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Nasal dosimetry of inspired naphthalene vapor in the male and female B6C3F1 mouse

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ABSTRACT

Naphthalene vapor is a nasal cytotoxicant in the rat and mouse but is a nasal carcinogen in only the rat. Inhalation dosimetry is a critical aspect of the inhalation toxicology of inspired vapors and may contribute to the species differences in the nasal response. To define the nasal dosimetry of naphthalene in the B6C3F1 male and female mouse, uptake of naphthalene vapor was measured in the surgically isolated upper respiratory tract (URT) at inspiratory flow rates of 25 or 50 ml/min. Uptake was measured at multiple concentrations (0.5, 3, 10, 30 ppm) in controls and mice treated with the cytochrome P450 inhibitor 5-phenyl-1-pentyne. In both sexes, URT uptake efficiency was strongly concentration dependent averaging 90% at 0.5 ppm compared to 50% at 30 ppm (25 ml/min flow rate), indicating saturable processes were involved. Both uptake efficiency and the concentration dependence of uptake were significantly diminished by 5-phenyl-1-pentyne indicating inspired naphthalene vapor is extensively metabolized in the mouse nose with saturation of metabolism occurring at the higher concentrations. A hybrid computational fluid dynamic physiologically based pharmacokinetic model was developed for nasal dosimetry. This model accurately predicted the observed URT uptake efficiencies. Overall, the high URT uptake efficiency of naphthalene in the mouse nose indicates the absence of a tumorigenic response is not attributable to low delivered dose rates in this species.

1. Introduction

Naphthalene is an inhalation carcinogen in the rat and mouse (NTP, 1992, 2000). Interestingly, this simple polycyclic aromatic hydrocarbon causes extensive cytotoxicity in the nose of both the mouse and rat, but causes nasal tumors only in the rat. Bronchiolar/alveolar lung tumors result from chronic inhalation exposure in the mouse. The reasons for this species difference are unknown, but could be due to diminished uptake of inspired naphthalene in the nose and enhanced penetration to the lower respiratory tract of the mouse compared to rat. The nasal dosimetry of naphthalene in the rat has been well defined (Morris and Buckpitt, 2009), but information on the mouse is lacking. The goal of the current study was to comprehensively characterize the nasal dosimetry of naphthalene vapor in the mouse including measurements of nasal absorption of naphthalene over a wide concentration range to fully define nasal dosimetry and to provide data for validation of a hybrid computational fluid dynamic-physiologically based pharmacokinetic (CFD-PBPK) model for nasal naphthalene dosimetry.

CFD-PBPK models have successfully described nasal dosimetry of a variety of vapors in the rat, including ethyl acrylate, acrylic acid, and diacetyl (Frederick et al., 1998, 2002; Gloede et al., 2011). An advantage of this modeling approach is that is allows for explicit inclusion of vapor solubility (as measured by blood:air partition coefficient) and tissue metabolism kinetics for nasal respiratory and olfactory mucosa. Thus, the model can predict non-linear uptake behavior due to saturation of metabolic pathways. This modeling approach has not been extensively applied to the mouse nose, but has been successfully in describing nasal dosimetry of styrene in that species (Sarangapani et al., 2002). In the current study, we developed a CFD-PBPK model for nasal dosimetry of naphthalene based on the structure of our most recently published model (Gloede et al., 2011). Information is available on female mouse nasal respiratory and olfactory mucosal metabolism of naphthalene (Lanosa et al., 2010), but data on the blood:air partition coefficient for the mouse are lacking. The current study included measurement of this parameter in mouse blood.

To fully characterize the nasal dosimetry of naphthalene in the mouse and provide robust model validation, uptake of this vapor was measured in the surgically isolated upper respiratory tract (URT) at two inspiratory flow rates (25 and 50 ml/min) in control mice and mice treated with the cytochrome P450 monooxygenase (CYP) inhibitor 5-phenyl-1-pentyne (PP) (Morris, 2009; Roberts et al., 1998). PP is a mechanism-based, "suicide" inhibitor (Beebe et al., 1996). These studies were performed at multiple concentrations: 0.5, 3, 10, 30 ppm; 10 and 30 ppm were the concentrations

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Table 1
Key model input data for the nasal CFD-PBPK model in female mice.

Body weight	23 g
Respiratory mucosal surface area	1.02 cm^2
Olfactory mucosal surface area	1.06 cm^2
Respiratory mucosal airspace	0.017 cm^3
Olfactory mucosal airspace	0.0059 cm^3
Cardiac output (ml/min)	14.2 ml/min
V_{max} (nmol/min-cm^2)	
Respiratory	3.3
Olfactory	15
Lower airways	15
K_m	20 nmol/ml

used in the NTP chronic bioassay (NTP, 1992, 2000) and 0.5 ppm was the lowest practicable concentration in our system. The study included male and female mice to delineate any sex differences that might exist. Results indicated that inspired naphthalene was efficiently absorbed in the nose, but that nasal uptake efficiencies decreased with increasing concentration, an effect greatly diminished by CYP inhibition. The CFD-PBPK model closely predicted the uptake behavior that was observed.

2. Materials and methods

Male and female B6C3F1 mice were obtained from Charles River Laboratories (Wilmington, MA). Animals were 6–8 weeks at time of purchase, were acclimated for at least 10 days, and were used within 6 weeks of arrival. At the time of use body weights averaged 23 ± 1.6 and 30 ± 2.3 g in female and male mice, respectively. All animal protocols were approved by the University of Connecticut IACUC.

Uptake of vapor was measured in the isolated URT of urethane-anesthetized mice as described previously (Morris, 1999). Briefly, after the onset of anesthesia, a tracheotomy was performed and an endotracheal tube inserted to the level of the larynx. The mouse was then placed in a nose-only inhalation chamber and chamber air was drawn continuously through the isolated URT at a constant flow rate of 25 or 50 ml/min. When administered, the CYP inhibitor PP was given at a dose of 100 mg/kg (10 mg/ml in olive oil, i.p.), 1 h prior to the initiation of measurement of uptake. Pilot studies indicated this inhibitor was without effect on URT uptake of acetone vapor. Specifically, URT acetone efficiency averaged 32 ± 2% in control compared to 31 ± 2% in PP treated mice (mean ± SEM). Acetone URT uptake kinetics has been extensively studied (Morris, 1991). Acetone is not extensively metabolized in the mouse nose; as in the rat nose, URT uptake in the mouse is solely dependent on nasal perfusion (Morris et al., 2010; Morris, 2012) The naphthalene blood:air partition coefficient was determined *ex vivo* as described for the rat (Morris and Buckpitt, 2009). For this purpose blood was drawn by cardiac puncture from control (non-naphthalene exposed, non-drug-pretreated) urethane-anesthetized mice into heparinized syringes and 0.2 ml was placed in a 300 ml glass vessel for partition coefficient determination by the vial equilibration technique.

Naphthalene atmospheres were generated by blowing filtered air over naphthalene crystals with airborne naphthalene concentrations being monitored by gas chromatography as described previously (Morris and Buckpitt, 2009). Chamber air was heated (38–40 °C) and humidified (>75% relative humidity) to prevent nasal dehydration. Naphthalene (>99% purity) was obtained from Sigma-Aldrich (St. Louis, MO), PP was obtained from (GFS Chemicals, Columbus, OH).

Data were analyzed by ANOVA followed by Newmans–Keuls test using XLSTAT software (Addinsoft, New York, NY). A $p < 0.05$ was required for significance. Data are reported as mean ± SD with the groups sizes (n) given parenthetically.

The modeling approach described by Gloede et al. (2011) was used to describe vapor uptake in the female mouse (metabolic data are not available for the male). A complete description of the model is provided in the appendix. This approach relies on modeling the disposition of vapor in "tissue stacks" consisting of airspace, mucus, epithelium and submucosa (see Fig. 1A). Blood is allowed to perfuse the submucosal space and metabolism is allowed in the tissue compartments. Separate stacks of tissue (Fig. 1B) were used to describe the nasal dorsal inspiratory flow pathway (passing over respiratory then olfactory mucosa) and the nasal ventral inspiratory flow pathway (passing over only respiratory mucosa). It was assumed that 15% of the inspired air followed the dorsal route and 85% of the inspired air followed the ventral route (Gloede et al., 2011; Sarangapani et al., 2002).

Key anatomical, physiological and metabolic parameters are shown in Table 1. Morphometric studies on rodent nasal structure (Gross et al., 1982) suggested that nasal surface area was related to the body weight to the ¾ power; linear regression of the surface areas versus body weight provided the following relationships: respiratory mucosal surface area = 17.3 $cm^2/g^{3/4} \times BW^{3/4}$ and olfactory mucosal surface area = 18.0 $cm^2/g^{3/4} \times BW^{3/4}$ (where BW is the body weight in grams). Nasal cavity airspace volume was linearly related to BW (volume = 0.983 $cm^3/g \times$ BW, with BW in grams) with 74% of the volume being over respiratory mucosa and 26% over olfactory mucosa (Teeguarden et al., 2008). These relationships were used to estimate nasal surface area and airspace volume in the 23 g female mice. Mouse nasal epithelial tissue thickness was assumed to be the same as in the rat (Sarangapani et al., 2002): 20 μm for respiratory epithelium; 40 μm for olfactory epithelium (Gloede et al., 2012). As in our previous models, the submucosa was modeled as being 20 μm thick (Morris et al., 1993; Gloede et al., 2011). The cardiac output was estimated based on the equation provided in Frederick et al. (1998), and it was assumed that 1% of the cardiac output perfused the nose with 1/3 of that perfusing the superficial capillaries which are in closest communication with the airspace (Gloede et al., 2011). The air-phase mass transfer coefficients for the mouse nose were assumed to be equal to those for the rat (Sarangapani et al., 2002). The only other input parameters were nasal metabolism kinetics (obtained from Lanosa et al., 2010), and blood:air partition coefficient. Model simulations were performed with ACSL Xsoftware (Aegis Technologies, Orlando, FL). Simulation were run at each of the exposure concentrations used in the uptake studies for both inspiratory flow rates (25, 50 ml/min) in metabolically competent and metabolically inhibited animals. Model output (fractional uptake) was compared to the experimental measurements. For comparative purposes simulations were also performed for cyclic respiration as described by Gloede et al. (2011). It was assumed the breathing frequency was 160 breaths/min

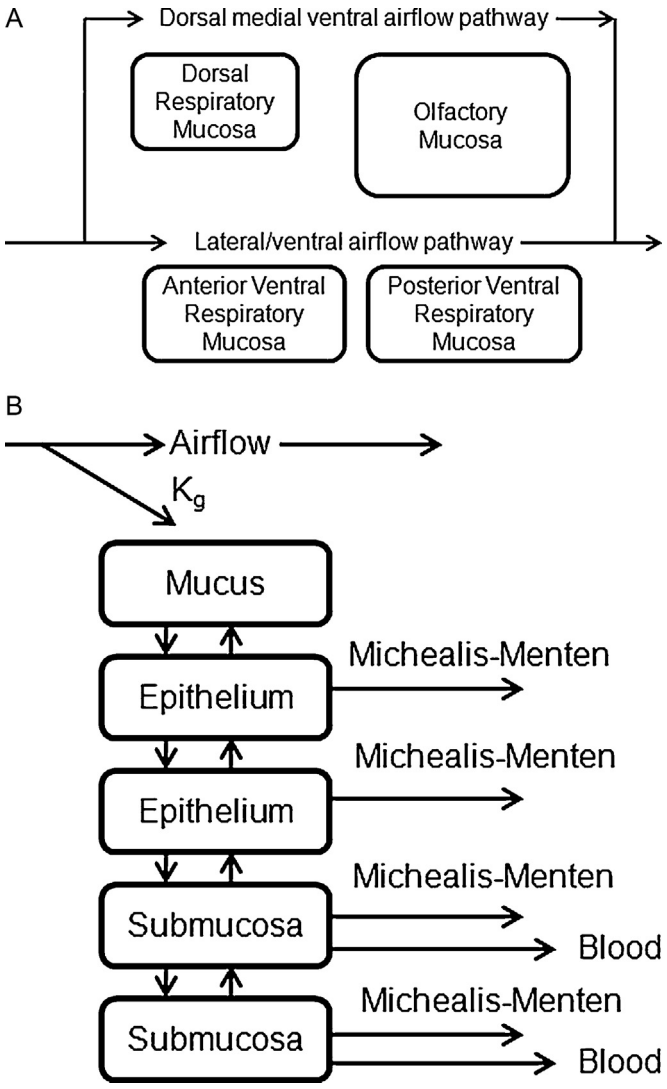

Fig. 1. (A) Schematic diagram of an airway tissue stack. Vapor is allowed to enter (or leave) the mucus lining layer an accordance with its mass transfer coefficient. Once in tissue vapor is allowed to diffuse to deeper tissue sites which are modeled as varying numbers of 10 μm thick epithelial or submucosal compartments. Vapor metabolism is modeled by Michealis–Menten kinetics in the tissue compartments. Blood perfuses the submucosal space (Gloede et al., 2011). (B) Schematic representation of the location of the model tissue stacks within the nose. It is assumed air follows a dorsal–medial pathway and passes over respiratory followed by olfactory mucosa. Air following the ventral pathway passes over only respiratory mucosa, which is modeled as two successive tissue stacks (Gloede et al., 2012).