overall rate		0/70 (0%)	66/69 (96%)	134/135 (99%)
average severity grade[a]		0	2.6	2.8
olfactory epithelium metaplasia				
overall rate		0/70 (0%)	66/69 (96%)	134/135 (99%)
average severity grade[a]		0	2.5	2.6
respiratory epithelium hyperplasia	female			
overall rate		0/69 (0%)	65/65 (100%)	135/135 (100%)
average severity grade[a]		0	2.5	2.7
olfactory epithelium metaplasia				
overall rate		0/69 (0%)	65/65 (100%)	135/135 (100%)
average severity grade[a]		0	2.5	2.4

a: Average severity grade based on l = minimal, 2 = mild, 3 = moderate, and 4 = marked.

Increased incidences of alveolar/bronchiolar adenomas and carcinomas were observed in male $B6C3F_1$ mice. Alveolar/bronchiolar adenoma or carcinoma incidences in the male mice as cited by NTP were 7/70, 17/69 and 31/135 for controls, and the 10 and 30 ppm exposure groups, respectively. The increased tumor incidences observed for the 10 and 30 ppm groups were significant when a pairwise comparison to control was performed using the Fisher exact test (p = 0.019 and 0.016 for the 10 and 30 ppm groups, respectively). However, NTP noted that an evaluation of the dose-response trend (p = 0.530) and pairwise comparisons between the controls and exposure groups (p = 0.212 and 0.394 for the 10 and 30 ppm exposure groups, respectively) using a logistic regression test indicated a lack of statistical significance. This was explained by NTP as being the result of the early control mortality due to fighting which lowered considerably the number of control animals at risk of developing lung tumors. NTP also noted that the alveolar/bronchiolar adenoma and carcinoma incidence (adjusted rate 26% in the high dose group) was within the historical control range for male $B6C3F_1$ mice (total incidence 19.7%, range 10-30%). NTP therefore concluded that the marginally increased alveolar/bronchiolar adenoma and carcinoma incidence in the male mice was more likely to be related to survival difference between exposed and control groups, than directly related to naphthalene exposure.

Increased incidences of alveolar/bronchiolar adenomas and carcinomas were also observed in female $B6C3F_1$ mice. The incidences of alveolar/bronchiolar adenoma or carcinoma, combined, in the female mice as cited by NTP were 5/69, 2/65 and 29/135 for controls, and the 10 and 30 ppm exposure groups, respectively. The tumors were primarily adenomas; one carcinoma was observed in high dose female mice. The increased tumor incidence in the 30 ppm exposure group females was statistically significant when compared to controls. NTP concluded that this provided *some evidence* of carcinogenicity.

These results were generally considered at the time to provide only equivocal evidence of carcinogenic activity, when considered in conjunction with earlier studies by various routes, which, although of lower power, also had negative or equivocal results (Adkins *et al*., 1986; Kennaway, 1930; Schmahl, 1955). However, the observation of possible tumor responses in the

mice prompted the National Toxicology Program to undertake naphthalene inhalation cancer studies in rats.

NTP (2000) exposed groups of 49 male and female Fischer 344N (F344) rats to naphthalene by inhalation at concentrations of 0, 10, 30 or 60 ppm for 6.2 hours/day, five days/week for 105 weeks. Survival of the male and female exposure groups were similar to that of controls.

These studies found clear evidence of carcinogenic activity in male and female rats, based on increased incidences of rare tumors, respiratory epithelial adenoma and olfactory epithelial neuroblastoma of the nose, in both sexes. Respiratory epithelial adenoma incidence occurred with a positive dose-response trend in male rats and was significantly increased in all exposed male rat groups. Male rat respiratory epithelial adenoma incidence as cited by NTP was 0/49, 6/49, 8/48 and 15/48 for controls, and the 10, 30 and 60 ppm exposure groups, respectively. Respiratory epithelial adenoma incidences in female rats exposed to 30 or 60 ppm were also increased, but the increase in the 60 ppm animals was not significant, and the increase in the 30 ppm animals was of borderline significance ($p = 0.053$ by Poly-3 test). Female rat respiratory epithelial adenoma incidence as cited by NTP was 0/49, 0/49, 4/49 and 2/49 for controls, and the 10, 30 and 60 ppm exposure groups, respectively.

Olfactory epithelial neuroblastomas occurred in males exposed to 30 and 60 ppm naphthalene and in all dose groups of naphthalene-exposed females. Neuroblastoma incidences occurred with positive dose-response trends in males and females. The incidence in females exposed to 60 ppm was significantly greater ($p < 0.001$ by Poly-3 test) than that in controls. Male rat olfactory epithelial neuroblastoma incidence as cited by NTP was 0/49, 0/49, 4/48 and 3/48 for controls, and the 10, 30 and 60 ppm exposure groups, respectively. Female rat olfactory epithelium neuroblastoma incidence as cited by NTP was 0/49, 2/49, 3/49 and 12/49 for controls, and the 10, 30 and 60 ppm exposure groups, respectively.

NTP also noted that nasal olfactory epithelial neuroblastomas and nasal respiratory epithelial adenomas have not been observed in male or female control rats in the NTP historical control database for animals fed NIH-07 feed in 2-year inhalation studies or in the more recent, smaller database for control rats fed NTP-2000 feed. Additionally, almost all of the male and female mice in the NTP 1992 inhalation studies demonstrated increased nasal respiratory epithelium hyperplasia and olfactory epithelium metaplasia (Table 1). These tissue types correspond to the tumor sites observed in rats exposed to naphthalene by inhalation.

Human Studies

Although a number of reports exist which describe non-cancer health effects in humans (OEHHA, 2000), no studies of carcinogenic effects in humans were identified.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Unit risk values for naphthalene were calculated based on data in female mice, male rats and female rats from the studies of NTP (1992, 2000). The mouse lung alveolar/bronchiolar adenoma or carcinoma incidence data, rat nasal respiratory epithelial adenoma data and nasal olfactory epithelial neuroblastoma data used to calculate unit risk values are listed in Tables 5, 6 and 7, respectively.

Table 5. Incidence of lung alveolar/bronchiolar adenoma or carcinoma in female $B6C3F_1$ mice exposed to naphthalene via inhalation (from NTP, 1992)

Chamber Concentration (ppm)	Average Concentration[a] (mg/m^3)	Lifetime Average Dose[b] (mg/kg-day)	Tumor Incidence[c] (%)	Statistical Significance[d]
0	0	0	5/67 (7)	$p < 0.001$
10	9.36	12.3	2/61 (3)	$p = 1$
30	28.1	36.8	29/129[e] (22)	$p < 0.01$

[a] Average concentration calculated by multiplying chamber concentration by six hours/24 hours, 5 days/7 days and 5.24 mg/m^3/ppm.

[b] Lifetime average doses were determined by multiplying the average concentrations during the dosing period by the female mouse breathing rate (0.038 m^3/day) divided by the female mouse body weight (0.029 kg). The dosing period of 104 weeks was equivalent to the standard lifespan of the test animals (104 weeks for rodents), so no correction for less than lifetime exposure was required.

[c] Effective rate. Animals that died before the first occurrence of tumor (day 471) were removed from the denominator.

[d] The *p*-value listed next to dose groups is the result of pairwise comparison with controls using the Fisher exact test. The *p*-value listed next to the control group is the result of trend tests conducted by NTP (1992) using the logistic regression, life table, and Cochran-Armitage methods (all three methods produced the same result).

[e] One carcinoma was observed in the high dose group.

Table 6. Incidence of nasal respiratory epithelial adenoma in male F344/N rats exposed to naphthalene via inhalation (from NTP, 2000)

Chamber Concentration (ppm)	Average Concentration[a] (mg/m^3)	Lifetime Average Dose[b] (mg/kg-day)	Tumor Incidence[c] (%)	Statistical Significance[d]
0	0	0	0/44 (0)	$p < 0.001$
10	9.67	5.69	6/42 (14)	$p < 0.05$
30	29.0	17.1	8/44 (18)	$p < 0.01$
60	58.0	34.1	15/41 (37)	$p < 0.001$

[a] Average concentration calculated by multiplying chamber concentration by 6.2 hours/24 hours, 5 days/7 days, and 5.24 mg/m^3/ppm.

[b] Lifetime average doses were determined by multiplying the average concentrations during the dosing period by the male rat breathing rate (0.262 m^3/day) divided by the male rat body weight (0.445 kg). The dosing period of 105 weeks was at least the standard lifespan of the test animals (104 weeks for rodents), so no correction for less than lifetime exposure was required.

[c] Effective rate. Animals that died before the first occurrence of tumor (day 552) were removed from the denominator.

[d] The *p*-value listed next to dose groups is the result of pairwise comparison with controls using the Fisher exact test. The p-value listed next to the control group is the result of the Poly-3 trend test, as reported by NTP (2000).

Table 7. Incidence of nasal olfactory epithelial neuroblastoma in F344/N rats exposed to naphthalene via inhalation (from NTP, 2000)

Chamber Concentration (ppm)	Average Concentration[a] (mg/m^3)	Lifetime Average Dose[b] (mg/kg-day)	Tumor Incidence[c] (%)	Statistical Significance[d]
Males				
0	0	0	0/49 (0)	$p = 0.027$
10	9.67	5.69	0/48 (0)	$p = 1$
30	29.0	17.1	4/48 (8)	$p = 0.056$
60	58.0	34.1	3/48 (6)	$p = 0.117$
Females				
0	0	0	0/49 (0)	$p < 0.001$
10	9.67	6.82	2/49 (4)	$p = 0.247$
30	29.0	20.4	3/49 (6)	$p = 0.121$
60	58.0	40.9	12/48 (25)	$p < 0.001$

[a] Average concentration calculated by multiplying chamber concentration by 6.2 hours/24 hours, 5 days/7days, and 5.24 mg/m^3/ppm.

[b] Lifetime average doses were determined by multiplying the average concentrations during the dosing period by the rat breathing rate (males: 0.262 m^3/day; females: 0.182 m^3/day) divided by the rat body weight (males: 0.445 kg; females: 0.258 kg). The dosing period was at least the standard lifespan of the test animals (104 weeks for rodents), so no correction for less than lifetime exposure was required.

[c] Effective rate. Animals that died before the first occurrence of tumor (males, day 399; females, day 429) were removed from the denominator.

[d] The *p*-value listed next to dose groups is the result of pairwise comparison with controls using the Fisher Exact test. The *p*-value listed next to the control group is the result of the Poly-3 trend test, as reported by NTP (2000).

Methodology

The default approach, as originally delineated by CDHS (1985), is based on a linearized form of the multistage model of carcinogenesis (Armitage and Doll, 1954). Cancer potency is estimated from the upper 95% confidence bound, q_1^*, on the linear coefficient q_1 in a model relating lifetime probability of cancer (p) to dose (d):

$$p = 1 - \exp[-(q_0 + q_1\, d + q_2\, d^2 + \ldots)] \qquad (1)$$

The parameter q_1^* is estimated by fitting the above model to dose response data using MSTAGE (Crouch, 1992).

For a given chemical, the model is fit to one or more data sets. The default approach is to select the data for the most sensitive species and sex. For carcinogens that induce tumors at multiple

sites, or at the same site but arising from different cell types, in a particular species and sex, cancer potency is taken to be the sum of potencies from the different sites or cell types. This approach assumes that tumors arising at different sites or from different cell types are independent. Because of the statistical uncertainty in individual estimates of potency, the terms are summed statistically as follows. A distribution of estimates corresponding to the 0.1 through 99.9 percentiles of the linear term (q_1) of the multistage model (Equation 1) is generated for each treatment-related tumor site in a given species and sex using the computer program MSTAGE (Crouch, 1992), modified to tabulate percentile values. (Distributional values stem from the assumption that twice the log likelihood function is χ^2 distributed). The discretized distributions were used to obtain a distribution of the sum of q_1s for each site affected by the chemical using Monte Carlo simulation (100,000 trials; Crystal Ball 2000 software, Decisioneering, Inc., Denver, Colorado). The upper 95 percent confidence bound on the summed q_1s is taken as q_1* for the combined tumor sites.

To estimate animal potency, q_{animal}, the parameter q_1* is adjusted to account for short duration of an experiment by assuming that the lifetime incidence of cancer increases with the third power of age. However, the durations of the studies examined here (NTP, 1992; 2000) were at least the standard lifespan of the test animals (104 weeks for rodents), so this correction was not required. Thus, for the calculations based on the NTP (1992; 2000) studies, q_1* is equivalent to q_{animal}.

Interspecies extrapolation from experimental animals to humans is normally based on the following relationship (Anderson *et al*., 1983), where bw_h and bw_a are human and animal body weights, respectively, and potency (e.g., q_{animal}) is expressed on a dose per body weight basis:

$$q_{human} = q_{animal} \times \left(\frac{bw_h}{bw_a} \right)^{\frac{1}{3}} \qquad (2)$$

Alternatively, when performing calculations based on applied dose in terms of air concentrations, the assumption has sometimes been made that air concentration values are equivalent between species (CDHS, 1985). However, using the interspecies scaling factor shown above is preferred because it is assumed to account not only for pharmacokinetic differences (*e.g.*, breathing rate, metabolism), but also for pharmacodynamic considerations. Therefore, lifetime average doses in mg/kg-day were determined (details provided below) and used in the calculation of q_{animal} in $(mg/kg\text{-}day)^{-1}$. The interspecies scaling factor was applied to q_{animal} to obtain q_{human} in $(mg/kg\text{-}day)^{-1}$. Unit risk in $(mg/m^3)^{-1}$was determined from q_{human} in $(mg/kg\text{-}day)^{-1}$ by applying a conversion factor (the ratio of human breathing rate [20 m^3/day] to human body weight [70 kg]).

Male and female rats (NTP, 2000) were exposed 6.2 hours/day, five days/week for 105 weeks. Female mice (NTP 1992) were exposed six hours/day, five days/week for 104 weeks. Average concentrations during the dosing period were calculated by multiplying the reported chamber concentrations by 6 or 6.2 hours/24 hours, five days/seven days and 5.24 mg/m^3/ppm. The average body weight of female mice was estimated to be approximately 0.029 kg based on data for controls reported by NTP (1992). The average body weights of male and female rats were calculated to be 0.445 kg and 0.258 kg, respectively, based on data for controls reported by NTP (2000). Inhalation rates (I) in m^3/day for mice and rats were calculated based on Anderson *et al.* (1983):

$$I_{mice} = 0.0345 \times (bw_{mice}/0.025)^{2/3} \quad (3)$$

$$I_{rats} = 0.105 \times (bw_{rats}/0.113)^{2/3} \quad (4)$$

Breathing rates were calculated to be 0.038 m^3/day for female mice, 0.262 m^3/day for male rats, and 0.182 m^3/day for female rats. Lifetime average doses were determined by multiplying the average concentrations during the dosing period by the appropriate animal breathing rate divided by the corresponding animal body weight. The dosing periods in the NTP studies were at least the standard lifespan of the test animals (104 weeks for rodents), so no correction for less than lifetime exposure was required.

An alternative dose description approach, using pharmacokinetic analyses based on models described in the literature (Willems *et al*., 2001; Quick and Shuler, 1999; Sweeney *et al*., 1996; Frederick *et al*., 1998, 2001; NTP, 2000) was evaluated. Although no data were available on the metabolism of naphthalene by rodent nasal tissues, simulations were conducted using parameters for rats and mice assuming either lung-like or liver-like scaling. The model predictions evaluated included amounts of naphthalene metabolized in each of the seven nasal compartments and their sum and the areas under the concentration × time curves (AUCs) for the olfactory and ventral respiratory compartments. Since all of these metrics appeared linear and in relative proportion to the applied doses, they did not indicate any substantial difference from the default potency analysis. If the assumptions used are correct, the concentrations used in the NTP studies were below those at which saturation of metabolism or other pharmacokinetic effects become important in the nasal and lung regions.

Application of an uptake rate for naphthalene was also considered. NTP (2000) estimated inhalation uptakes of 22 to 31 percent for rats and 65 to 73 percent for mice based on pharmacokinetic data and PBPK modeling. However, in the subsequent publication of NTP's PBPK modeling of inhaled naphthalene, uptakes are estimated to be 85 to 94 percent in rats and 92 to 96 percent in mice (Table 3, Willems *et al*., 2001). Until more reliable estimates become available we assume there are no significant differences in uptake between mice and rats used in the NTP bioassay. Also we assume similar uptake in humans exposed to low levels of naphthalene.

Table 8 provides the q_{animal}, q_{human} and unit risk values, calculated using the linearized multistage procedure as described above, based on data for female mice (NTP, 1992) and male and female rats (NTP, 2000).

Table 8. Cancer potency and unit risk values for naphthalene derived using the linearized multistage procedure based on data from NTP (1992) and NTP (2000).

Sex, Species	Site, Tumor Type	q_{animal}	q_{human}[a]	Human Unit Risk Value[b]	Goodness-of-Fit Test[c]
		$(mg/kg\text{-}day)^{-1}$	$(mg/kg\text{-}day)^{-1}$	$(mg/m^3)^{-1}$	
Female mice	Lung alveolar/bronchiolar adenoma/carcinoma	0.004382	0.059	0.017	$p = 0.1428$
Male rats	Nasal respiratory epithelial adenoma	0.01919	0.10	0.030	$p = 0.4192$
	Nasal olfactory epithelial neuroblastoma	0.004651	0.025	0.0072	$p = 0.4224$
	All naphthalene-related tumor sites in male rats	0.02219	0.12	0.034	NA[d]
Female rats	Nasal olfactory epithelial neuroblastoma	0.007636	0.049	0.014	$p = 0.6342$

[a] The interspecies extrapolation was applied to q_{animal} in $(mg/kg\text{-}d)^{-1}$ to determine q_{human} $(mg/kg\text{-}day)^{-1}$, as described above.

[b] Unit risk was determined by multiplying the human cancer potency in $(mg/kg\text{-}day)^{-1}$ by the human breathing rate divided by human body weight, as described above.

[c] A *p*-value of greater than 0.05 for the chi-square goodness-of-fit test indicates an adequate fit.

[d] Not applicable.

U.S. EPA (2003) and others (*e.g.* Gaylor *et al.*, 1994) have more recently advocated a benchmark dose method for estimating cancer risk. This involves fitting an arbitrary mathematical model to the dose-response data. A linear or multistage procedure is often used, although others may be chosen in particular cases, especially where mechanistic information is available which indicates that some other type of dose-response relationship is expected, or where another mathematical model form provides a better fit to the data. A point of departure on the fitted curve is defined: for animal carcinogenesis bioassays this is usually chosen as the lower 95% confidence bound on the dose predicted to cause a 10% increase in tumor incidence (LED_{10}). Linear extrapolation from the point of departure to zero dose is used to estimate risk at low doses either when mutagenicity or other data imply that this is appropriate, or in the default case where no data on mechanism are available. The slope factor thus determined from the experimental data is corrected for experimental duration and interspecies extrapolation in the same way as the q_1^* adjustments described for the linearized multistage procedure. In the exceptional cases where data suggesting that some other form of low-dose extrapolation, such as the assumption of a threshold, is appropriate, a reference dose method with safety factors as required may be used instead.

The benchmark dose methodology was applied to the tumor incidence data for naphthalene in the NTP (1992; 2000) studies. Genetic toxicology results for naphthalene are mixed: *Salmonella* reverse mutation assays were generally negative, but some test results with eukaryotic systems *in vivo* or *in vitro* were positive (NTP, 2000). However, it was considered on balance that the weight of evidence, including metabolism to 1-naphthol via an epoxide intermediate (NTP, 1992, citing Bock *et al.*, 1976 and others; NTP, 2000), and the reactivity of naphthoquinones to cellular components (Zheng *et al.*, 1997) favors the interpretation that the mechanism of naphthalene

carcinogenicity likely involves a reactive metabolic intermediate which causes direct damage to DNA. A low dose linearity assumption is therefore appropriate when extrapolating from the point of departure to obtain an estimate of the cancer risk at low doses.

Model fits, points of departure and unit risks calculated using the benchmark methodology and U.S. EPA's Benchmark Dose Software version 1.3 are shown in Table 9. In all three cases, the model used was either a multistage polynomial, or a quantal linear model, which is identical to the multistage procedure in cases where the higher terms are not significant.

Table 9: Unit risk and human cancer potency values for naphthalene based on NTP (1992) and NTP (2000), derived using benchmark methodology.

Sex, Species	Site, Tumor Type	Model Fit:[a]	LED_{10} [ED_{10}] (mg/m^3)	Animal Unit Risk Value[b] $(mg/m^3)^{-1}$	Human Unit Risk Value[c] $(mg/m^3)^{-1}$ [Human Cancer Potency][c] $(mg/kg\text{-}d)^{-1}$
Female mice	Lung alveolar/bronchiolar adenoma/carcinoma	$\chi^2 = 1.42$ $p = 0.23$	17.1 [22.8]	0.0058	0.017 [0.059]
Male rats	Nasal respiratory epithelial adenoma	$\chi^2 = 2.82$ $p = 0.42$	9.3 [12.5]	0.0108	0.028 [0.099]
	Nasal olfactory epithelial neuroblastoma	$\chi^2 = 2.82$ $p = 0.42$	38.5 [67.6]	0.0026	0.0068 [0.024]
	All naphthalene-related tumor sites in male rats	NA	8.1[d] [10.6]	0.012	0.031 [0.11]
Female rats	Nasal olfactory epithelial neuroblastoma	$\chi^2 = 1.73$ $p = 0.63$	18.1 [26.4]	0.0055	0.014 [0.050]

[a] A *p*-value of greater than 0.05 for the chi-square goodness-of-fit test indicates an adequate fit.

[b] Animal unit risk was calculated using the relationship $0.1/LED_{10}$.

[c] The interspecies extrapolation from rodent unit risks to human unit risks was based on the $(mg/kg\text{-}d)^{-1}$ equivalents of the animal unit risks, as described above. The following parameters were used to derive the $(mg/kg\text{-}day)^{-1}$ equivalents of the animal unit risks: bw_{animal} = 0.029 kg for female mice, 0.445 kg for male rats, and 0.258 kg for female rats; I_{animal} = 0.038 m^3/d for female mice, 0.262 m^3/d for male rats and 0.182 m^3/d for female rats. Human cancer potency was derived by applying the interspecies scaling factor to the $(mg/kg\text{-}day)^{-1}$ equivalents of the animal unit risks. The interspecies scaling factor is $(bw_{human}/bw_{animal})^{1/3}$, or 13.4 for female mice, 5.4 for male rats, and 6.5 for female rats. Human unit risks were then derived by multiplying human cancer potency by the human breathing rate (20 m^3/day) divided by the human body weight (70 kg).

[d] The LED_{10} in mg/m^3 for the multi-tumor analysis in rats was calculated by assuming a linear dose response relationship: $LED_{10} = -\ln(0.9)/(q_{animal} \times [I_{animal}/bw_{animal}])$. By inspection, this assumption appears reasonable for the dose-response curves considered, in the ED_{10} range. For the current case, q_{animal} is the cancer potency in rats (0.02219 $[mg/kg\text{-}day]^{-1}$) generated using the multi-tumor analysis described above, I_{animal} is the breathing rate in male rats (0.262 m^3/day), and bw_{animal} is the male rat body weight (0.445 kg).

Using either of these methodologies, the 95% upper confidence bound on the unit risk value for purposes of calculating cancer risks associated with exposure to naphthalene is in the range 0.014-0.034 $(mg/m^3)^{-1}$, based on the incidence data in female mice and male and female rats from the NTP (1992; 2000) studies.

The male rat was the most sensitive sex and species tested by NTP (1992; 2000) in the inhalation

carcinogenesis studies of naphthalene. NTP considered the increased incidences of nasal respiratory epithelial adenoma and nasal olfactory epithelial neuroblastoma, which are rare tumors, to provide clear evidence of the carcinogenic activity of naphthalene. The unit risk value of 0.034 $(mg/m^3)^{-1}$, or 3.4 x 10^{-5} $(\mu g/m^3)^{-1}$, based on the tumor incidence data in male rats, is therefore considered the most appropriate for use in risk assessment.

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