TOXICOLOGICAL SCIENCES **111(2)**, 383–391 (2009)
doi:10.1093/toxsci/kfp138
Advance Access publication July 31, 2009

Upper Respiratory Tract Uptake of Naphthalene

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Received February 16, 2009; accepted June 18, 2009

Naphthalene is a nasal toxicant and carcinogen in the rat. Upper respiratory tract (URT) uptake of naphthalene was measured in the male and female F344 rat at exposure concentrations of 1, 4, 10, or 30 ppm at inspiratory flow rates of 150 or 300 ml/min. To assess the potential importance of nasal cytochrome P450 (CYP) metabolism, groups of rats were pretreated with the CYP inhibitor 5-phenyl-1-pentyne (PP) (100 mg/kg, ip). *In vitro* metabolism of naphthalene was similar in nasal tissues from both genders and was reduced by 80% by the inhibitor. URT uptake in female rats was concentration dependent with uptake efficiencies (flow 150 ml/min) of 56, 40, 34, and 28% being observed at inspired concentrations of 1, 4, 10, and 30 ppm, respectively. A similar effect was observed in male rats (flow 150 ml/min) with uptake efficiencies of 57, 49, 37, and 36% being observed. Uptake was more efficient in the male than female rat, likely due to their larger size (226 vs. 144 g). Uptake of naphthalene was significantly reduced by inhibitor pretreatment with the effect being greater at the lower inspired concentrations. Specifically, in pretreated female rats (150 ml/min), URT uptake averaged 25, 29, and 26% at inspired concentrations of 4, 10, and 30 ppm, respectively. Thus, the concentration dependence of uptake was virtually abolished by PP pretreatment. These results provide evidence that nasal CYP metabolism of naphthalene contributes to URT scrubbing of this vapor and is also involved in the concentration dependence of uptake that is observed.

Key Words: **naphthalene; upper respiratory tract; nose; inhalation dosimetry.**

Naphthalene is a ubiquitous air pollutant that is derived from a variety of sources including petroleum, coal, and tobacco smoke (Agency of Toxic Substances and Disease Registry [ATSDR], 2005; Buckpitt *et al*., 2002). Inhalation toxicity testing has revealed that naphthalene is a nasal toxicant and carcinogen in the rat. Chronic (2 year) exposure to naphthalene at concentrations of 10, 30, or 60 ppm results in marked inflammation and cytotoxicity in both the respiratory and the olfactory epithelium of the nasal cavity of the F-344 rat. Increased incidence of respiratory adenomas was observed in male rats; in female rats, an increased incidence of respiratory adenomas was observed as well as an increased incidence of olfactory neuroblastomas (Abdo *et al*., 2001; National Toxicology Program [NTP], 2001). The latter is considered to be an extremely rare response (North *et al*., 2008). NTP (2001) reported a historical incidence in 2-year studies of 0%. The mechanism(s) responsible for the toxicity, carcinogenesis, and the gender differences in these responses are not fully understood. Inhalation dosimetric patterns are often critical in influencing the response of the respiratory tract to inspired toxicants (Morris, 2006; US Environmental Protection Agency [US EPA], 1994), but there is currently no information on the nasal dosimetry of naphthalene.

Naphthalene is a low volatility, lipophilic, hydrophobic molecule with a vapor pressure of 0.09 torr at 25°C, a log octanol:water partition coefficient of 3.3, and an aqueous solubility of 32 mg/l (ATSDR, 2005). A relatively large database exists on nasal uptake efficiencies of vapors (e.g., Frederick *et al*., 2002; Hinderliter *et al*., 2005; Morris 1993, 1999a; Morris *et al*., 1993). However, to our knowledge, the existing data set does not extend to vapors with the limited volatility and high lipophilicity of naphthalene. Similarly, physiologically based pharmacokinetic (PBPK) models for nasal vapor uptake and disposition have been developed but not for compounds with the physical chemical characteristics of naphthalene. As indicated by Barton *et al*. (2007), there is a need to extend PBPK modeling approaches to a broader range of structures and properties. The overall goal of the current study was to provide data on nasal uptake of naphthalene to allow determination of whether or not our current understanding of nasal vapor dosimetry extends to vapors with the physical/chemical properties of naphthalene and to also provide data to support the development of PBPK modeling approaches for this ubiquitous pollutant.

The capacity of the nose to scrub vapor is dependent on inspiratory flow rate, vapor solubility (as measured by the tissue:air or blood:air partition coefficient) and the potential for the vapor to directly react with tissue substrates, and/or be metabolized *in situ* in the nasal mucosa (Morris, 2001, 2006). A blood:air partition coefficient of 571 has been estimated for naphthalene (NTP, 2000), suggesting that it should be scrubbed with moderate efficiency in the rat upper respiratory tract (URT) (Morris *et al*., 1993). This relatively high partition coefficient is

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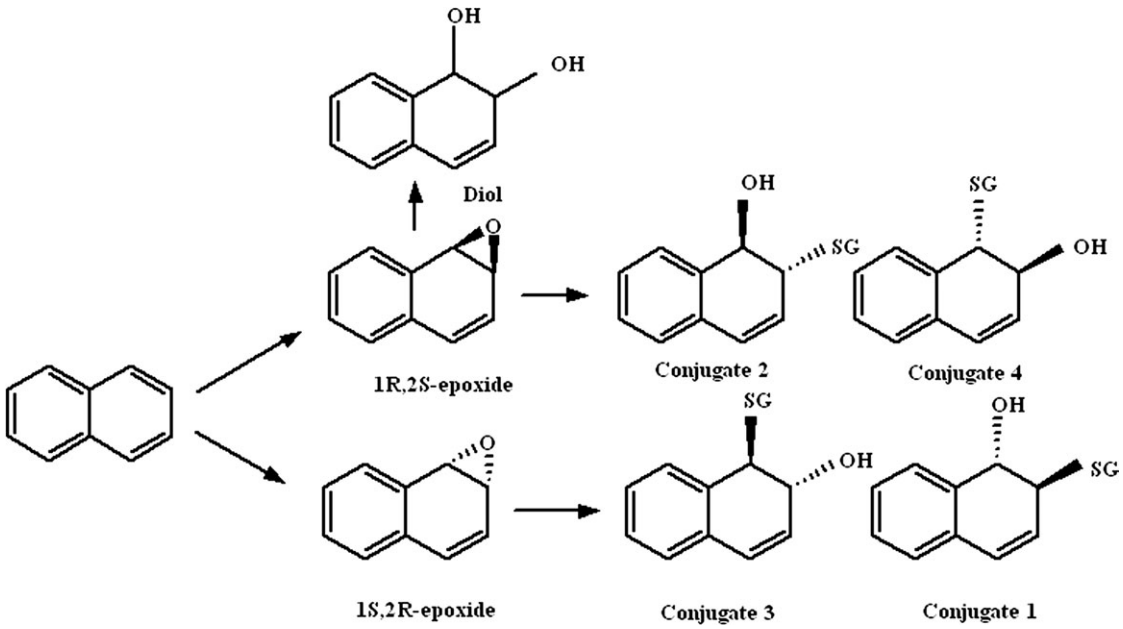

FIG. 1. Schematic diagram showing the metabolism of naphthalene to diol and glutathione (GSH) conjugates. The epoxides are unstable and are trapped as GSH adducts.

reflective of the lipophilicity not aqueous solubility because naphthalene is essentially insoluble in water (ATSDR, 2005). Because the blood:air partition coefficient of lipophilic vapors may be strongly dependent on the diet (Kaneko *et al*., 2000), the current study also included direct measurement of the naphthalene blood:air partition coefficient to confirm the partition coefficient in the rats used in the current uptake study.

The nasal metabolic pathways of naphthalene are well investigated (Fig. 1; Buckpitt *et al*., 2002; Lee *et al*., 2005), but there are limited data on gender differences in regional metabolism patterns within the rat nose, a potentially important consideration relative to the gender difference in induction of olfactory neuroblastomas. Naphthalene is metabolized within both the respiratory and the olfactory mucosa of the rat; the activity in the olfactory mucosa exceeds that in the respiratory mucosa by at least 40-fold (Lee *et al*., 2005). Naphthalene is oxidized, primarily by CYP2F, to naphthalene oxide (Baldwin *et al*., 2005; Bogen *et al*., 2008; Shultz *et al*., 1999). This process is thought to be critical in the cytotoxicity and carcinogenicity of this vapor (Bogen *et al*., 2008; Buckpitt *et al*., 2002). While systemically administered naphthalene can induce nasal olfactory toxicity, the pattern of injury among the ethmoid turbinates differs after systemic versus inhalation exposure (Lee *et al*., 2005). The induction of olfactory lesions after systemic exposure suggests that local activation is key in the toxic process (Lee *et al*., 2005; Morris, 2006). The differing patterns of injury after systemic versus inhalation exposure point to the importance of localized deposition patterns within the nose with respect to the injury resulting from inhalation exposure.

The specific aims of the current investigation were to provide data in both genders of rats on nasal uptake and metabolism and to define the contribution, if any, of local metabolism in the uptake process. The focus of this study was on olfactory metabolism because metabolism rates are highest in this tissue, and olfactory neuroblastomas represent such a rare response. Toward these ends, URT uptake efficiency of naphthalene was measured at two inspiratory flow rates in control rats and in rats pretreated with the cytochrome P450 (CYP) suicide inhibitor 5-phenyl-1-pentyne (PP) (Roberts *et al*., 1998). This inhibitor has been shown to prevent nasal toxicity of naphthalene in the mouse (Genter *et al*., 2006). To confirm that PP was without nonspecific effects on nasal uptake, uptake of acetone (a nonmetabolized vapor; Morris *et al*., 1986) was measured in control and PP-treated mice. The effect of PP on naphthalene metabolism was assessed by determination of naphthalene metabolic activity *in vitro*, after ip administration of the suicide inhibitor. URT uptake of naphthalene was measured at exposure concentrations of 1, 4, 10, and 30 ppm to cover a range of concentrations including those used in the chronic inhalation bioassay (Abdo *et al*., 2001; NTP, 2000). Results indicated that naphthalene was scrubbed from the air with moderate efficiency in both the male and the female rats URT with metabolism contributing to the uptake process, particularly at low exposure concentrations.

MATERIALS AND METHODS

Animals and reagents. Male and female F344 rats (VAF/Plus Crl:CDBR) used in the nasal uptake studies were obtained from Charles River

(Wilmington, MA). Rats were 5–6 weeks old at delivery and were acclimated for at least 1 week prior to use. Animals were housed over hard wood bedding in rooms maintained at 22°C–25°C with a 12-h light cycle (lights on at 0630 h). Food (Lab Diet; PMI Nutrition International, Brentwood, MO) and tap water were provided *ad libitum*. Body weights of the male and female rats averaged 226 and 144 g, respectively, at the time of use. Some animals were pretreated with the suicide CYP 450 inhibitor, PP (Roberts *et al*., 1998). The inhibitor was dissolved in sesame oil (5 mg/ml) and was administered at a dose of 100 mg/kg by ip injection 1–2 h prior to uptake measurement or tissue collection. When collected, blood was obtained via cardiac puncture of anesthetized rats with heparinized syringes. All protocols were approved by the University of Connecticut IACUC.

Animals used to demonstrate that the doses and dose timing of the PP would produce adequate inhibition of naphthalene metabolism were F344 from Harlan Laboratories, Indianapolis, IN, and were 7–8 weeks of age at delivery. All animals were housed in high efficiency particulate air filtered cage racks for at least 1 week in animal facilities at UC Davis prior to use. Animals were provided food and water *ad libitum* and were used under protocols approved by the IACUC at UC Davis.

Partition coefficient determination. Partition coefficients were determined in a 300-ml glass vial with headspace air being drawn off the vial through stainless steel tubing directly into a gas sampling valve for analysis by gas chromatography (see below). To minimize adsorption, no plastic was present anywhere within the system. Recovery of added naphthalene in this apparatus averaged 96.4 ± 3.4%. Naphthalene (3 μl sample dissolved in ethanol) was added to either empty vials or vials containing 10 ml water or 0.30 ml of freshly drawn rat blood. Similar values for partition coefficient were obtained if differing volumes of fluid were used. Blood was used on the day of collection and stored on ice. After addition of naphthalene, vials were incubated for at least 1 h at 37°C and headspace air was injected into a gas chromatograph for determination of naphthalene concentration (see below). Partition coefficients were calculated based on the difference in airborne concentration between empty and water- or blood-containing vials assuming mass balance.

Naphthalene metabolism and analysis. Following euthanasia, ethmoid samples were combined from two animals each to assess naphthalene metabolic profiles. A total of eight animals of each sex were administered vehicle alone (control) or PP intrapertinoneally 2 h prior to euthanasia. Microsomes were prepared by differential centrifugation as described by Lee *et al.* (2005). Microsomes were incubated with 0.25mM naphthalene in the presence of optimal amounts of glutathione and glutathione transferase for 15 min at 37°C, and reactions were quenched by the addition of ice-cold methanol. Precise determinations of the K_m for naphthalene metabolism are not available but estimates based on trapping of the intermediate epoxide are ~50μM (Lee *et al*., 2005). Based on the velocity-substrate concentration curves, concentrations of 160μM were at or near maximal velocity (V_{max}). Samples were centrifuged to remove protein, and the supernatants were evaporated to dryness under reduced pressure and reconstituted in water for high-performance liquid chromatography analysis. Rates of formation of 1,2-dihydro-1,2-dihydroxynaphthalene and four diastereomeric glutathione conjugates were measured as described earlier (Shultz *et al*., 1999). Microsomal yields from septal tissue containing respiratory mucosa were low, and rates of metabolism could be measured in only a few samples.

URT uptake measurement. The surgical procedure and protocols for uptake measurement have been described in detail (Morris, 1999b). Briefly, animals were anesthetized with urethane (1.3 g/kg ip). After the onset of anesthesia, the trachea was isolated, incised ~3 mm below the larynx, and an endotracheal tube (PE205 tubing) was inserted anteriorly until its tip was just past the larynx and was ligated in place. This served to isolate the URT, defined as all regions of the respiratory tract anterior to larynx. URT uptake was then measured as described below. After uptake measurement, animals were killed by exsanguination.

To measure URT uptake, the surgically prepared animal was placed in a nose-only exposure chamber in a supine position and the endotracheal tube was connected to an air sampling train. This sampling train served to draw chamber-laden air through the isolated URT under constant velocity flow conditions for 1 h. This is of sufficient duration for attainment of steady-state uptake conditions. Flow rates of 150 and 300 ml/min were used for both male and female rats. The minute ventilation rates of the male and female rats differed because they were of differing body weight (but of the same age). Rather than using differing flows based upon constant fractions of estimated minute ventilations, it was deemed preferable to use identical flows to avoid reliance on algorithm-based estimates of minute ventilation. For comparative purposes, it can be noted that the 150 ml/min flow rate corresponds to ~130 and 190% of the predicted minute ventilation (Brown *et al*., 1997) of male and female rats, respectively, while the 300 ml/min flow rate corresponds to ~270 and 380%. To facilitate gender comparisons, results were normalized to body weight to the three-quarters power. This normalization was used because it has long been appreciated that rates correlate closely with the three-quarters power of body weight (Brown *et al*., 1997; Guyton, 1947; US EPA, 1994).

The sample line consisted of stainless steel tubing with a tee. Air was bled off the tee and drawn through the gas sampling valve of the gas chromatograph (see below) through stainless steel tubing at a flow of 20 ml/min. Total flow rates were maintained at 150 and 300 ml/min via rotameters that had been calibrated in the sample line with a bubble meter. Immediately before and after animal exposures, the sample train was connected directly to the nose-only inhalation chamber to measure inspired air (Cin) naphthalene concentrations. During animal exposures, the sample train was connected to the endotracheal tube for measurement of naphthalene concentrations in air exiting the isolated URT (Cex). The ratio of the before and after Cin concentrations was 101.1 ± 8.3% (mean ± SD), indicating that chamber concentration remained constant. Uptake efficiency was calculated from the Cin and Cex values for each rat as described previously (Morris, 1999b).

To determine if PP exerted nonspecific effects on the nasal uptake process, uptake of acetone was measured in control and PP-pretreated male rats at a flow rate of 150 ml/min using a methodology identical to that used as for naphthalene uptake measurement. Acetone was selected for this purpose because it has been previously studied in our laboratory and is not significantly metabolized in the URT of the rat (Morris *et al*., 1986).

Atmosphere generation and analytical procedures. Naphthalene atmospheres were generated by passing clean air over crystalline naphthalene in controlled temperature (22°C) glass flasks. This naphthalene-laden air was mixed with heated humidified air and passed into a nose-only inhalation chamber. Chamber air temperature was ~38°C and relative humidity exceeded 75%. Airlines and the chamber walls were heated and humidified to prevent condensation of water vapor. Nominal exposure concentrations were 1, 3, 10, and 30 ppm. These concentrations were used to cover the range of concentrations used in the inhalation bioassays for naphthalene (Abdo *et al*., 2001; NTP, 2001) and included 1 ppm as it was the lowest concentration at which uptake could be easily quantitated by the analytical methodology. Measured exposure concentrations were: 0.9 ± 0.1, 4 ± 0.7, 10 ± 1.2, and 34 ± 5.0 (mean ± SD). These are referred to subsequently as the 1-, 4-, 10-, and 30-ppm groups, respectively. Airborne vapor concentration was measured by gas chromatography with flame ionization detector detection (Varian model 3600; Varian Inc., SugarLand, TX) equipped with a gas sampling valve with two 0.5-ml sample loops. The gas chromatograph was programmed to inject sample onto the column (DB-WAX megabore; Agilent, Santa Clara, CA) at 3-min intervals to provide continuous sampling. The carrier gas was nitrogen. Peak areas were converted to airborne concentration on the basis of standard curves that were generated by injecting naphthalene (dissolved in ethanol) or acetone (dissolved in water) into a 4.3-l gas bottle, allowing 1 h for equilibration, and drawing air from the bottle through the sample train.

Statistical analysis. Data are reported as mean ± SD unless otherwise indicated. Data were analyzed by linear regression or multifactorial ANOVA followed by Newman-Keuls test. Calculations were performed with Statistica software (StatSoft, Tulsa, OK). A $p < 0.05$ was required for statistical significance.

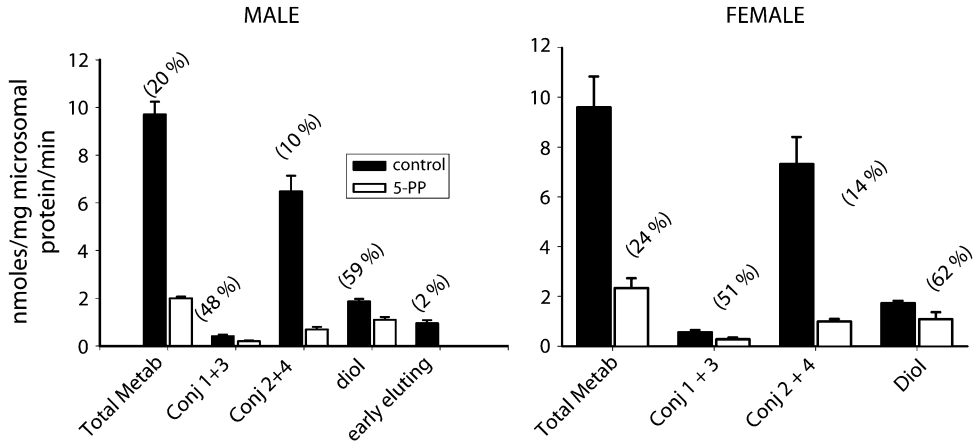

FIG. 2. Naphthalene metabolism rates in olfactory mucosa of the male and female rats. Data are shown as mean ± SD of an *n* of 4 separate incubations with micromes prepared from two animals per pool. The values in parenthesis represent the activity in PP-treated samples expressed as a percent of control activity.

RESULTS

Partition Coefficient

The measured values for water:air partition coefficient averaged 14 ± 4 ($n = 9$). Similar values were obtained regardless of the amount of water or the amount of naphthalene added to the vial. The blood:air partition coefficients were similar in both genders, averaging 700 and 760 in samples from five males and five females, respectively. The overall average was 725 ± 124.

PP Characterization Studies

In vitro studies were performed to characterize naphthalene metabolism in nasal tissues of male and female rats and confirm that the inhibitor PP diminished metabolism rate. In addition, *in vivo* studies were performed to confirm that PP did not alter URT uptake of a nonmetabolized vapor. Shown in Figure 2 are the naphthalene metabolism rates in olfactory epithelia of control and PP-pretreated male and female rats. PP pretreatment decreased the overall rates of naphthalene metabolism by nearly 80% in both genders but appeared to have a differential effect on the formation of the 1R,2S-epoxides (90% compared to 50% for the 1S,2R-epoxide). This is also reflected in less inhibition of the formation of naphthalene diol which is generated selectively from the 1S,2R-epoxide. The rates of naphthalene metabolism are almost identical in males and females (9.7 vs. 9.5 nmol/mg microsomal protein/min, respectively), the metabolite profile was similar in both genders as well. Assuming microsomal protein accounts for one fifth of the total protein content and nasal olfactory mucosal tissue wet weight is ~100 mg (Casanova-Schmitz *et al.*, 1984), it can be estimated that the total metabolic activity of the olfactory mucosa is ~20 nmol/min. The total activity in respiratory mucosal microsomes was at least 40-fold lower than that in microsomes from olfactory epithelia and although detectable was too low to allow for accurate speciation and quantitation of specific metabolites (data not shown). Activity was not detectable in respiratory mucosal microsomes from PP-pretreated rats.

The effects of PP pretreatment on uptake of a nonmetabolized vapor (acetone) were assessed to provide information on the potential for the inhibitor to produce nonspecific effects on nasal vapor uptake processes. URT uptake of acetone, a nonmetabolized vapor (Morris *et al.*, 1986), was measured in control and PP-pretreated male rats at an inspiratory flow rate of 150 ml/min. Uptake efficiencies averaged 25 ± 4.4 ($n = 8$) and 25 ± 4.8 ($n = 6$) in the control and pretreated rats, respectively ($p > 0.5$). Nasal airflow resistance was measured in these animals during acetone exposure and was unaltered by the inhibitor (data not shown).

Naphthalene Uptake Studies

Shown in Figure 3 are the URT uptake efficiencies for naphthalene concentrations in control and PP-pretreated female

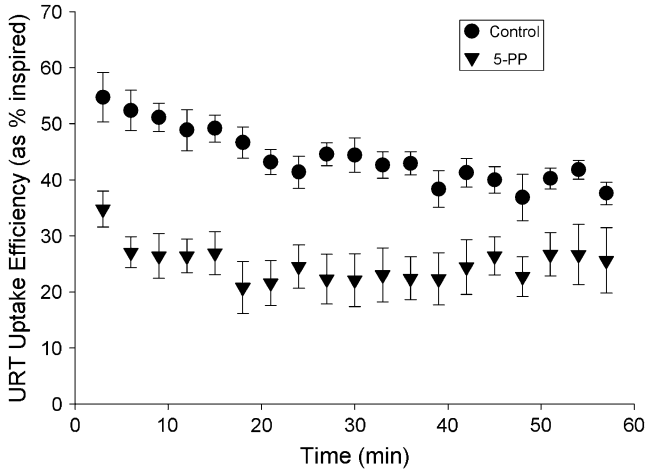

FIG. 3. Time course of naphthalene concentration in URT exiting air during the 1-h exposure to 4 ppm naphthalene in control (circles) and PP-pretreated (triangles) female rats at a flow rate of 150 ml/min. Data are shown as mean ± SD.

TABLE 1
URT Naphthalene Uptake in the Female Rat[a]

	Female rats				
	1 ppm	3 ppm	10 ppm	30 ppm	PP pretreated
150 ml/min	56.1 ± 13.1	40.0 ± 5.7	34.6 ± 5.1	28.4 ± 7.7	26.2 ± 8.7
300 ml/min	31.7 ± 7.8	24.0 ± 6.9	21.3 ± 6.4	16.3 ± 4.2	14.5 ± 7.6[b]

[a]Data are presented as mean ± SD. Each concentration group contained 7–8 rats and the pooled PP-treated groups contained 28–29 rats.

[b]Data were analyzed by two-factor ANOVA which revealed a significant difference between flow rates ($p < 0.001$), a significant difference among groups ($p < 0.001$) and no interaction between factors ($p = 0.2$). Underscoring indicates the results of Newman-Keuls *post-hoc* test, groups which did not differ significantly from each other ($p > 0.05$) share the same underscore, groups that differed at the $p < 0.05$ level do not share the same underscore. Since a statistical interaction was not observed, the underscoring reflects the relationships at both flow rates.

TABLE 2
URT Naphthalene Uptake in the Male Rat[a]

	Male rats				
	1 ppm	3 ppm	10 ppm	30 ppm	PP pretreated
150 ml/min	57.4 ± 13.1	49.3 ± 9.0	37.0 ± 3.0	36.1 ± 9.3	33.0 ± 7.8
300 ml/min	32.9 ± 13.4	29.0 ± 8.6	20.4 ± 5.6	20.0 ± 4.4	16.3 ± 7.3[b]

[a]Data are presented as mean ± SD. Each concentration group contained 6–12 rats and the pooled PP-treated groups contained 29–30 rats.

[b]Data were analyzed by two-factor ANOVA which revealed a significant difference between flow rates ($p < 0.001$), a significant difference among groups ($p < 0.001$) and no interaction between factors ($p = 0.5$). Underscoring indicates the results of Newman-Keuls *post-hoc* test, groups which did not differ significantly from each other ($p > 0.05$) share the same underscore, groups that differed at the $p < 0.05$ level do not share the same underscore. Since a statistical interaction was not observed, the underscoring reflects the relationships at both flow rates.

rats during exposure to 4 ppm naphthalene. Exiting air naphthalene concentrations reached a plateau within 25 min of the onset of exposure. Linear regression analysis revealed that percent uptake did not change significantly during the last one half of exposure (30–60 min); the slope of the uptake efficiency versus time averaged −0.2 ± 0.1 and 0.002 ± 0.002 (mean ± SEM) in the control and PP animals, respectively. Similar results were observed at other exposure concentrations in both male and female rats. Since uptake efficiency remained steady, the average uptake efficiency during the last one half of exposure was averaged for each animal for subsequent analysis.

Shown in Table 1 are the average URT uptake efficiencies in the female rat uptake studies. Uptake efficiency was measured at both flow rates (150 and 300 ml/min) in PP-pretreated rats at all exposure concentrations. In PP-pretreated rats, uptake efficiency at 300 ml/min averaged 8–18% in the 1-, 4-, 10-, and 30-ppm groups; the values did not differ significantly from each other ($p > 0.05$); at 150 ml/min, uptake efficiency averaged 24–29% which, again, did not differ significantly from each other ($p > 0.05$). Since uptake efficiency did not differ significantly among the concentration groups, the data from each concentration were pooled to form a single PP-pretreated group. The pooled data are shown in Table 1. Two-factor ANOVA revealed a significant effect of flow rate ($p < 0.0001$) and a significant difference among concentration groups ($p < 0.0001$), a statistical interaction between flow rate and concentration group was not detected ($p > 0.05$). As can be seen, uptake efficiency decreased with increasing exposure concentration in metabolically competent (nonpretreated) controls. The uptake efficiency in the 1- and 4-ppm group exceeded that in the 10- and 30-ppm groups ($p < 0.05$, Newman-Keuls test). Uptake efficiency in the 1-, 4-, and 10- but not 30-ppm group exceeded that in the PP-treated animals ($p < 0.05$, Newman-Keuls test). Delivered dosage rates can be calculated from the product of the inspired concentration, flow rate, and uptake efficiency. These averaged ~3, 10, 20, and 60 nmol/min in the 1, 4, 10, and 300-ppm control female rat groups, respectively. Expressed per gram body weight to the three-quarters power, these values were 15, 50, 90, and 240 nmol/min-kg$^{0.75}$.

Shown in Table 2 are the average uptake efficiencies in the male rat uptake studies. Uptake efficiency in the male rats was higher ($p < 0.001$, multifactorial ANOVA) than in the female rats (see Table 1). These data were analyzed similarly to the female rat data. Uptake efficiency was measured at both flow rates (150 and 300 ml/min) in PP-pretreated rats at all exposure concentrations. In PP-pretreated rats, uptake efficiency at 150 ml/min averaged 29–39% in the 1-, 4-, 10-, and 30-ppm groups; the values did not differ significantly from each other ($p > 0.05$); at 300 ml/min uptake efficiency averaged 14–18% which, again, did not differ significantly from each other ($p > 0.05$). Since uptake efficiency did not differ significantly among the concentration groups, the data from each concentration were pooled to form a single pooled PP-pretreated group. The pooled data are shown in Table 2. Two-factor ANOVA revealed a significant effect of flow rate ($p < 0.0001$) and a significant difference among concentration groups ($p < 0.0001$). A statistical interaction between flow rate and concentration group was not detected ($p > 0.05$). As can be seen, uptake efficiency decreased with increasing exposure concentration in metabolically competent (nonpretreated) controls. The uptake efficiency in the 1- and 4-ppm group exceeded that in the 10- and 30-ppm groups ($p < 0.05$, Newman-Keuls test). Uptake efficiency in the 1- and 4- but not the 10- and 30-ppm group exceeded that in the PP-treated animals ($p < 0.05$, Newman-Keuls test). Comparison of the male to female data reveals subtle statistical differences exist.

In the female rat, uptake efficiency at 10 ppm was greater than at 30 ppm or in the PP-treated rats, whereas in the male rats this was not the case.

Delivered dosage rates can be calculated from the product of the inspired concentration, flow rate, and uptake efficiency. These averaged ~3, 12, 25, and 70 nmol/min in the 1-, 4-, 10-, and 300-ppm control male rat groups, respectively. These values are somewhat higher than in the female rat but never more than by 25%. Expressed per gram body weight to the three-quarters power, these values were 10, 40, 80, and 220 nmol/min-kg$^{0.75}$. These values are somewhat lower than those in the female rats, but the differences were not great, particularly at the higher exposure concentrations.

DISCUSSION

Delivery of airborne toxicants to target tissues within the respiratory tract is critical in determining the response to such materials (US EPA, 1994). Thus, quantitative and theoretical perspectives on dosimetry patterns of inhaled materials represent an essential component of quantitative inhalation risk assessment. A large database on URT deposition of inspired vapors has been obtained and used to support development of a variety of inhalation dosimetry models (Frederick *et al*., 2002; Hinderliter *et al*., 2005; Morris *et al*., 1993; Schroeter *et al*., 2008; Teeguarden *et al*., 2008). This body of work has indicated the importance of tissue solubility (as determined by tissue:air or blood:air partition coefficient) in controlling URT vapor deposition (Medinsky *et al*., 1999; Morris, 2001; Morris *et al*., 1993;), a concept perhaps first proposed by Haggard (1924). Local metabolism of inspired vapors within nasal tissues is also a critical factor in determining uptake in that site; without exception, pretreatment with metabolic inhibitors has been shown to diminish nasal scrubbing capacity of metabolized vapors (e.g., Bogdanffy *et al*., 1999; Frederick *et al*., 2002; Morris *et al*., 1993;), indicating that local nasal metabolism of inspired vapors occurs and serves to enhance nasal scrubbing capacity.

Naphthalene is a low volatility highly lipophillic vapor that is a nasal cytotoxicant and carcinogen in the rat. It is known to be metabolized by CYP 450 in rat nasal tissue *in vitro*, and the regional injury patterns within olfactory mucosa after acute inhalation or parenteral exposure suggest that local activation of inspired naphthalene plays a critical role in the toxic process (Lee *et al*., 2005). It is not known with certainty if inspired naphthalene is metabolized in the nose or is quantitative information on the degree of metabolism available. The current *in vitro* metabolism studies confirm that naphthalene is rapidly metabolized by olfactory mucosa. The olfactory mucosa is well known to express high levels of CYP 450 (Thornton-Manning and Dahl, 1997). Gender differences in olfactory CYP 450 activation of naphthalene were not apparent. Both the specific activity and the spectrum of metabolites in olfactory mucosa in male and female rats were virtually identical. In both genders, PP treatment resulted in ~80% inhibition of activity. This gender similarity is in contrast to the mouse lung in which gender differences in naphthalene metabolic activation are apparent (Van Winkle *et al*., 2002).

By analogy to other metabolized vapors, inhibition of nasal naphthalene metabolism by PP would be expected to diminish nasal scrubbing capacity of naphthalene. This was observed (Tables 1 and 2) providing strong evidence that inspired naphthalene is, in fact, metabolized within the nasal mucosa. That the inhibitor was without effect on nasal uptake of acetone, a nonmetabolized vapor (Morris *et al*., 1986), indicates that its effects on naphthalene uptake cannot be attributed to nonspecific effects (e.g., altered nasal perfusion) on the nasal uptake process. Naphthalene metabolism rates are ~40-fold higher in olfactory than septal nonolfactory epithelium (Lee *et al*., 2005). This is thought to be the reason why parentally administered naphthalene produces damage in olfactory but not respiratory mucosa of the nose (Buckpitt *et al*., 2002; Genter *et al*., 2006; Lee *et al*., 2005;). It is known that only ~15% of the inspired air passes over the olfactory mucosa of the rat (Kimbell *et al*., 1993; Morris *et al*., 1993). Since the airborne delivery rate is lower and the metabolic capacity is higher in olfactory mucosa, it is quite likely that inspired naphthalene is much more extensively activated in olfactory than respiratory tissues, perhaps offering an explanation for the extensive olfactory injury resulting from naphthalene inhalation.

URT uptake efficiency of naphthalene was concentration dependent, with lower uptake efficiencies being observed at higher exposure concentrations, a behavior observed in control but not PP-pretreated rats. This underscores the importance of metabolism in the nonlinear concentration-dependent behavior. Similar patterns were observed in both genders. This pattern has been observed for a variety of vapors and is thought to be due to saturation and/or capacity limitation of metabolic pathways (Morris, 1993, 2001; Stanek and Morris, 1999). It is important to recognize that nasal uptake is a highly dynamic process. Inhibition of metabolic pathways results in reduced uptake efficiency, reduced tissue clearance rates, and higher tissue concentrations. For this reason, *in situ* metabolism rates cannot be assessed simply by subtracting uptake efficiencies in PP-pretreated rats from those in control rats. A validated model for nasal uptake of naphthalene would be required to provide detailed insights into precise metabolism rates and the degree of metabolic saturation and/or capacity limitation at high concentrations. The data in the current study will allow development of such a model. Nonetheless, it is interesting to note that total URT delivery rates ranged between 3 and 70 nmol/min compared to estimated total nasal olfactory metabolic capacity ~20 nmol/min, suggesting that capacity limitation would be anticipated in the concentration range used in this study.

Neuroblastomas were significantly increased in female but not male rats (Abdo *et al.*, 2001) chronically exposed to naphthalene. The gender difference may be reflective of pharmacokinetic and/or pharmacodynamic differences. The potential for gender differences in nasal vapor scrubbing have not been extensively examined, but the available data suggest only minimal, if any, gender differences exist (Morris *et al.*, 1991). The current study allows for detailed examination of potential pharmacokinetic gender differences. As noted above, metabolic patterns appeared identical in males and females. Naphthalene was scrubbed from the inspired air with less efficiency in female than male rats; however, due to differing body weights, the flow rates represent differing ratios of the minute ventilation in each gender. In males, 150 and 300 ml/min represent 130 and 270% of the predicted minute ventilation rates compared to 190 and 380% in females. For all vapors examined to date (including naphthalene), uptake has been shown to be strongly dependent on inspiratory flow rate with lower uptake efficiencies being observed at higher flows (see Morris *et al.*, 1993). Thus, the lower uptake in females likely reflects the proportionately higher flow rate relative to minute ventilation in this gender, a hypothesis that could be examined via a model for naphthalene uptake in the nose.

The delivered dosage rates differed slightly between genders. Based on nanomoles per minute normalized to kilogram body weight, the delivered dosage rate tended to be higher in male rats. Based on nanomoles per minute normalized to body weight to the three-quarter power, delivered dosage rates tended to be higher in female rats. Importantly, at the 10 and 30 ppm level (the concentrations used in the chronic inhalation bioassays; Abdo *et al.*, 2001; NTP, 2001), the differences were not major, with the delivered dosage rates being within a factor of 1.25 of each other in the two genders. The most appropriate normalization procedure is not known, although body weight to the three-quarter power is often used for such normalizations because ventilation rate and other rate phenomena relate to body weight three-quarter power rather than simply body weight (Brown *et al.*, 1997; Guyton, 1947; US EPA, 1994). Since the difference in delivered dosage rate between genders was within a factor of 1.25-fold, it is difficult to attribute the differing incidence of neuroblastomas in male and female rats (Abdo *et al.*, 2001; NTP, 2000) to difference in delivered dosage rate. The other aspects of nasal doismetry that were examined in the current study appear to be similar in both genders as well. Specifically, in both genders, uptake efficiencies were decreased at higher compared to lower concentrations with the concentration dependence on uptake being abolished by PP pretreatment. Moreover, the *in vitro* naphthalene metabolic patterns were virtually identical in both genders.

These data suggest that differences in delivered dose or activation pathways may not offer a likely explanation for the gender difference in the neuroblastoma response. This conclusion is made with one important caveat. The rats used in the current study were not previously exposed to naphthalene. Under chronic exposure conditions, a variety of changes might occur (e.g., inflammation, enzyme induction), which could alter dosimetry. For example, studies in mice have demonstrated relatively rapid tolerance to naphthalene administered via either parenteral or inhalation routes (West *et al.*, 2002, 2003), and this appears to be related to altered rates of glutathione turnover in tolerant animals. It might be argued that such changes, if gender specific, would represent a gender difference in pharmacodyamics, but nonetheless, there would be a resultant gender difference in dosimetry. The possibility of gender differences in phase II metabolic pathways represents another possible mechanism for the gender difference in the tumorigenic response. Differences in phase II metabolism of styrene are thought to be important relative to the species difference in nasal response to this solvent (Green *et al.*, 2001). Future studies would be needed to examine these possibilities.

Naphthalene is virtually insoluble in water yet its blood:air partition coefficient was found to be ~750, a value slightly higher than that estimated by NTP (2000). This value is relatively high and is between that of acetone (275) and ethanol (1710) (Morris *et al.*, 1986). Naphthalene partitioning into blood must be reflective of partitioning into the lipophilic components rather than simple partitioning into the aqueous phase of whole blood. In this regard, naphthalene appears to differ from the other vapors whose URT dosimetry has been evaluated. Naphthalene uptake in PP-pretreated male rats averaged 33% (at 150 ml/min flow rate) and reflects partitioning into blood because metabolic clearance pathways were inhibited in these animals. Based on partition coefficients, it would be expected that this value would lay between that of acetone and ethanol. In the current study, acetone uptake efficiency averaged 25% (at 150 ml/min flow rate) and ethanol uptake efficiency has been reported to be 59% (at a similar flow rate; Morris *et al.*, 1986). Thus, despite its markedly different physical chemical properties from the water-soluble vapors examined previously, naphthalene scrubbing in the URT appears to fall within the range predicted by its partition coefficient, suggesting that our theoretical understanding of URT uptake processes extends to vapors with high lipid but low water solubility.

FUNDING

Naphthalene Coalition, including contributions from the Asphalt Institute, American Petroleum Institute, Electric Power Research Institute, Naphthalene Council, and Utility Solid Waste Advocacy Group.

ACKNOWLEDGMENTS

The expert technical assistance of Barbro Simmons and Dexter Morin is gratefully acknowledged.

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