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TOXICOLOGICAL REVIEW

OF

NAPHTHALENE

(CAS No. 91-20-3)

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

August 1998

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(Inhalation cancer assessment and other selected text, as indicated)

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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 assay, 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	<i>S. typhimurium</i> TA1535, TA1537, TA98, TA100	100 µg/plate ± S9 activation	100	Negative	McCann et al., 1975
	<i>S. typhimurium</i> TA1535, TA1537, TA98, TA100	0.3-100 µg/plate ± S9 activation	100	Negative	Mortelmans et al., 1986
	<i>S. typhimurium</i> TA1535, TA1537, TA98, TA100	0.3-100 µg/plate ± S9 activation	100	Negative	NTP, 1992a
	<i>S. typhimurium</i> TA1537, TA1538	10-200 µg/plate ± S9 activation	100	Negative, toxic above 100 µg/plate	Gatehouse, 1980
	<i>S. typhimurium</i> TA98, TA100	10-50 µg/plate ± S9 activation	50	Negative	Bos et al., 1988
	<i>S. typhimurium</i> TA1535, TA1537, TA98, TA100	0.03-30 µmol/plate ± S9 activation	3	Negative, toxic above 3 µmol/plate	Florin et al., 1980
	<i>S. typhimurium</i> TA1535, TA1537, TA98, TA100	250 µg/plate ± S9 activation	250	Negative	Sakai et al., 1985
	<i>S. typhimurium</i> TA1535, TA1537, TA1538, TA98, TA100	3-300 µg/plate ± S9 activation	300	Negative, toxic above 300 µg/plate	Godek, 1985
	<i>S. typhimurium</i> TM677	1-2 mM ± S9 activation	2	Negative	Kaden et al., 1979
	<i>S. typhimurium</i> 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
	<i>S. typhimurium</i> UTH8413, UTH8414, TA98, TA100	100-2000 µg/plate ± S9 activation	2000	Negative	Connor et al., 1985
	<i>S. typhimurium</i> TA1535, TA1537, TA98, TA100	1000 µg/plate ± S9 activation	1000	Negative (1-naphthol)	McCann et al., 1975
	<i>S. typhimurium</i> TA98, TA1535	5-1000 µg/plate ± S9 activation	1000	Negative (1-naphthol)	Narbonne et al., 1987
	<i>S. typhimurium</i> TA1535, TA1537, TA98, TA100	250 µg/plate ± S9 activation	250	Negative (1,4-naphthoquinone)	Sakai et al., 1985
	<i>S. typhimurium</i> 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	<i>S. typhimurium</i> TA1535/p5K1002 (uMuC-lacZ)	83 µg/mL ± S9 activation	83	Negative	Nakamura et al., 1987
SOS chromotest	<i>E. coli</i> K12 inductest (λ lysogen GY5027; uvrB ⁻ , envA ⁻)	2000 µg/plate ± S9 activation	2000	Negative	Mamber et al., 1984
	<i>E. coli</i> PQ37 (sfiA::lacZ fusion)	0.156-10.0 µg/assay ± S9 activation	10	Negative	Mersch-Sundermann et al., 1993
Pol A ⁻ or Rec assay	<i>E. coli</i> WP2/WP10 (uvrA ⁻ , recA ⁻)	2000 µg/mL ± S9 activation	2000	Negative	Mamber et al., 1983
	<i>E. coli</i> 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	<i>E. coli</i> WP2/WP3478 (pol A ⁻)	dose not specified ± S9 activation	NS	Negative	Mamber et al., 1983
Mutatox (reversion to luminescence)	<i>V. fischeri</i> M169	Up to 5000 µg/tube ± S9 activation	0.203 0.625	Negative without S9 activation Positive with S9 activation	Arfsten et al., 1994
<i>In vitro</i> 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
<i>In vitro</i> eukaryotic gene mutation, cytogenetic, or DNA damage assays (continued)					
Alkaline elution (<i>in vitro</i>)	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 (<i>in vitro</i>)	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
<i>In vivo</i> eukaryotic gene mutation, cytogenetic, or DNA damage assays					
Somatic mutation, recombination	<i>D. melanogaster</i>	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
<i>In vivo</i> eukaryotic gene mutation, cytogenetic, or DNA damage assays (continued)					
Micronuclei induction	Salamander larvae (<i>Pleurodeles waltl</i>): 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 (<i>in vivo</i>)	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 (<i>in vivo</i>)	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 (<i>in vivo</i>)	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, cause 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 be 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-1,2,3,4-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 ($\geq 50\%$) 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 Mice	400; 90 d 133-200; 90 d	BCL, 1980a 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 Mice	400; 90 d 120-200; 60-90 d	BCL, 1980a Shichi et al., 1980; BCL, 1980b; Shopp et al., 1984
Decreased body weight Effect observed	Rats Pregnant rats	200; 90 d 150; 10 d	BCL, 1980a NTP, 1991
No effect observed	Rats Mice Pregnant rats Pregnant rabbits Pregnant rabbits	100; 90 d 133-200; 90 d 50; 10 d 400; 12 d 120; 13 d	BCL, 1980a BCL, 1980b; Shopp et al., 1984 NTP, 1991 Naismith and Matthews, 1985 NTP, 1992b
Nervous system depression Effect observed (clinical signs including lethargy)	Rats Mice Pregnant rats Pregnant rabbits	400; 90 d 200; 90 d 50; 13 d 200; 12 d	BCL, 1980a BCL, 1980b NTP, 1991 Naismith and Matthews, 1986
No effect observed	Rats Mice Pregnant rabbits	200; 90 d 100-133; 90 d 40; 12 d	BCL, 1980a BCL, 1980b; Shopp et al., 1984 Naismith and Matthews, 1986

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 Pregnant rabbits Pregnant rabbits	300; 8 d 150; 13 d 630; 12 d	Plasterer et al., 1985 NTP, 1990 Naismith and Matthews, 1985
No effect observed	Pregnant rabbits Pregnant rabbits Pregnant rats	400; 12 d 120; 13 d 450; 10 d	Naismith and Matthews, 1986 NTP, 1992b NTP, 1991

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