

Inhalation dosimetry of hexamethylene diisocyanate vapor in the rat and human respiratory tracts

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Abstract

Hexamethylene diisocyanate (HDI) is a reactive chemical used in the commercial production of polyurethanes. Toxic effects in rodents exposed to HDI vapor primarily occur in the nasal passages, yet some individuals exposed occupationally to concentrations exceeding current regulatory limits may experience temporary reduction in lung function and asthma-like symptoms. Knowledge of interspecies differences in respiratory tract dosimetry of inhaled HDI would improve our understanding of human health risks to this compound. HDI uptake was measured in the upper respiratory tract of anesthetized Fischer-344 rats. Nasal uptake of HDI was >90% in rats at unidirectional flow rates of 150 and 300 ml/min and a target air concentration of 200 ppb. Uptake data was used to calibrate nasal and lung dosimetry models of HDI absorption in rats and humans. Computational fluid dynamics (CFD) models of the nasal passages were used to simulate inspiratory airflow and HDI absorption. Transport of HDI through lung airways was simulated using convection-diffusion based mass transport models. HDI nasal uptake of 90% and 78% was predicted using the rat and human nasal CFD models, respectively. Total respiratory tract uptake was estimated to be 99% in rats and 97% in humans under nasal breathing. Predicted human respiratory uptake decreased to 87% under oral breathing conditions. Absorption rates of inhaled HDI in human lung airways were estimated to be higher than the rat due to lower uptake in head airways. Model predictions demonstrated significant penetration of HDI to human bronchial airways, although absorption rates were sensitive to breathing style.

Keywords

Computational fluid dynamics, dosimetry, hexamethylene diisocyanate, inhalation, lung, nose

History

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Introduction

Hexamethylene diisocyanate (HDI) is an aliphatic diisocyanate primarily used in the production of polyisocyanate products, the latter used, for example, as a hardening agent in automobile polyurethane paints. There is potential for inhalation and dermal exposures to HDI during the manufacturing process or during spray applications that may cause irritation to the skin or respiratory tract. Inhaled HDI reacts rapidly with proteins and cells lining the respiratory tract and to a lesser degree may undergo hydrolysis forming hexamethylene diamine (HDA), thereby greatly limiting HDI delivery to the systemic circulation (Berode et al., 1991). Urinary excretion of HDI metabolites hydrolyzed to HDA occurs in workers exposed by inhalation to HDI, indicating absorption in the respiratory tract (Brorson et al., 1990; Gaines et al., 2010). Respiratory tract retention of inhaled HDI was >60% in healthy and asthmatic human volunteers (Monso et al., 2000). However, retention sites and dosimetry estimates within the respiratory system are unknown.

Human health concerns following HDI inhalation focus on tracheobronchial or pulmonary airway responses including occupational asthma (Vandenplas et al., 1993). Due to its widespread use in automobile spray painting, there are numerous occupational studies of exposure and health effects from inhaled HDI (Cassidy et al., 2010; Hathaway et al., 1999) and from HDI-based polyisocyanates (Alexandersson et al., 1987; Tornling et al., 1990). Health effects associated with occupational exposure to HDI at air concentrations higher than the occupational exposure limits (threshold limit value = 5 ppb) include nasal and lung irritation, airway hypersensitivity, and a temporary reduction in lung function. These findings are suggestive of HDI retention in the human nasal and bronchial regions (ATSDR, 1998; Musk et al., 1988; Tornling et al., 1990).

Animal studies have been conducted to further characterize the respiratory toxicity of HDI following inhalation exposure. Histopathological effects in the nasal cavities of rats following a 2 year exposure to HDI concentrations of 5, 25 and 175 ppb include squamous hyperplasia/metaplasia, inflammation, hyaline droplet degeneration and degeneration of olfactory epithelium (Miles, Inc., 1989). The latter effect was used to derive an inhalation reference concentration (RfC) for HDI

(RfC = ~ 1.45 ppt) based on a NOAEL of 5 ppb (Foureman et al., 1994). In contrast to humans where the predominant HDI-related health effects involve the lower respiratory tract, adverse effects in rodents were limited to the nasal cavity under the exposure conditions of the study.

Differences in response patterns observed in rodents and humans following HDI inhalation suggest that regional deposition within the respiratory tract is a critical determinant of the locations of airway responses. Morphological differences between rat and human respiratory airways lead to differences in airflow and vapor uptake patterns. Mathematical dosimetry models have helped elucidate the effects these differences have on regional uptake of inhaled gases. These models include computational fluid dynamics (CFD) models of the nasal passages (Kimbell et al., 2001; Schroeter et al., 2008), physiologically based pharmacokinetic (PBPK) models of nasal and lung regions (Frederick et al., 1998; Gloede et al., 2011; Morris & Hubbs, 2009), and mass transport models of lung airways (Bush et al., 2001; Miller et al., 1985; Overton et al., 1987).

The goal of this study was to predict respiratory tract absorption of inhaled HDI vapor in rats and humans. To this end, uptake of HDI vapor was experimentally measured in the isolated upper respiratory tract (URT) of anesthetized rats. Nasal uptake data was then used to calibrate rate constants in nasal and lung dosimetry models governing absorption of inhaled HDI vapor in respiratory airways. Anatomically accurate CFD models of the rat and human nasal passages were used to predict upper airway absorption of inhaled HDI. Lung dosimetry models were developed using an approach based on earlier modeling efforts with other reactive gases.

Methods

Animals

This study was approved by the Hamner Institutes for Health Sciences Institutional Animal Care and Use Committee. Nasal uptake studies were performed on male Fischer-344 rats, 8 weeks of age at purchase (Charles River Laboratory, Raleigh, NC). Vapor uptake was measured in the isolated URT as previously described (Morris, 1999; Schroeter et al., 2006b; Struve et al., 2008). Rats were anesthetized with urethane (1.3 g/kg ip), placed in a supine position, and the trachea was surgically exposed. A 2.5 cm polyethylene endotracheal tube was positioned toward the lungs while a 14 G Teflon catheter was inserted into a second tracheal incision so that the catheter tip was positioned near the larynx. The head was placed in a nose-only cone and vapor-laden air was drawn through the isolated URT with a rotary-vane vacuum pump for up to 90 min under constant uni-directional flow rates of 150 and 300 ml/min. HDI concentrations were measured at the breathing zone of the animal (pre-nose) and after exiting the URT through the endotracheal tube (post-nose). Animals were killed by exsanguination immediately after exposure.

Exposure system

Liquid HDI (CAS # 822-06-0) was purchased from Sigma-Aldrich (Allentown, PA). HDI vapor was generated by the diffusion of HDI from a diffusion vial in a constant

temperature oven (Dynacalibrator, VICI Metronics Model 340, Santa Clara, CA) set at 91.5 °C. HDI vapor in nitrogen was mixed with dilution air drawn from an instrument grade compressed air supply to achieve a target concentration of 200 ppb. Humidified air at 40–55% relative humidity was used to minimize nasal mucosal dessication. Laboratory studies indicate that isocyanates in the vapor state do not react with atmospheric water vapor, so the presence of humidified air should not affect HDI concentration levels (Holdren et al., 1984). The exposure system was pre-conditioned prior to exposures to ensure that exposure lines were saturated and to achieve stable target concentrations at the pre- and post-nose sampling locations. No adjustments were made to the generation system once an exposure had begun.

For the exposures at a 150 ml/min flow rate, HDI concentrations were measured using a Hewlett Packard 5890 Series II gas chromatograph (GC) (Agilent Technologies, Palo Alto, CA) equipped with a nitrogen phosphorus detector (NPD). An eight-position gas sampling valve was used by the GC to sample at a programmed sequence of locations from both pre- and post-nose locations, immediately after the stream exited the dynacalibrator oven, and the room air. Samples were injected into the GC/NPD from the gas-sampling valve approximately every three minutes to provide near continuous sampling. The GC/NPD was calibrated by entering a concentration based on the analysis of filter samples by high-pressure liquid chromatography (HPLC) versus the mean area count of three analyses by the GC. Prior to sampling, HDI concentrations were determined according to OSHA method 42 for the analysis of diisocyanates in air. Briefly, a known volume of HDI containing air was passed through a glass fiber filter coated with 1-(2-pyridyl)piperazine (1-2PP). Filters were then extracted with an acetonitrile/DMSO solution to remove derivatized HDI. The 1-2PP derivatized HDI in the extraction solution was quantitatively analyzed using HPLC. The filter samples were taken at the same time as the GC analyses at each concentration level and the concentration was manually entered during the calibration procedure. The concentration was changed by adjusting the dilution flow from the dynacalibrator. The limit of detection for these exposures was 17 ppb.

At a 300 ml/min flow rate HDI concentrations were monitored using a Shimadzu QP2010 GC mass spectrometer (GC/MS, Shimadzu Scientific Instruments, Kyoto, Japan) to provide more accurate measurements of low HDI post-nose concentrations. The exposure setup was identical to that described previously using the GC. Comparisons between HPLC analysis of glass fiber filter extracts and peak areas obtained during the analysis of HDI by GC/MS were conducted for the purpose of generating HDI calibration curves for the GC/MS. The calibrated GC/MS was used to measure pre- and post-nose concentrations. For these exposures, the limit of detection was 4 ppb.

Data analysis

The concentration of HDI vapor entering the isolated URT (pre-nose) and exiting the URT via the Teflon catheter (post-nose) were measured every 3–5 min through the end of the 90 min exposures. URT uptake as a percentage was calculated

as $100 * [(pre\text{-}nose\ sample - post\text{-}nose\ sample)/pre\text{-}nose\ sample]$. Time-averaged nasal uptake was calculated as the mean of all samples during the exposure period.

Nasal dosimetry models

Anatomically accurate 3D reconstructions of the rat and human nasal passages were developed in earlier studies (Kimbell et al., 1997; Subramaniam et al., 1998). The rat model reconstruction consisted of the right nasal passages of a mature F344 rat from tracings of serial histological cross-sections; the human model was reconstructed from MRI scan tracings of the complete nasal passages of a healthy adult male. The original models were used in studies investigating nasal dosimetry of inhaled reactive gases, including formaldehyde, hydrogen sulfide and acrolein (Kimbell et al., 2001; Schroeter et al., 2006a, 2008). For this study, the surfaces from the rat and human nasal models were imported into the medical imaging software Mimics™ (Materialise, Inc., Leuven, Belgium), where the surface contours were smoothed to generate higher-fidelity models (Figure 1). High-density, unstructured tetrahedral meshes were generated in both models using the commercial software package ICFM-CFD™ (ANSYS, Inc., Canonsburg, PA). Mesh elements were smoothed until the aspect ratios were greater than 0.3 to ensure that there were no distorted elements in the meshes. The computational meshes for the rat and human nasal models contained approximately 4.4 and 3.5 million elements, respectively. Element sizes were selected to ensure that there were a suitable number of elements spanning all airways. The mesh density study conducted by Garcia et al. (2009) showed that tetrahedral meshes of this size are sufficient for mesh independence of airflow and gas uptake solutions.

Steady-state inspiratory airflow was simulated in the nasal models using the commercial software package Fluent™ (ANSYS, Inc., Canonsburg, PA) to solve the Navier-Stokes equations governing viscous incompressible flow. Boundary conditions consisted of a no-slip condition on all airway walls, a mass-flow condition at the nostril(s) and a zero pressure condition at the nasopharyngeal outlet. Airflow simulations

were conducted with an air density of 1.204 kg/m^3 and a dynamic viscosity of $1.8 \times 10^{-5}\text{ kg/m}\cdot\text{s}$. Inspiratory airflow rates were determined by dividing the amount of inspired air (tidal volume) by the estimated time involved in inhalation (half the time of one breath), which is equal to twice the minute volume. The flow rate used for the rat was 434 ml/min , based on an estimated minute volume of 217 ml/min for a 315 g rat (U. S. EPA, 1994). Airflow rates used for the human model were 15 l/min (twice the estimated minute volume of 7.5 l/min) for resting breathing and 30 l/min to simulate moderate nasal breathing. Although transient cyclical flow is more physiologically realistic, steady-state flow rates were used for their manageable computational cost, to be able to directly compare results with the experimental nasal uptake data, and to be able to link nasal results with the lung dosimetry models.

Transport of HDI vapor through nasal airways was governed by the convection-diffusion equation:

$$u \cdot \nabla C = D_a \nabla^2 C, \quad (1)$$

where u is the air velocity, $D_a = 0.038\text{ cm}^2/\text{s}$ is the air-phase diffusivity of HDI and C is the HDI concentration in the inspired air. At low concentrations, the presence of HDI vapor will not affect the airflow, so the gas transport simulations were solved decoupled from the airflow. The boundary condition at the nostril(s) was defined to be the inhaled HDI exposure concentration.

As inhaled vapor passes through the respiratory airways, it will diffuse to the airway tissue and react with mucus and tissue constituents in the epithelial lining. The absorption rate is determined by the concentration gradient between air and tissue at the air:tissue interface. The mass transfer boundary condition on airway walls was defined so that vapor flux was proportional to the air-phase concentration near the wall, given by the following expression:

$$flux = h \cdot C. \quad (2)$$

The proportionality constant, h , is referred to as a mass transfer coefficient and can be determined from the air-phase resistance and biochemical behavior of HDI in respiratory tissues. To account for these effects, the kinetic behavior of absorbed HDI in the nasal lining composed of the mucosal and epithelial tissues was simulated, following the approach of Schroeter et al. (2006b). Due to its high reactivity, absorbed HDI was modeled according to the processes of tissue diffusion and first-order clearance, which governs the reactivity of HDI with water and tissue constituents. These transport processes in nasal tissues were described by the reaction-diffusion equation:

$$D_t \frac{d^2 C_t}{dx^2} - k_f \cdot C_t = 0, \quad (3)$$

where C_t is the HDI tissue concentration, $D_t = 7.17 \times 10^{-6}\text{ cm}^2/\text{s}$ is the tissue diffusivity of HDI (obtained from The Risk Assessment Information System website: www.ra.is.ornl.gov), x is the distance within the tissue layer from the air:tissue interface, and k_f is a first-order rate constant governing elimination of the parent compound from nasal tissues. Assuming the equilibrium condition across the air:tissue

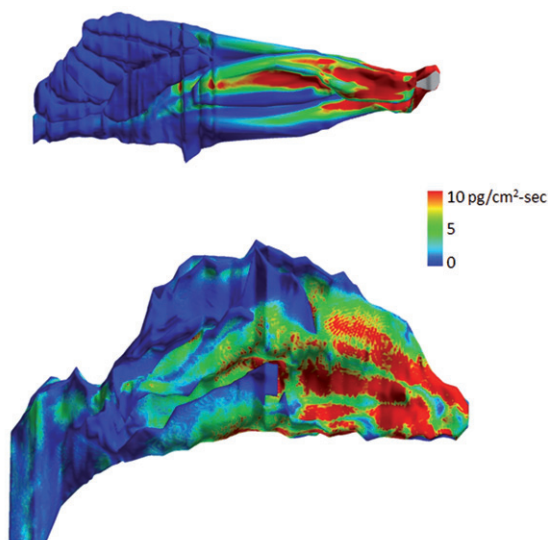


Figure 1. Wall mass flux contour plots of inhaled HDI vapor in the rat and human nasal CFD models at an inhalation exposure concentration of 1 ppb. Model sizes are not to scale.

interface, the Henry's Law constant can be used to define the boundary condition $C_t = PC_a$, where P is the liquid:air partition coefficient of HDI and C_a is the concentration in air. The solubility of HDI has not been reported due to its rapid reaction with water, so a partition coefficient of 2 was selected since this value represents the approximate blood:air partition coefficient of medium chain length hydrocarbons such as hexane, heptane, and octane. A $C_t = 0$ boundary condition was implemented at the tissue:blood interface. Absorbed HDI vapor is unlikely to reach the systemic circulation due to its high reactivity and has never been detected in the blood of animals or humans (ATSDR, 1998). With these boundary conditions, Equation (3) was solved analytically. Equating the flux from the air phase (Equation (2)) with the flux into the tissue as determined from the analytical solution to Equation (3), we arrive at an expression for the mass transfer coefficient, h , given by:

$$h = P\sqrt{k_f D_t} \coth\left(\sqrt{\frac{k_f}{D_t}} L_t\right), \quad (4)$$

where L_t is the nasal tissue depth. Since the clearance rate of HDI in respiratory tissues (k_f) is unknown, this parameter was calibrated in the rat nasal CFD model to the experimentally measured HDI nasal uptake data. The same value of the first-order rate constant was used in the human nasal CFD model. The mass transfer boundary condition was applied to all nasal airway walls and was implemented in the nasal CFD models through the use of User Defined Functions in Fluent. Unlike previous CFD nasal dosimetry modeling efforts with soluble gases (Kimbell et al., 2001; Schroeter et al., 2008), the boundary condition on squamous epithelium at the anterior nose was not distinguished from other epithelial types due to the low solubility and high reactivity of HDI.

Lung dosimetry models

Dosimetry models were developed to describe the transport and absorption of inhaled HDI vapor in rat and human lungs. An approach was taken that was based on previously published models describing dosimetry of other highly reactive gases such as ozone and formaldehyde that are capable of estimating concentration gradients throughout lung airways (Miller et al., 1985; Overton et al., 1987, 2001). A brief description of the model development is given here; further details can be found in the referenced publications.

The airways of the lower respiratory tract were described by a single-path model of sequential generations of circular cylinders to simplify the lung geometry from millions of airways to a manageable size for modeling efforts. The branching structure of the rat lung airways was described in a monopodial fashion using the anatomic model of Yeh et al. (1979). The human lung was assumed to be symmetric and dichotomously branching using the anatomical description provided by Yeh & Schum (1980). The comprehensive descriptions of airway parameters such as lengths, diameters and alveolar volume by airway generation from these morphometric studies provide the necessary anatomical detail to develop mathematical models of vapor dosimetry in rat and human lungs. A typical-path model of the whole lung, where one airway is representative of an entire

generation, was used starting with the trachea (generation 0) and ending with the fully alveolated airways (generation 23). Alveolar volume (generation 24) was uniformly divided among the pulmonary airways (generations 17–23).

The airway lengths and diameters given by Yeh et al. (1979) and Yeh & Schum (1980) correspond to a lung volume at total lung capacity (TLC). Since respiration begins at functional residual capacity (FRC), airway dimensions were isotropically scaled to 60% and 30% of TLC for the human and rat, respectively (Anjilvel & Asgharian, 1995; Asgharian et al., 2001). To account for dynamic changes in airway size during a breathing cycle, airway dimensions were further adjusted to $FRC + 0.5 * (\text{tidal volume})$ (Anjilvel & Asgharian, 1995). To complete the respiratory system, nasopharynx and larynx components were added to the rat lung dosimetry model and were also represented as circular cylinders. The lengths and diameters were measured from a naso-laryngeal mold and were 1.3 cm and 0.23 cm, respectively for the nasopharynx, and 0.8 and 0.23 cm, respectively, for the larynx. Oral and pharynx/larynx components were added to the human model with lengths of 10 and 15 cm and diameters of 1.784 cm (Overton et al., 2001). The nasopharyngeal and laryngeal airways were not assumed to change size with breathing. An oral pathway was not included in the rat lung dosimetry model since rats are obligate nasal breathers. Nasal pathways were not needed in the lung models since results with nasal breathing were normalized to reflect nasal absorption as determined from the CFD models.

The breathing cycle consisted of inspiratory and expiratory phases of equal duration. One complete breath was simulated with the lung dosimetry model. Steady breathing profiles were used to be consistent with the nasal airflow simulations. A breathing rate of 118 breaths/min was used for the rat (Guyton, 1947). With a minute volume of 217 ml/min, this corresponds to an inhaled tidal volume of 1.84 ml. Human lung dosimetry simulations were conducted at flow rates of 15 and 30 l/min for resting and moderate breathing, which correspond to tidal volumes of 500 and 882 ml with breathing rates of 15 and 17 breaths/min, respectively. Nasal and oral breathing conditions were used for the human lung model to compare lung dosimetry profiles using these different breathing routes. Volumetric flow rates in the nasopharynx, larynx and trachea were identical; volumetric flow rates in subsequent generations were defined to be proportional to the lung volume distal to the proximal end of the airway.

Transport of HDI vapor in lung airways was simulated by the convection-diffusion equation:

$$\frac{\partial C}{\partial t} + u \frac{\partial C}{\partial x} = D \frac{\partial^2 C}{\partial x^2} - KC, \quad (5)$$

where C is the concentration of HDI in air at airway position x and time t , u is the cross-sectional average airway velocity, D is the axial dispersion coefficient and KC is a sink term governing radial flux of HDI vapor into lung tissues (Asgharian et al., 2012; Miller et al., 1985). Equation (5) is a 1-D equation in space, and therefore the concentration at any point in the lung is estimated by the average cross-sectional concentration of HDI vapor at that point. The position, x , begins at the entrance to the oral or nasal

airways and ends at the alveolar sacs. The boundary conditions at the entrance consist of a constant concentration on inhalation and zero concentration gradient on exhalation. At the end of the alveolar space, a zero flux condition was imposed for both inhalation and exhalation phases. Concentrations were continuous and mass flux was preserved between airway generations. Mucus/surfactant and tissue thicknesses were taken from Miller et al. (1985).

The dispersion coefficient, D , was used in place of the molecular diffusion coefficient to account for airflow turbulence and secondary flows during inhalation and exhalation in the conducting lung airways and alveolar mixing in pulmonary airways. It is defined in the tracheobronchial airways by the empirical formula:

$$D = D_a + \frac{\gamma Lu}{3}, \quad (6)$$

where D_a is the air-phase molecular diffusion coefficient, L is the airway length and γ is an empirical parameter (1.08 and 0.37 for inspiration and expiration, respectively) determined from experiments in a tube model of the upper airways (Scherer et al., 1975). In alveolar airways, where airflow velocities are small, the dispersion coefficient is defined as

$$D = \frac{V_c}{V} D_a, \quad (7)$$

where V_c is the nonalveolar volume and V is the total airway volume of the airway generation (Miller et al., 1985). This form of the axial dispersion coefficient has been used in previous lung dosimetry modeling efforts (Miller et al., 1985; Overton et al., 1987, 2001).

The sink term, KC , accounts for losses of HDI from the air phase due to absorption into lung tissues. K is referred to as an overall mass transfer coefficient, defined as:

$$K = \frac{2}{R} \cdot \frac{k_l k_g}{k_l + H k_g}, \quad (8)$$

where R is the airway radius, H is the unitless Henry's Law constant (i.e. the air:tissue partition coefficient), and k_g and k_l are the air and liquid phase mass transfer coefficients, respectively. As with the nasal models, the mucus (or surfactant) and all epithelial and submucosal layers were lumped into a single tissue compartment, where mass transport processes were governed by the steady-state reaction diffusion equation and clearance was governed by the first-order rate constant, k_f , which accounts for the reactivity of HDI in lung tissues. The liquid phase mass transfer coefficient, k_l , was defined by Equation (4) and is a function of the physicochemical and reactivity properties of HDI in respiratory tissues (Miller et al., 1985). Defined in this manner, a consistent description of HDI absorption was applied to the nasal and lung dosimetry models.

The gas phase mass transfer coefficient, k_g , was defined as

$$k_g = \frac{Sh \cdot D_a}{2R}, \quad (9)$$

where Sh is the Sherwood number, empirically defined as (Ranz & Marshall, 1952):

$$Sh = 2 + 0.6 \left(Re^{1/2} Sc^{1/3} \right), \quad (10)$$

where Re is the Reynolds number and Sc is the Schmidt number, defined as the ratio of Reynolds to Peclet numbers.

A fully implicit finite difference scheme was implemented to numerically solve the convection-diffusion Equation (5) for one inspiratory/expiratory breathing cycle with oral or nasal breathing. All lung dosimetry simulations were conducted using Matlab™ (Mathworks, Inc., Natick, MA).

Results

Nasal uptake studies

Uptake of HDI vapor in the isolated URT of anesthetized rats quickly reached values near 100%. At a steady flow rate of 150 ml/min, HDI uptake was >80% for the duration of the exposure period. Time-averaged nasal uptakes rates ranged from 95% to 97%; average quantifiable uptake was approximately 92% within limits of detection. At a 300 ml/min flow rate HDI uptake was consistently at or near 100%. Within limits of detection, these studies demonstrate that 90–100% of inhaled HDI is absorbed by the rat nasal passages at an exposure concentration of approximately 200 ppb.

HDI nasal dosimetry

Uptake of inhaled HDI vapor was simulated in the rat and human nasal CFD models. Due to the high uptake of HDI vapor demonstrated in the nasal uptake studies, a $C=0$ boundary condition was implemented in the rat CFD model at a flow rate of 300 ml/min to describe the maximal uptake scenario. This yielded a nasal uptake fraction of 93%, which is in the range of uptake values shown from the nasal uptake studies. Although the “ $C=0$ ” boundary condition could also be used for the human nasal and rat and human lung dosimetry calculations, the flux boundary condition (Equation (2)) was preferred to test the sensitivity of various tissue parameters and since it can easily be modified in future studies to incorporate more information on HDI reactivity as it becomes available. Using the boundary condition from Equations (2) and (4), the value of the first-order rate constant, k_f , was increased until 93% uptake was obtained in the rat model at a flow rate of 300 ml/min. This value of the first-order rate constant was $k_f = 5.0 \times 10^6$ /s. At a flow rate of twice minute volume (434 ml/min in the rat, 15 l/min in the human), predicted nasal uptake from the rat and human nasal CFD models was 90% and 78%, respectively. Nasal uptake of HDI in the human model was 64% at a flow rate of 30 l/min. Lower uptake at higher flow rates was due to less residence time of the gas in the nose.

Wall mass flux contour plots demonstrate the presence of high absorption rates of inhaled HDI vapor in the anterior regions of the rat and human nasal passages (Figure 1). Cross-sectional area averages of wall mass flux show larger fluxes in the extreme anterior region of the rat nose compared to the human (Figure 2). Wall mass flux values in the rat model decreased rapidly due to decreased HDI airway concentrations in the distal nasal cavity. The rostral-caudal concentration gradient seen in the rat nasal cavity was steeper than that seen in humans, i.e. air HDI concentrations drop dramatically within the anterior 20% of the rat nose.

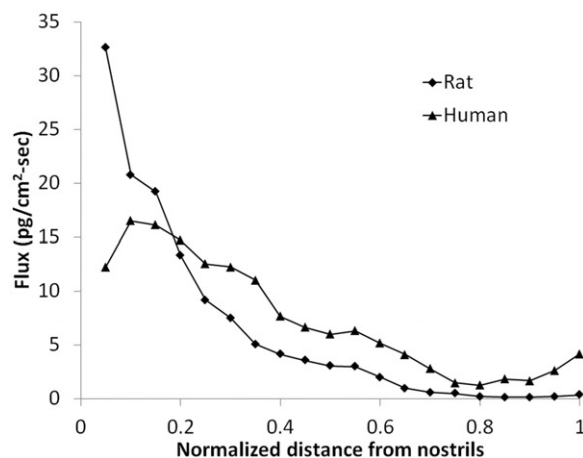


Figure 2. Cross-sectional averages of wall mass flux of inhaled HDI vapor in the rat and human nasal CFD models at an inhalation exposure concentration of 1 ppb. Nasal lengths were normalized to 1 for comparison of intranasal flux values between species.

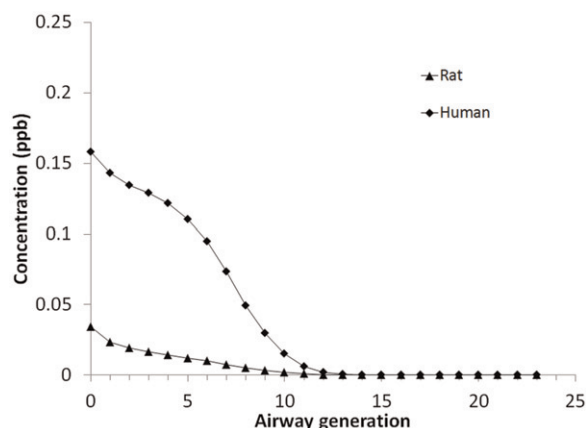


Figure 3. Predicted concentration of inhaled HDI vapor in rat and human lung airways at the end of the inhalation period with nasal breathing at twice minute volume (434 ml/min for the rat; 15 l/min for the human). Concentration levels reflect absorption in nasal airways and an exposure concentration of 1 ppb.

HDI lung dosimetry

Lung dosimetry calculations were conducted in the rat and human lung models with nasal breathing for both species at twice minute volume to coincide with the nasal dosimetry calculations. All lung dosimetry simulations were conducted with an inhalation exposure concentration of 1 ppb and were scaled accordingly to account for nasal absorption. The average tracheal airway concentration of HDI vapor was <0.04 ppb in the rat at the end of inhalation due to high absorption in the URT (Figure 3). Airway HDI concentrations decreased further as the remaining HDI vapor was absorbed in the upper lung airways of the rat. The average tracheal airway concentration of inhaled HDI vapor at the end of inhalation (0.16 ppb) as predicted by the human lung model was much higher than the rat due to less nasal absorption in humans. But, HDI airway concentrations decreased rapidly in the upper- and mid-bronchial airways with ongoing absorption/deposition at these sites. The predicted HDI vapor concentration was <0.02 ppb in generation 10 at the end of inhalation. Predicted total respiratory tract uptake of inhaled

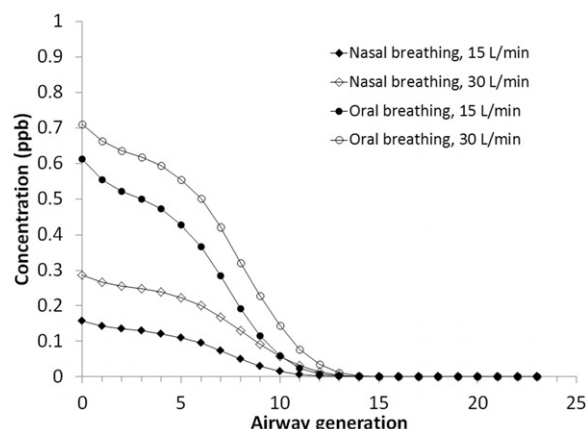


Figure 4. Predicted concentration of inhaled HDI vapor in human lung airways under nasal and oral breathing conditions. Concentration levels reflect absorption in nasal or oral airways and an exposure concentration of 1 ppb.

HDI vapor under nasal breathing was 99% in the rat and 97% in the human model. Predicted respiratory tract uptake was independent of exposure concentration due to the linear boundary condition used in the dosimetry models.

Breathing dynamics had a profound influence on HDI concentration in human lung airways (Figure 4). Tracheal airway concentrations increased with higher flow rates due to decreased absorption in head airways. Despite these higher tracheal concentrations, HDI did not penetrate much deeper into the lung. Higher HDI concentration levels were observed under oral breathing compared to nasal breathing, with concentrations >0.5 ppb in generations 0–2 and concentrations >0.1 ppb down to generations 9–10. Under the breathing scenarios considered here, HDI concentrations in distal alveolar airways were $<2.0 \times 10^{-6}$ ppb, indicating virtually no HDI vapor reaches the deeper pulmonary airways of the human lung. Total respiratory tract uptake of inhaled HDI in the human lung using oral breathing was estimated to be 87%, which was in the range of measurements (61–90%) of respiratory retention of HDI vapor from volunteers in inhalation chambers exposed to concentrations of 5–15 ppb (Monso et al., 2000).

The maximum wall mass flux occurred at the end of the inhalation phase. Predicted rat lung airway flux was approximately 0.56 pg/cm²/s in the trachea and decreased to <0.1 pg/cm²/s in generation 8 (Figure 5). Human lung airway flux values were significantly higher than the rat under nasal breathing scenarios due to higher HDI concentration levels in lung airways. Flux values ranged from 0.7 to 0.9 pg/cm²/s in generations 0–5 under nasal breathing at twice minute volume (Figure 5). Flux values decreased rapidly with further penetration into the lung and were <0.1 pg/cm²/s in generation 11. The slight decrease in flux between the trachea and main bronchi followed by a slight increase in flux in generations 2–4 is due to differences in surface area between upper airway generations.

Predicted wall mass flux values in human lung airways under oral breathing were consistently higher than nasal breathing due to higher HDI airway concentrations (Figure 6). Flux values from 2.8 to 3.5 pg/cm²/s were predicted in generations 0–5 under oral breathing at 15 l/min and from 4.7

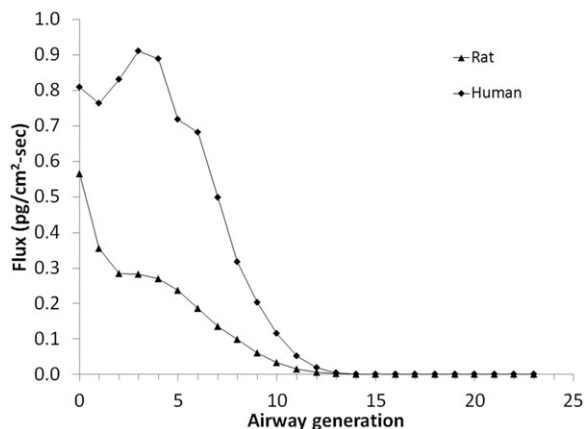


Figure 5. Predicted airway wall mass flux in rat and human lung airways at the end of the inhalation phase with nasal breathing at twice minute volume. The exposure concentration was 1 ppb.

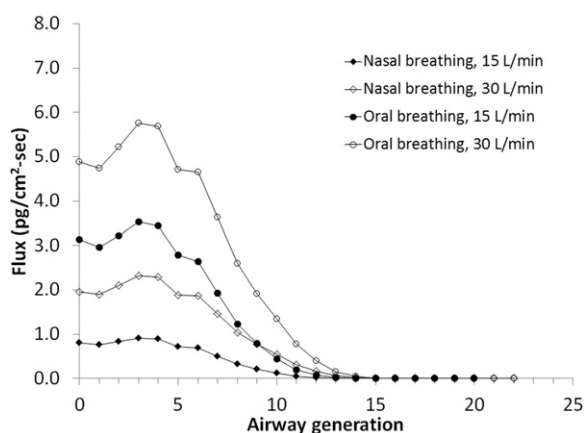


Figure 6. Predicted airway wall mass flux in human lung airways at the end of the inhalation phase under nasal and oral breathing conditions. The exposure concentration was 1 ppb.

to 5.9 pg/cm²/s in generations 0–5 at 30 l/min. Flux values decreased rapidly after generation 6 regardless of breathing route. Flux values under all breathing conditions considered in this study were <1.0 pg/cm²/s in generation 11 and decreased to <1.0 × 10^{−4} pg/cm²/s in the pulmonary region (generation 17). Wall mass flux values can be scaled accordingly for other exposure concentrations.

A sensitivity analysis on the human lung dosimetry model indicated that tracheal flux values were most sensitive to air-phase parameters (Table 1). The sensitivity coefficient (SC) for tracheal flux values was defined as

$$SC = \frac{|\text{Flux}_1 - \text{Flux}_0|/\text{Flux}_0}{|x_1 - x_0|/x_0},$$

where x_0 and x_1 are the original and changed model parameter, and Flux_0 and Flux_1 are the original and updated flux values, respectively.

This differs from the sensitivity results of Asgharian et al. (2012), where they found that lung uptake of soluble vapors was sensitive to the tissue diffusion coefficient. This is likely due to the high reactivity of HDI, which results in absorbed vapor being cleared rapidly from respiratory tissues, thereby rendering results insensitive to tissue-phase properties. Lung flux results were also sensitive to breathing parameters.

Table 1. Sensitivity analysis of tracheal flux values from the human lung dosimetry model at an exposure concentration of 1 ppb HDI.

Parameter	Symbol	SC	
		±50%	±10%
Partition coefficient	P	0.06	0.03
Air-phase diffusivity	D_a	0.37	0.34
Tissue-phase diffusivity	D_t	0.01	0.01
Air-phase mass transfer coefficient	k_g	0.53	0.50
Tissue-phase mass transfer coefficient	k_t	0.02	0.02
Pseudo first-order rate constant	k_f	0.01	0.01
Tidal volume	TV	0.71	0.68
Breathing frequency	f	0.74	0.72

SCs reflect the average of a ±50% or ±10% change in model parameters.

Discussion

Inhalation dosimetry patterns play an important role in determining the locations of airway injury sites to inhaled gases. Lesions observed in HDI-exposed rodents were largely confined to the nasal cavity, indicating significant absorption in the upper airways. In contrast, occupational HDI exposure predominantly affects the human bronchial airways. To investigate interspecies differences in HDI absorption, we used experimental methods to determine the uptake efficiency of inhaled HDI in the rat nasal passages and dosimetry models to extrapolate these results to the full respiratory systems of rats and humans. This approach allowed us to account for species differences in oronasal breathing and airway morphology that affect HDI absorption. For example, rats are obligate nasal breathers and their nasal airways generate complex airflow patterns that lead to higher filtering efficiencies of inhaled particles and gases when compared to humans. Additionally, human oral airways may filter less inhaled material than the nasal passages, leading to higher chemical delivery to deeper lung airways when breathing through this route. It is therefore critical to consider regional airway dosimetry when assessing health risks of inhaled reactive gases and extrapolating nasal effects from animal studies to humans, where pulmonary effects may be more prevalent.

URT uptake of inhaled HDI vapor in rats was in excess of 90%, which was consistent with pathology results from a chronic inhalation study (rats exposed for 6 h/d, 5 d/week for 2 years at concentrations up to 175 ppb) where numerous lesions were observed throughout the noses of HDI-exposed rats, yet no effects were observed in airways immediately distal to the nose such as the larynx, trachea and bronchi (Miles, Inc., 1989). Respiratory tract absorption of inhaled gases is governed by diffusion in the airspace, partitioning into respiratory tissues and diffusion and clearance from respiratory tissues. Rapid reaction with proteins and other nucleophiles (and water to a lesser extent) in the mucus-coated epithelium of the respiratory tract is the primary determinant of HDI uptake. To simulate this clearance process, a pseudo first-order rate constant (k_f) was calibrated by fitting rat nasal uptake in an anatomically accurate CFD model to the experimentally derived uptake value. This rate constant was also used in the rat lung and human nasal and lung dosimetry models, assuming that HDI clearance due to

inherent chemical reactivity would not change between species or with location in the respiratory tract. Inter- or intra-species differences in distribution of glutathione and other proteins, lining the respiratory tract may affect the uptake of HDI but were not accounted for in this modeling effort.

The approach used for simulating HDI uptake was also used in dosimetry modeling efforts of ozone, chlorine and formaldehyde, which are also compounds that react with the moist environment in respiratory mucus and tissues (Bush et al., 2001; Cohen Hubal et al., 1996; Miller et al., 1985). Absorption of ozone in the rat nasal passages was estimated to be ~50% (Hatch et al., 1989) and uptake of inhaled chlorine was estimated to be 95% in the mouse nasal passages (Morris et al., 2005). Using the bolus inhalation method, Nodelman & Ultman (1999) showed that >95% of inspired chlorine and 50–95% of inhaled ozone is absorbed in the upper airways, depending on flow rate. Based on these observations, the respiratory absorption patterns of inhaled HDI are similar to other reactive gases and are characterized by high absorption in head airways with slight penetration into the upper airways of the lung. Differences in respiratory uptake patterns between these reactive gases can be attributed to differences in hydrolysis rates and reaction rates with other nucleophiles. The first-order reaction rate derived for HDI ($k_f = 5 \times 10^6/s$) was similar to values derived for ozone and chlorine. Miller et al. (1985) estimated a chemical rate constant for ozone of $5 \times 10^4/s$ in the lung tissue compartment, although Bush et al. (2001) achieved better agreement with ozone lung penetration fraction using values of $2\text{--}8 \times 10^6/s$. Bush et al. (2001) showed good agreement with chlorine lung penetration data when the first-order rate constant was infinite (i.e. there was no diffusion resistance).

Absorption patterns in human lungs depend on the breathing route (oral versus nasal) and ventilation rate. Predicted HDI vapor concentrations during oral breathing were much higher in upper lung airways and resulted in slightly further penetration beyond generation 10 when compared with nasal breathing. Furthermore, lung penetration of HDI was increased at higher flow rates due to less absorption in the URT and deeper penetration of tidal air into lung airways. Human lung fluxes were much higher than those predicted in the rat lung, mainly due to differences in nasal absorption, but were comparable with predicted flux values in the mid-nose regions of the rat.

Degeneration of olfactory epithelium was observed in rodents chronically exposed to HDI vapor at a concentration of 25 ppb (with a NOAEL of 5 ppb) (Miles, Inc., 1989). This response was selected as the critical effect for an RfC calculation due to its clear concentration–response relationship (Foureman et al., 1994). Other effects such as inflammation, hyperplasia, and squamous metaplasia were also observed following exposure to 25 ppb, although the incidence and severity of these responses showed less distinct dose–response relationships with exposure level. These effects were observed in the caudal rat nose (at an approximate normalized distance of 0.6–0.65 from the nares, see Figure 2). CFD-predicted flux values at these locations averaged $1\text{--}2\text{ pg/cm}^2/s$ at 1 ppb, which yields flux values of $5\text{--}10\text{ pg/cm}^2/s$ at a 5 ppb exposure concentration.

According to dosimetry estimates, individuals under resting or moderate breathing conditions would have to be exposed to 1–10 ppb HDI to receive flux values in the upper bronchial airways (generations 2–4) that are comparable to flux values in the caudal rat nose at the NOAEL of 5 ppb, although the exposure range is sensitive to the breathing route and ventilation rate. These estimates are in agreement with observations of the absence of exposure-related respiratory symptoms in HDI-exposed workers after exposures in the 1–5 ppb range (Cassidy et al., 2010; Hathaway et al., 1999).

The respiratory tract modeling approach used for HDI was patterned after that of another reactive gas, formaldehyde, which consisted of anatomically accurate CFD models of the nasal passages coupled with mass transport models of lung dosimetry (Kimbell et al., 2001; Overton et al., 2001). This approach allowed for incorporation of the complex structure of the nasal passages to examine interspecies differences in HDI uptake, which is high at the portal of entry, and be able to extend simulations to all airways of the lung to characterize vapor penetration into lung airways under different breathing conditions. The nasal CFD and lung dosimetry models have differences related to their structure and details of airflow calculations, but they share common absorption and clearance processes. CFD models use a mechanistic approach to solve the Navier–Stokes and convective-diffusion equations to obtain velocity, pressure and vapor concentration information at millions of nodal points in the nasal airspace. Absorption of vapor from the air into respiratory tissues is governed by concentration gradients at the air:tissue interface. The lung dosimetry models, on the other hand, use a mass transfer coefficient approach to describe vapor uptake since predicted airway concentrations are averaged over the radial space. This approach for modeling lung airways allowed us to simplify the lung structure to a typical-path model from the trachea to alveoli to predict HDI concentration levels in all airway generations.

There have been several other recent efforts modeling lung dosimetry of inhaled gases (Asgharian et al., 2012; Corley et al., 2012; Gloede et al., 2011). Gloede et al. (2011) developed a hybrid CFD-PBPK model for inhaled diacetyl in rats and humans that contained compartments for nasal regions and lung airways. Model predictions were able to correlate diacetyl tissue concentrations in rat nasal airways, where effects were seen *in vivo*, to lower bronchial airways in humans, where bronchiolitis was observed in workers exposed to diacetyl vapor. Diacetyl represents another vapor where rodent nasal effects can be translated to distal airway effects in humans based on dosimetry predictions. In the study by Asgharian et al. (2012), a new model was developed to describe human lung uptake of soluble and reactive vapors. The model was applied to predict uptake efficiency and tissue disposition of acetaldehyde, acrolein and formaldehyde. In their approach, a tissue layer was implemented so that the transient behavior of absorption and tissue clearance could be simulated and the effect of vapor desorption from respiratory tissues could be quantified. Due to the rapid interaction of HDI with nucleophiles in the respiratory tract and the chemical nature of this reaction, it is unlikely that any absorbed HDI vapor would be released from tissues to the air phase during exhalation.

Some of the limitations of this study include the use of simplified models for the oral airways and branching lung structure, which are not capable of evaluating the effects of anatomical details on site-specific dose. Recently, Corley et al. (2012) developed CFD models of the respiratory tracts of a rat, monkey and human from high-resolution imaging data from the nose (and mouth in the human model) to the lung. These models were used to analyze interspecies differences in inhalation airflow patterns and uptake of acrolein vapor. The advantage of their approach was that a contiguous model capturing the anatomical details of the entire respiratory tract was used for the upper and lower airways of each species. Use of a model of this form would improve estimates of HDI dosimetry in oral and upper lung airways, where geometric and turbulence effects could be more accurately simulated. However, personalized models such as these yield dosimetry predictions for a single subject, whereas typical-path models of the lung, as we have used, are more representative of a population average.

In conclusion, dosimetry models were developed to examine differences in respiratory tract absorption of inhaled HDI between rats and humans. High nasal uptake efficiencies of HDI vapor were measured in the rat, which led to reduced concentrations in rat lung airways. Nose-breathing humans were predicted to have higher absorption rates in lung airways compared to those in the rat. In comparison to nose-breathing, in mouth-breathing individuals HDI concentrations and absorption rates were significantly higher in bronchial airways. Results from the interspecies dosimetry models indicate that, based on dosimetry estimates, nasal responses in exposed rats may be indicative of pulmonary responses that might occur in humans. Additionally, it should be noted that the results from this study relate exclusively to HDI monomer vapor and do not address HDI partitioned to the liquid aerosol that is commonly encountered with the use of HDI-based products. Further studies of liquid/vapor partitioning of the polyisocyanate form and its transport and deposition would have to be conducted to study respiratory deposition of this isocyanate form.

Declaration of interest

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