


Spring 5-1-2014

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Baldino, Joshua Baruch, "Assessing the Relationship Between the Blood-Air Partition Coefficient and Fractional Uptake of Inspired Halothane, Acetone, and Ethanol Vapors in the Airways of the C57BL/6J Mouse" (2014). *Honors Scholar Theses*. 355.
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Assessing the Relationship Between the Blood-Air Partition Coefficient and Fractional Uptake of Inspired Halothane, Acetone, and Ethanol Vapors in the Airways of the C57BL/6J Mouse

Joshua Baruch Baldino

ABSTRACT

The following series of studies investigates the elimination and uptake trends of halothane, acetone, and ethanol vapors in the airways of C57BL/6J mice. These vapors were chosen because they span a wide range of solubilities, as indicated by their blood-air partition coefficients, and are not associated with any significant airway metabolism or reactivity with tissue substrates *in vivo*. Mice were exposed to a homogeneous vapor mixture containing a 1:1:1 ratio of halothane, acetone, and ethanol at relative concentrations of approximately 10 ppm. Exposure studies were performed with mice in two states, conscious and deceased, in order to provide control against adsorption to superficial anatomical features. Elimination rates were measured by recording airborne vapor concentrations at discrete intervals via gas chromatography. Decay constants were obtained through log-linear regression analysis, and were used to calculate clearances and fractional uptake ratios. The average clearance per mouse for halothane, acetone, and ethanol vapors was 20.9 ± 1.70 mL/min, 51.9 ± 1.88 mL/min, and 52.2 ± 1.54 mL/min (normalized mean \pm standard deviation), respectively. Fractional uptake values for each vapor were, in the same order, 0.238 ± 0.053 , 0.589 ± 0.123 , and 0.593 ± 0.123 . A non-linear relationship between the blood-air partition coefficient and airway extraction was

observed, suggesting that vapor solubility is correlated with absorption in a complex manner that requires advanced, highly compartmentalized models to accurately predict.

INTRODUCTION

"All substances are poisons: there is none which is not a poison. The right dose differentiates a poison and a remedy." – Paracelsus (1493 – 1541)

Background

Paracelsus is considered by many to be the founder of modern toxicology and is well-recognized for his observation that toxicity is determined by the dose of a substance, not merely its chemical identity (Borzelleca, 2000). This fundamental premise of toxicology is often concisely summarized in the adage: “the dose makes the poison”. Indeed the difference between a substance as a source of medicinal relief or of toxicity often bounds a very fine line; one that must be carefully considered in the pharmacologic management of disease. To advance this principle, the effect of a substance on a particular biological target, whether that be a protein, individual cell, tissue, or organ system, is chiefly determined by the *delivered dose* at that target and the innate sensitivity of that target to the toxicant. In other words, for a chemical to exert some desired or undesired effect, it must be present in sufficient quantity at the site at which the pharmacodynamic outcome is to be realized. The superficial dose of a substance (e.g. amount inhaled, swallowed, or injected) does not necessarily allow for an accurate prediction of *where* that effect will occur, though the magnitude of any effect is proportional to dose.

With this perspective in mind, the dose or concentration of a substance alone is meaningless for predicting where a xenobiotic will exert its effect. Delivered dosimetry is the study of the disposition patterns of foreign substances within a biological organism in an effort to provide insight into the relationship between exposure to exogenous chemicals and the localized effects thereof. Respiratory toxicologists are particularly interested in delivered dosimetry studies as they help to quantify the regional injury patterns of an inhaled toxicant within the airways. The respiratory system is a complex of anatomical structures and organs whose biological purpose is to provide the essential framework for the efficient exchange of oxygen and carbon dioxide gases, a process critical to sustaining cellular function and systemic homeostasis. With respect to pulmonary toxicology and pharmacology, the respiratory system also provides a means of entry for toxic and therapeutic agents.

Interspecies differences can make direct comparisons between experimental models (e.g. rats, mice) and humans quite difficult, or even useless, for toxicologists given the dramatic differences in anatomy and physiology. As an example, a questionnaire of 117 workers at a microwave-popcorn plant in Missouri found that the workers had twice the rate of physician-diagnosed asthma and chronic bronchitis than the national average (Kreiss *et al.*, 2000). Analysis of air samples in the plant identified diacetyl (2,3-butanedione) as the predominant compound. Diacetyl is an important ingredient in the butter flavoring of popcorn and was used extensively at the site. The injury patterns in the workers suggest that diacetyl causes injury primarily in the smaller, lower airways of humans and penetrates significantly to these areas. Subsequent exposure studies in rats showed that diacetyl induced necrotic injury in the epithelium of the nose, larynx, trachea, and bronchi. (Hubbs *et al.*, 2002; Hubbs *et al.*, 2008). Airway damage was confined entirely to the upper airways of the rats, with no injury noted in the alveoli. The rat

nasal passage is known to be able to extract diacetyl in its entirety before it can penetrate to the deeper airways (Gloede *et al.*, 2011). These results suggest that the upper airways themselves are not inert, and may represent sites of significant uptake, which can lead to localized toxicity.

Physical Principles

At the most abstract level, absorption of inhaled vapors is dependent upon the concentration gradient between the lumen of the airway and the superficial tissue layers, including the mucosa. Vapor flux will always occur from a region of high concentration (e.g. lumen) to a region of lower concentration (e.g. tissue) as described by Fick's first law of diffusion for a one-dimensional system (**Equation 1**), where J is net flux in $\frac{\text{amount}}{\text{m}^2 \cdot \text{s}}$, D is the diffusion coefficient in $\frac{\text{m}^2}{\text{s}}$, and $\frac{\partial C}{\partial x}$ represents the concentration gradient.

$$J = -D \frac{\partial C}{\partial x} \quad (1)$$

Fick's law tells us that flux is directly proportional to the magnitude of the concentration gradient at steady state. Any process that increases the concentration of vapor in the lumen relative to tissue such as cellular metabolism, direct reaction with tissue substrates, or elimination via the circulating blood flow, will promote flux of vapor into tissue. Conversely, vapor may desorb back into the airstream from the tissue layers if the concentration gradient is reversed (Morris, 2012). Although Fick's law indicates in which direction and to what extent vapor will move, it does not provide a precise description of overall mass transport at the air-tissue interface. Because the concentration gradient is only linear in idealized or highly specific real-world situations, advanced mass transport equations are required to accurately model flux patterns *in vivo*. The derivation of these equations is beyond the scope of this paper; however,

Fick's law does include the principle driving force of mass flux (concentration differentials) and can be used as a simplified proxy for understanding this phenomenon.

Vapor molecules diffuse through the air by Brownian motion until they contact the mucosa. The rate at which diffusion occurs through the air and tissue is described mathematically by Graham's law. Graham's law states that the rate of diffusion of a vapor is inversely proportional to the square root of the vapor's molecular mass (M) (**Equation 2**). For instance, a vapor four times as massive as the reference will diffuse at half the rate.

$$rate \propto \frac{1}{\sqrt{M}} \quad (2)$$

At the interface of the lumen and tissue, vapor solubility determines affinity for the deeper layers of the epithelium, and eventually the circulation. The relative concentration of vapor between liquid and air phases can be described by Henry's law, which says that the concentration of vapor in the liquid at steady state is directly proportional to the partial pressure of the vapor in the air. A partition coefficient is the ratio of the concentration of vapor in one phase over another at equilibrium and can be experimentally determined for various interfaces. For instance, a vapor with a high blood-air partition coefficient, will have greater concentration in the blood at steady state than a vapor with a small blood-air partition coefficient. Inert (non-metabolized, non-reactive) vapors follow Henry's law as a good approximation of solubility. However, reactive vapors deviate from Henry's law because more vapor will enter the fluid phase due to elimination in the tissue. Metabolism, direct reaction, and removal of vapor via the circulation will maintain the concentration gradient. If these factors are significant enough, the elimination of vapor could be essentially complete, allowing the tissue to serve as an infinite sink and to increase uptake dramatically.

The purpose of the following studies was to determine the elimination patterns of halothane, acetone, and ethanol vapors for C57BL/6J mice *in vivo* with respect to vapor solubility. These vapors were chosen as they span a high range of blood-air partition coefficients (halothane 2.5, acetone 260, ethanol 1800) are not known to undergo extensive metabolism, and are not appreciably reactive with tissue substrate (Gloede *et al.*, 2011). Outcomes are reported as clearance and fractional uptake, and trends are compared with previous experimental results.

MATERIALS AND METHODS

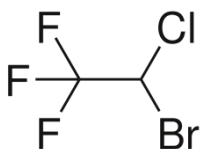
Animals

Female C57BL/6J mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA) and used in all exposure studies. The mice were housed over hardwood shavings (Sani-Chip Dry, P. J. Murphy Forest Products, Montville, NJ, USA) under standard environmental conditions with temperature maintained from 22°C to 25°C and a 12-hour light-dark cycle (lights on at 06:30 hours). Food (LabDiet® by PMI Nutrition International, Brentwood, MO, USA) and tap water were provided *ad libitum*. Mice were acclimated to this environment for at least 2 weeks prior to exposure and were used within 14 weeks of arrival. The average weight of the mice was 20 g at the time of use. All experimental procedures were approved by the University of Connecticut Institutional Animal Care and Use Committee.

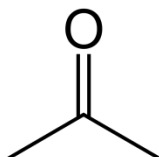
Chemicals

Halothane (2-bromo-2-chloro-1,1,1-trifluoroethane; $\geq 99\%$ purity), acetone ($\geq 99.9\%$ purity), and ethanol ($\geq 99.5\%$ purity) stock solutions were obtained from Sigma-Aldrich (St.

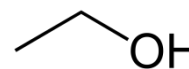
Louis, MO, USA). Because very small volumes of these stock solutions (5 μL) were used to create a homogeneous vapor atmosphere for animal exposure studies, all volume measurements and transfers were performed using an adjustable (1 – 10 μL) air-displacement micropipette.



Halothane (2.5)



Acetone (260)



Ethanol (1800)

Table 1: The molecular structures of halothane, acetone, and ethanol are shown with corresponding blood-air partition coefficients in parentheses.

Vapor Generation

Acetone, Halothane, and Ethanol Atmospheres

Prior to vaporization of stock halothane, acetone, and ethanol samples, one or more 4.3 L inert glass laboratory bottles were cleaned and heated in an oven for at least one hour to remove any residue from previous experiments. Vapor atmospheres were generated by transferring 5 μL of each of the stock solutions into a clean 4.3 L bottle (1:1:1). A tin foil hat was secured to the top of the bottle with an elastic band prior to reattaching the bottle cap to prevent vapor adsorption to the plastic in the cap. The bottle was heated in the oven for a minimum of 30 minutes to allow for complete evaporation of the liquids. A 40 mL sample of the homogeneous vapor mixture was extracted from the bottle and injected into the experimental uptake chamber (see below) to measure clearance. If multiple runs were performed, one bottle was prepared for each run. No bottles were immediately reused for subsequent runs to minimize vapor loss and inconsistent atmosphere generation. The final concentration ratio of each vapor in the uptake chamber was approximately 10 ppm throughout the exposure studies.

Uptake Chamber

Vapor clearance was measured in an inert 4 L stainless steel and glass cylindrical container. Conscious or deceased mice were placed into the container followed by injection of the 1:1:1 homogeneous mixture. After several trial runs without animals, it was determined that a paraffin wax film (Parafilm M®) seal around the crease of the lid and the container prevented overt leaking of vapors and adsorption to less inert materials. The lid was secured over the chamber by three adjustable locking pliers to ensure an airtight seal. Vapor was extracted from the uptake chamber through a metal column attached to the line sampler of the gas chromatograph. The column was inserted to the geometric center of the uptake chamber through a pinhole in the cover. The hole was small enough to account for negligible vapor loss as confirmed by trial runs prior to the animal exposures. If multiple runs were performed in succession, the uptake chamber was cleaned and dried before reuse.

Respiratory Exposure Protocols

Mice were subjected to vapor atmospheres in two states, conscious and deceased, to allow for normalization of exposure data against control. Mice were exposed to equal volume concentrations of halothane, acetone, and ethanol in a 1:1:1 ratio over the course of an analytical exposure run.

Conscious Mice

Three conscious, female, C57BL/6J mice were placed in the uptake chamber, followed by injection of 40 mL of the halothane, acetone, and ethanol mixture. The chamber was sampled at 3-minute intervals via the gas chromatograph through the duration of vapor exposure. Each run was performed with fresh mice not previously exposed to vapor. After completion of the

sampling run, the mice were euthanized via intraperitoneal urethane injections at a dose of 1.3 g/kg and exsanguinated for dissection and subsequent tissue studies (not reported).

Deceased Mice

To provide control against vapor loss not attributable to reactive or metabolic processes (e.g. adsorption to hair); three previously unexposed, female, C57BL/6J mice were euthanized via urethane overdose (1.3 g/kg, IP) prior to the run. Atmospheres within the uptake chamber were created as described above, and the mice were exsanguinated and dissected post-exposure for further analysis (not reported).

Analysis

Physical Methods

Vapor concentrations were measured using a Varian CP-3800 gas chromatograph (Varian, Sugarland, TX, USA) with a gas-sampling valve, 15-meter DB-Wax column, and a flame ionization detector (FID). Vapor samples were injected into the gas chromatograph at discrete, 3-minute intervals with each exposure lasting a minimum of 30 minutes. Appropriate temperatures, pressures, and carrier gas flow rates were selected to allow for adequate separation of vapor samples in the line column. The order of elution was previously determined so that the elution peaks of halothane, acetone, and ethanol could be identified. Output from the FID was obtained as numerical data representing the integrated area under the curve (AUC) for the elution peaks of each vapor. Flow rate was measured at multiple intervals during the sampling run using a bubble meter such that the velocity of the bubble indicated a flow rate of approximately 20 mL/min (e.g. 1 mL/3 sec) over the duration of the run.

Statistical Methods

Output from the gas chromatograph was used to generate plots of concentration against time. These plots were noted to be linear following logarithmic transformation of AUC values, and simple linear regression using the method of least squares was performed to obtain a line of best fit for the data points. The slope of this line was taken to be the rate constant of elimination over the course of the run. Results are described in terms of clearance (mL/min) by multiplying the rate constant of elimination (min^{-1}) by the volume of the uptake chamber (4000 mL), and in terms of fractional uptake by dividing the total airway clearance (mL/min) by the average minute ventilation rate (mL/min) of the animals. Statistical calculations and data presentation were performed with STATISTICA 12 software developed by StatSoft (Tulsa, OK, USA).

RESULTS

Preliminary runs with an empty uptake chamber were completed prior to animal exposures. In these experiments, the clearance values obtained were similar to the sampling flow rate of 20 mL/min used in all studies.

Halothane

Halothane exposure runs ($n = 2$) with deceased mice were performed and concentration versus time scatterplots are shown in **Figure 1**. Halothane clearance differed negligibly from the vapor-sampling rate suggesting limited adsorption to the anatomical features of the mice. The mean elimination rate constant was determined to be $0.0063 \pm 0.0002 \text{ min}^{-1}$ with an associated mean clearance of $25.2 \pm 1.13 \text{ mL/min}$. Exposure outcomes for conscious mice ($n = 4$) are shown in **Figure 2**. The elimination rate constant in these runs averaged $0.0220 \pm 0.0012 \text{ min}^{-1}$

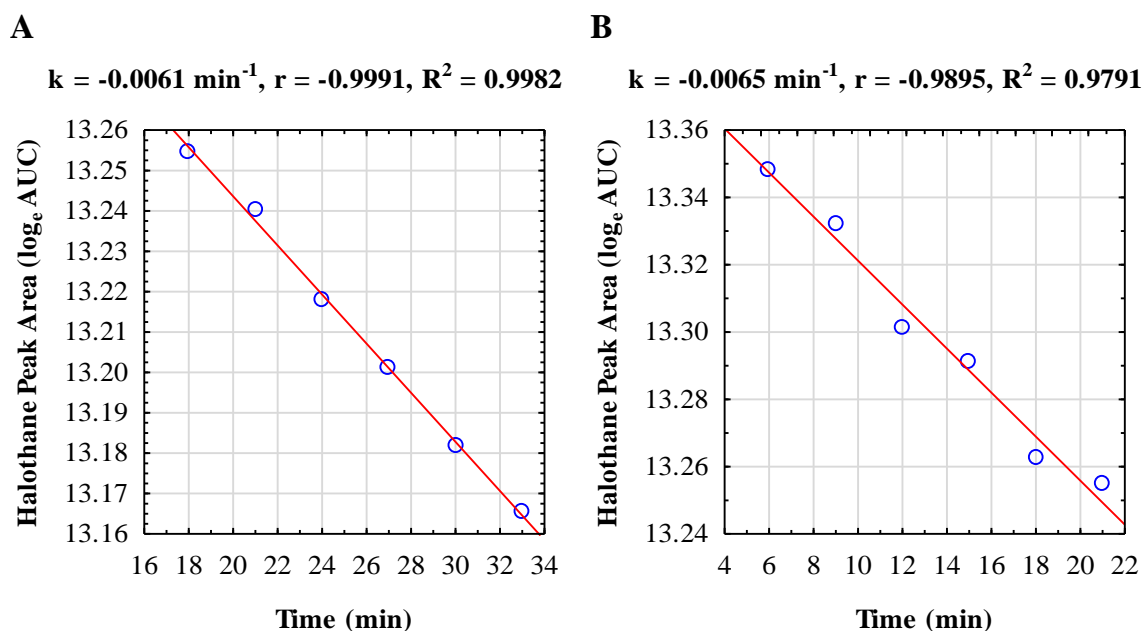


Figure 1: Shown are log-linear plots ($n = 2$) of concentration against time for exposures of **deceased** C57BL/6J mice to **halothane** atmospheres. Halothane concentration is represented as the log-transform of FID signal peaks (AUC) from the GC. Samples were injected at discrete 3-minute intervals. Linear regression was performed in STATISTICA with the slope of the line representing the rate constant of elimination, k , in min^{-1} . Also shown are the linear correlation coefficients, r , and the coefficients of determination, R^2 , for the plots of A and B.

giving a mean clearance of $88.0 \pm 4.96 \text{ mL/min}$. Halothane clearance in the conscious groups exceeded elimination rates in the deceased groups by at least 3-fold. With consideration for the control group data, overall airway clearance was calculated as the difference between the mean clearances, equal to $62.8 \pm 5.09 \text{ mL/min}$. This data was normalized per mouse (3 per group) to yield a clearance estimate of $20.9 \pm 1.70 \text{ mL/min}$. In order to determine the fractional uptake of halothane vapor, experimental minute ventilation rates for female C57BL/6J mice were obtained from the Mouse Phenome Database ($88.0 \pm 18.1 \text{ mL/min}$, $n = 7$). Fractional uptake was calculated as the normalized clearance (mL/min) divided by the average minute ventilation rate (mL/min). A value of 0.238 ± 0.053 was determined for halothane vapor in the sample groups.

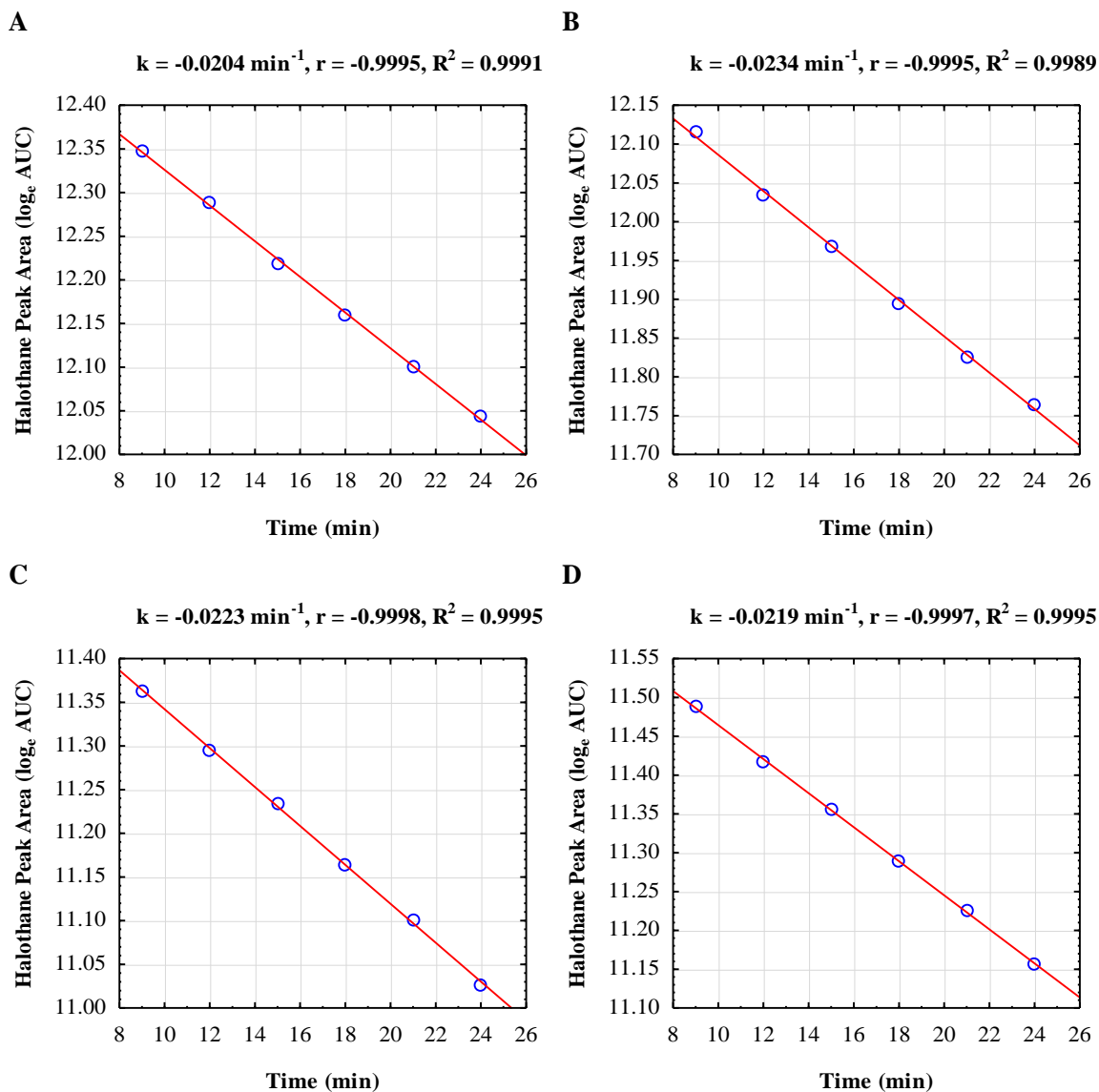


Figure 2: Shown are log-linear plots ($n = 4$) of concentration against time for exposures of **conscious** C57BL/6J mice to **halothane** atmospheres. Halothane concentration is represented as the log-transform of FID signal peaks (AUC) from the GC. Samples were injected at discrete 3-minute intervals. Linear regression was performed in STATISTICA with the slope of the line representing the rate constant of elimination, k , in min^{-1} . Also shown are the linear correlation coefficients, r , and the coefficients of determination, R^2 , for the plots of A, B, C, and D.

Acetone

Graphical plots of acetone concentration versus time for deceased and conscious mice are shown in **Figure 3** and **Figure 4**, respectively. The mean elimination rate constant and mean clearance for exposure runs with deceased mice ($n = 2$) were determined to be 0.0080 ± 0.0007

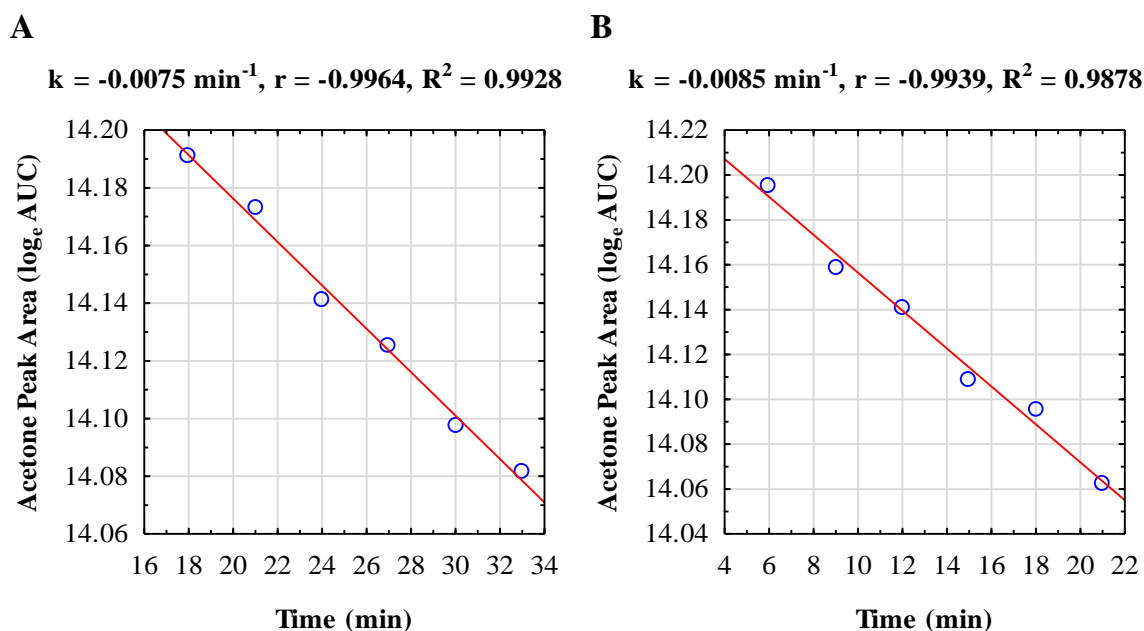


Figure 3: Shown are log-linear plots ($n = 2$) of concentration against time for exposures of **deceased** C57BL/6J mice to **acetone** atmospheres. Acetone concentration is represented as the log-transform of FID signal peaks (AUC) from the GC. Samples were injected at discrete 3-minute intervals. Linear regression was performed in STATISTICA with the slope of the line representing the rate constant of elimination, k , in min^{-1} . Also shown are the linear correlation coefficients, r , and the coefficients of determination, R^2 , for the plots of A and B.

min^{-1} and $32.0 \pm 2.83 \text{ mL/min}$. Exposures with conscious mice ($n = 4$) showed significantly greater elimination profiles. The mean elimination rate constant and clearance for these runs were $0.0470 \pm 0.0012 \text{ min}^{-1}$ and $187.6 \pm 4.88 \text{ mL/min}$. Although acetone demonstrated greater adsorption affinity than halothane, there was at least a 5-fold difference in observed clearance rates between deceased and conscious exposure groups. Adjusting for control, overall airway clearance of acetone averaged $155.6 \pm 5.64 \text{ mL/min}$ for each group. Normalizing clearance by mouse (3 per group) gave an average clearance of $51.9 \pm 1.88 \text{ mL/min}$. Calculating fractional uptake using minute ventilation data from the Mouse Phenome Database data yielded a value of 0.589 ± 0.123 .

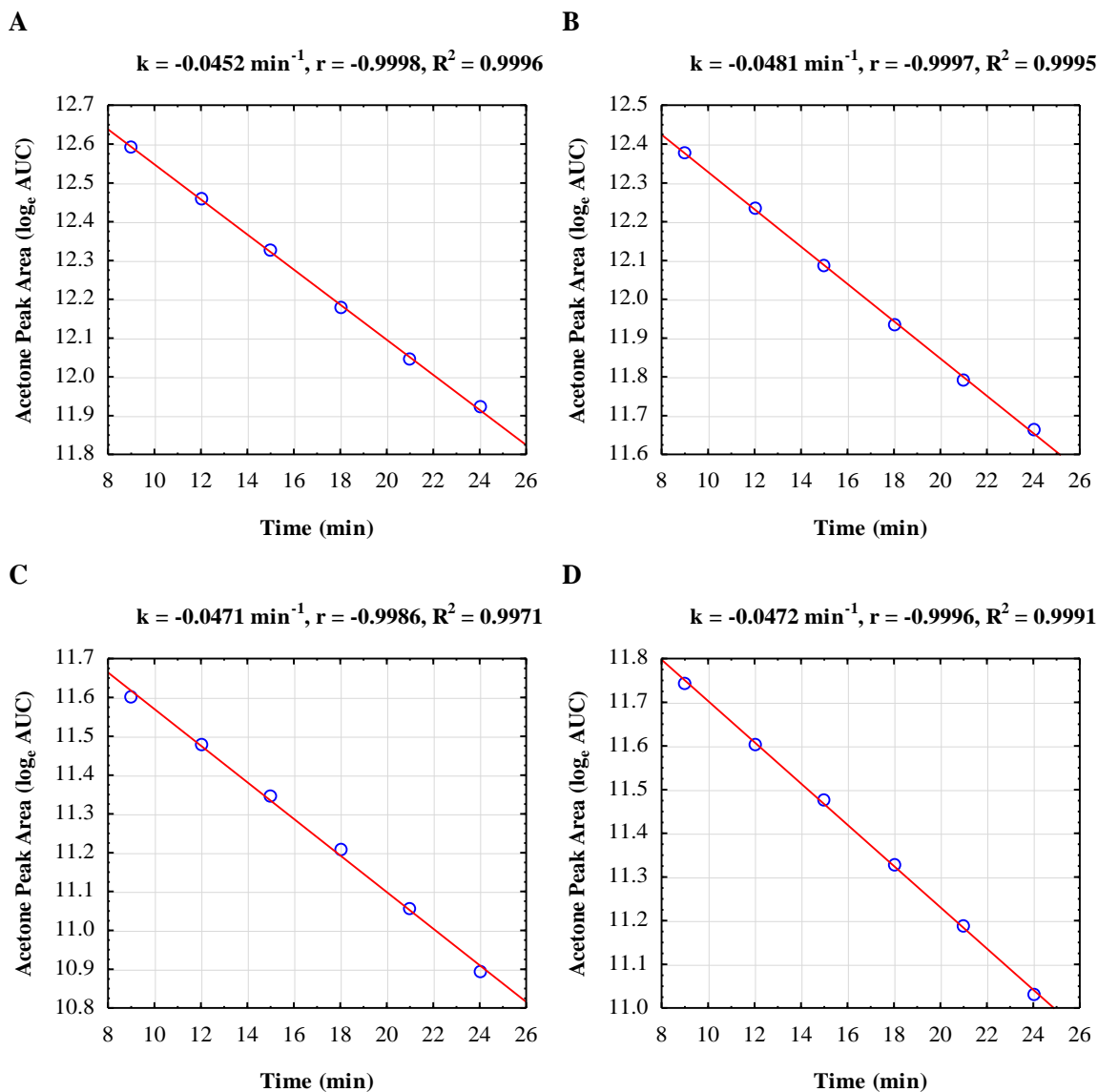


Figure 4: Shown are log-linear plots ($n = 4$) of concentration against time for exposures of **conscious** C57BL/6J mice to **acetone** atmospheres. Acetone concentration is represented as the log-transform of FID signal peaks (AUC) from the GC. Samples were injected at discrete 3-minute intervals. Linear regression was performed in STATISTICA with the slope of the line representing the rate constant of elimination, k , in min^{-1} . Also shown are the linear correlation coefficients, r , and the coefficients of determination, R^2 , for the plots of A, B, C, and D.

Ethanol

Ethanol exposures with deceased ($n = 2$) and conscious ($n = 4$) mice are summarized in **Figure 5** and **Figure 6**, respectively. The average elimination rate constant and clearance with deceased mice were $0.0098 \pm 0.00007 \text{ min}^{-1}$ and $39.0 \pm 0.28 \text{ mL/min}$. Conscious exposure

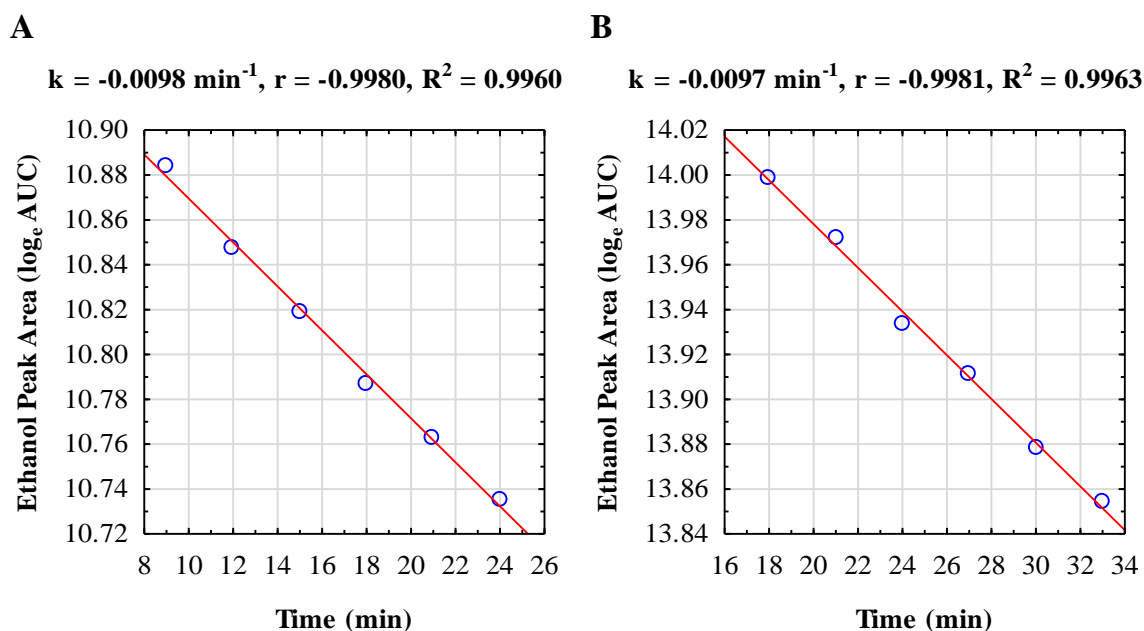


Figure 5: Shown are log-linear plots ($n = 2$) of concentration against time for exposures of **deceased** C57BL/6J mice to **ethanol** atmospheres. Ethanol concentration is represented as the log-transform of FID signal peaks (AUC) from the GC. Samples were injected at discrete 3-minute intervals. Linear regression was performed in STATISTICA with the slope of the line representing the rate constant of elimination, k , in min^{-1} . Also shown are the linear correlation coefficients, r , and the coefficients of determination, R^2 , for the plots of A and B.

groups generated a mean elimination rate constant of $0.0490 \pm 0.0012 \text{ min}^{-1}$, equivalent to a mean clearance of $195.6 \pm 4.61 \text{ mL/min}$. Even more so than acetone, ethanol showed significant adsorption affinity, nearly double the airflow sampling rate (20 mL/min). However, clearances for conscious mice exceeded those of the deceased mice by at least 4-fold. The overall airway clearance given control data was equal to $156.6 \pm 4.62 \text{ mL/min}$. Adjusted per mouse (3 per group), clearance was determined to be $52.2 \pm 1.54 \text{ mL/min}$. This value leads to a calculated fractional uptake value of 0.593 ± 0.123 using minute ventilation rates from the Mouse Phenome Database.

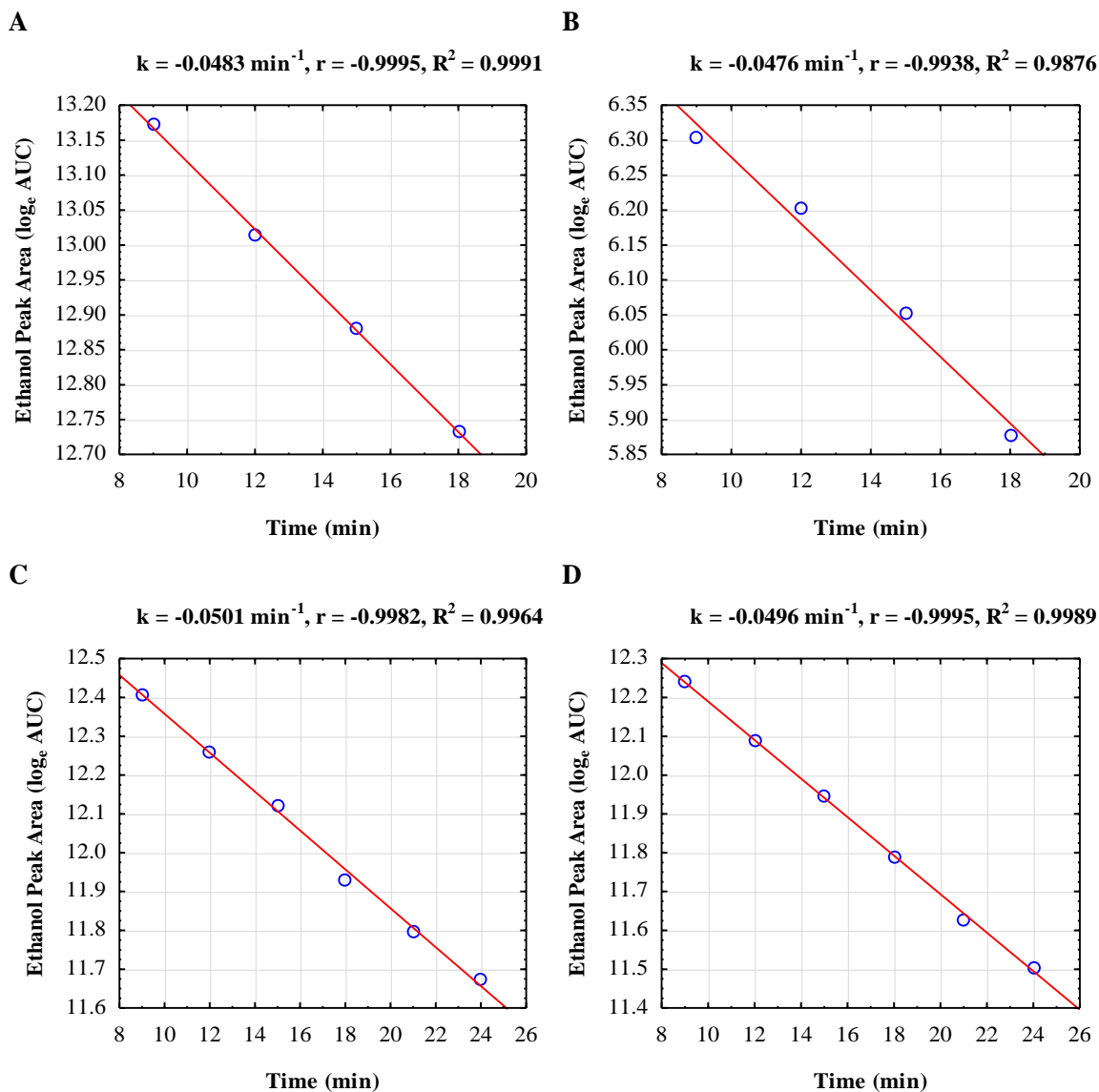


Figure 6: Shown are log-linear plots ($n = 4$) of concentration against time for exposures of **conscious** C57BL/6J mice to **ethanol** atmospheres. Ethanol concentration is represented as the log-transform of FID signal peaks (AUC) from the GC. Samples were injected at discrete 3-minute intervals. Linear regression was performed in STATISTICA with the slope of the line representing the rate constant of elimination, k , in min^{-1} . Also shown are the linear correlation coefficients, r , and the coefficients of determination, R^2 , for the plots of A, B, C, and D.

DISCUSSION

In the Varian CP-3800 gas chromatograph equipped with a flame ionization detector (FID), a hydrocarbon sample (eluent) is mixed with oxygen before being passed through a burner jet. The combustion of the hydrocarbon mixture generates negative ions that are driven toward

an electrode with a positively biased potential difference (voltage). When these anions strike the electrode detector plate, they generate a current proportional to the mass-amount of eluent injected into the FID. The current is measured by an extremely sensitive picoammeter and fed through a current integrator that computes the area under the curve (AUC) of the elution peak. Raw numerical output from the gas chromatograph represents the time course of elimination for a particular compound over the duration of an analytical run. The elimination patterns in the presented series of experiments were observed to follow an exponential decay curve when peak AUC was plotted against time, as described by **Equation 3**.

$$A(t) = A_0 e^{-kt} \quad (3)$$

Here, $A(t)$ is the amount of substance at time t (min), A_0 is the initial quantity of the substance, e is Euler's constant, and k is the rate constant of elimination (min^{-1}). The goal of these studies was to determine the value of k for each vapor in each run in order to calculate clearance and fractional uptake. Unfortunately, exponential plots do not show an easily identifiable relationship between the amount of a substance and time, should one exist.

Logarithmic transformation of y-axis data points serves as an effective means of overcoming the analytical difficulties in visualizing these relationships. The log-transform of **Equation 3** is shown below as **Equation 4**, and resembles the generic first-order polynomial $y(t) = mt + b$, where m is the slope of the line and b is the y-intercept.

$$\ln A(t) = -kt + \ln A_0 \quad (4)$$

Here, the rate constant, k , is easily identified as the slope (m) of the semi-log plot of $\ln A(t)$ against time. Data from the experiments presented herein were observed to follow a linear relationship when plotted in this semi-log fashion. Simple linear regression via the least squares method was performed with STATISTICA software and used to generate trend lines in

the form of **Equation 4**. The associated linear correlation coefficients, r , and coefficients of determination, R^2 , for each semi-log plot suggest minimal deviation from the calculated trend lines (**Table 2**).

	Halothane			Acetone			Ethanol		
	k (min ⁻¹)	r	R ²	k (min ⁻¹)	r	R ²	k (min ⁻¹)	r	R ²
Deceased	-0.0061	-0.9991	0.9982	-0.0075	-0.9964	0.9928	-0.0098	-0.9980	0.9960
	-0.0065	-0.9895	0.9791	-0.0085	-0.9939	0.9878	-0.0097	-0.9981	0.9963
Conscious	-0.0204	-0.9995	0.9991	-0.0452	-0.9998	0.9996	-0.0483	-0.9995	0.9991
	-0.0234	-0.9995	0.9989	-0.0481	-0.9997	0.9995	-0.0476	-0.9938	0.9876
	-0.0223	-0.9998	0.9995	-0.0471	-0.9986	0.9971	-0.0501	-0.9982	0.9964
	-0.0219	-0.9997	0.9995	-0.0472	-0.9996	0.9991	-0.0496	-0.9995	0.9989

Table 2: Shown are the rate constant of elimination, k , linear correlation coefficient, r , and coefficient of determination (R^2) for all analytical runs subjecting deceased ($n = 2$) and conscious ($n = 4$) mice to halothane, acetone, and ethanol atmospheres.

Mean clearance values for each vapor in each exposure group (conscious and deceased) were also uniform, yielding adjusted airway clearances with minimal deviation (**Figure 7**). Unfortunately, direct observation of ventilation rates was not possible as exhaled and humidified air from the mice condensed on the glass lid of the uptake chamber preventing an interior view of the specimens. In order to compute fractional uptake, empiric minute ventilation data from the Mouse Phenome Database was used as a proxy. It is unknown how much variance in baseline minute ventilation could exist between batches of C57BL/6J mice, or how exposure to the vapors in these experiments may alter minute ventilation. In addition, only female mice were tested and a small number of analytical runs were performed. Any discussion of the presented data should be discussed with respect to these limitations, all of which may affect external validity.

Vapor uptake in the airways is dependent upon the concentration gradient between the lumen of the airways and the interface of the mucosa and underlying tissue layers, as well as the interfacial transport across the air-fluid barrier. Diffusion will always occur in the direction of decreasing concentration; thus, processes that eliminate vapor from the superficial airway

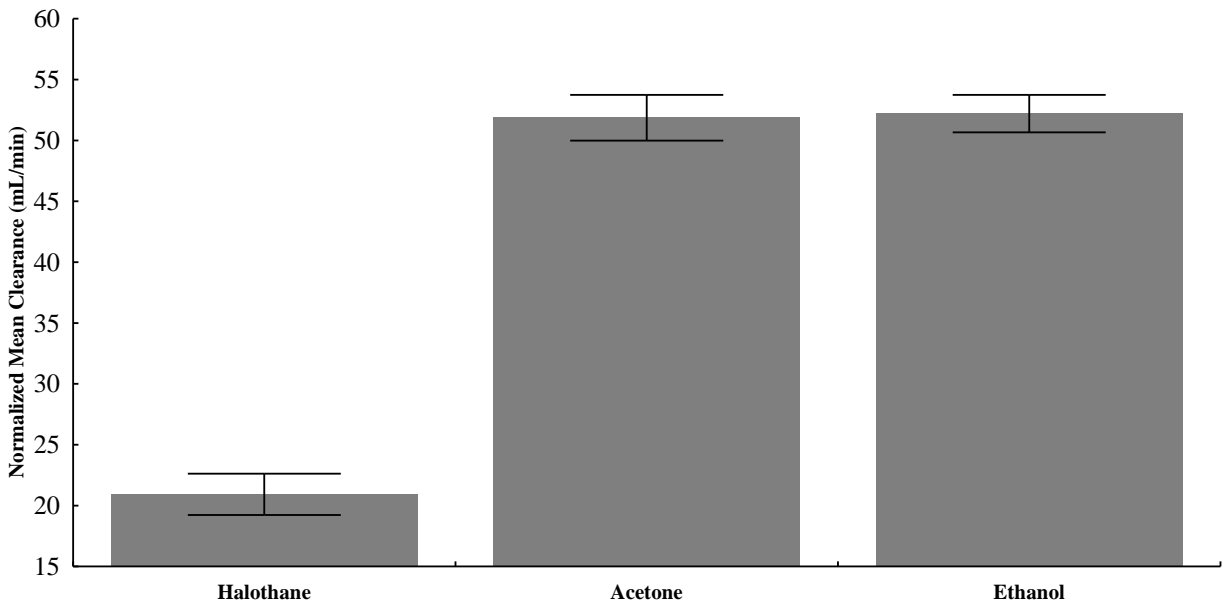


Figure 7: Total airway mean clearance for halothane, acetone, and ethanol per mouse after adjusting for control values. Standard deviations are shown as whisker plots above each column. The blood-air partition coefficients of halothane, acetone, and ethanol are 2.5, 260, and 1800, respectively. Though not plotted, fractional uptake values are directly proportional to clearance.

structures will serve to maintain a gradient to drive uptake. Metabolic, reactive, and perfusion qualities of these structures will determine the rate of absorption and uptake, while solubility will determine the extent of deposition. Vapor that is highly metabolized and highly soluble would predictably see rapid absorption and high degrees of accumulation within the tissue. Conversely, less metabolized and poorly soluble vapors are not likely to experience appreciable deposition over a similar exposure period (Morris, 2012).

In the present series of experiments, the relationship between the blood-air partition coefficient and fractional uptake in the airways was non-linear. Experiments by Gloede *et al.* (2011) showed that the plot of fractional uptake of vapors in the lower respiratory tract of humans against the blood-air partition coefficient demonstrated a complex polynomial relationship (**Figure 8**). Vapors with low blood-air partition coefficients (< 10) had increased uptake with increased solubility. Partition coefficients in a transitive range (10 – 1000) were associated with less efficient uptake than might be expected if absorption was solely dependent

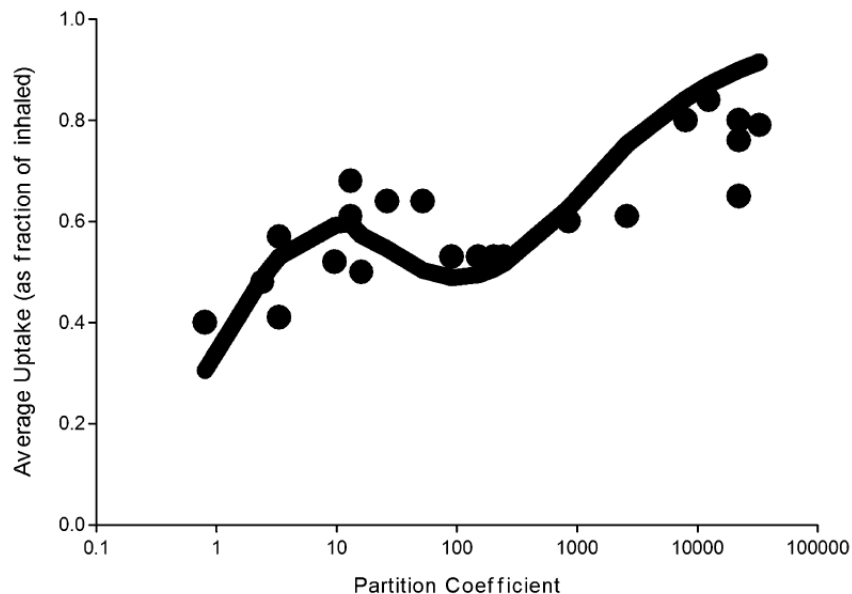


Figure 8: Model output and measured values for lower respiratory tract vapor uptake in humans from Gloede *et al.*, 2011. Measured values are depicted as filled circles, model estimates are shown by the solid line.

on solubility. For vapors with partition coefficients greater than 1000, uptake again increases nearly proportionally with solubility. This complex uptake pattern can be attributed to biphasic breathing cycles and the resulting absorption and desorption of vapor during inhalation and exhalation, respectively (see below). In these studies, halothane demonstrated minimal uptake (0.238 ± 0.053) as would be predicted for a low blood-air partition coefficient. However, uptake values for acetone (0.589 ± 0.123) and ethanol (0.593 ± 0.123) were essentially identical despite a 7-fold difference in their blood-air partition coefficients.

Because breathing in mammals is cyclic, absorption occurs in two phases. At the start of inhalation, vapor-laden air is brought into the airways. The vapor is at a higher concentration in the air than in the tissue, establishing a concentration gradient that drives vapor into the tissue. As vapor diffuses into tissue, the concentration gradient decreases until the start of exhalation, where air that has been stripped of vapor passes back over the tissue. The concentration gradient is now reversed, and desorption can occur into the unsaturated air. For vapors with small partition coefficients (halothane), there is minimal desorption because very little vapor is

absorbed to begin with. Hence, airway uptake increases with partition coefficient until a critical point is reached. As the partition coefficient continues to increase, more vapor is absorbed into tissue, leaving little in the expired air. A steeper concentration gradient is established with respect to the exhaled air, which pushes vapor back into the air stream. Due to significant desorption, uptake for vapors in this transitive range (acetone) is less than what would be predicted if uptake were linearly correlated with solubility. Finally, vapors with large partition coefficients (ethanol) are soluble enough so as to minimize the desorptive process. These vapors are sequestered with great efficiency in the airways and may be almost completely absorbed.

As a final word, the results observed for acetone and ethanol are closer than expected. Acetone uptake was predicted to be less efficient than ethanol as was observed for F344 rats (Gloede *et al.*, 2011), yet the results reported in this investigation suggest that acetone uptake is identical to that of ethanol. Previous research has shown that, similar to rats, acetone is not metabolized appreciably *in vivo* in the upper respiratory tract of mice (Morris, 1991). Therefore, metabolism should not have contributed significantly to acetone uptake, and it is not clear what could have resulted in deviation from expected values. Further experimentation would be necessary to confirm the results observed for acetone in these studies.

CONCLUSION

Vapor absorption and deposition patterns involve complex, multi-factorial processes that cannot be adequately predicted via simple compartmental modeling. The purpose of the present experiments was to determine the association between the blood-air partition coefficient and airway extraction of halothane, acetone, and vapors in C57BL/6J mice. These vapors were

chosen as they spanned a wide range of blood-air partition coefficients (2 – 2000), and are not known to be metabolized or directly reactive within airway tissue. Fractional uptake was observed to follow a non-linear relationship with the blood-air partition coefficient *in vivo*. Comparatively, the present results show congruence with previous experimental outcomes and modeling estimates observed in rats and humans (Gloede *et al.*, 2011). This increases confidence that the model accurately describes crucial physical variables *a priori* for predicting uptake patterns in the C57BL/6J mouse. As with any scientific model, it is important to remember that, though the structure and mathematical framework are based upon sound experimental observations, it is still a predictive model and not equivalent to empiric observation. Dosimetry modeling is an extremely valuable tool for respiratory toxicologists and medicinal scientists as it helps to predict the toxicologic effect of xenobiotic exposure on the airways in a well-defined population, as well as to compare injury patterns between species. However, output from computational models should not be taken as a valid substitute for the scientific method, no matter how tempting it may be.

ACKNOWLEDGEMENTS

I would like to extend my gratitude to Dr. John Morris for mentoring me on this project. I have met few individuals who are as passionate about teaching as he is, and I would not have been able to complete my research without his enthusiastic support. I also want to thank my honors advisors Dr. Gerald Gianutsos, Dr. Amy Anderson, and Dr. Brian Aneskievich for their guidance. Finally, Joe Cichocki and Greg Smith were the wonderful post-graduate students with whom I have spent numerous hours in the laboratory and were of great help and encouragement.

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