A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR DECANE

by

ULRICH REIKO PERLEBERG

(Under the Direction of Jeff Fisher)

ABSTRACT

Decane, a 10-carbon \( n \)-alkane, was selected to represent the semi-volatile fraction for the initial development of a physiologically-based pharmacokinetic (PBPK) model for Jet Propellant-8. Rats were exposed to decane vapors at time weighted average concentrations of 1200, 781, or 273ppm in a 32L leach chamber for 4hrs. Time course samples for 1200ppm and end of exposure samples for 781 and 273ppm decane exposures were collected from blood, brain, liver, fat, bone marrow, lung, skin, and spleen. A PBPK model for decane was developed using flow-limited and diffusion-limited equations to describe the uptake and clearance of decane in the blood and tissues. Model predictions were adequate in most tissues and blood. For model validation, the model had mixed successes at predicting tissue and blood concentrations for lower concentrations of decane vapor, suggesting that further improvements in the model may be necessary for extrapolating to concentrations less than 100ppm.

INDEX WORDS: Decane, PBPK Modeling, JP-8
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May 2004
DEDICATION

This is dedicated to all my friends and family, who have supported and inspired me throughout my life, enabling me to achieve my aspirations. But above all else, this is dedicated to my lord and savior, Jesus Christ, who has given me life through his sacrifice and blessed me with his love, mercy, and grace.
ACKNOWLEDGEMENTS

Funding from Air Force Office of Science Research supported the research; contract No. F49620-0300157. The animal used described in this research was conducted in accordance with the National Institutes of Health guidelines for the care of laboratory animals.

Special thanks goes to Jerry Campbell, Andy Smith, Tara Almekinder, Wilson Everett, John Swint, Deidre Mahle and Kathy Frank for their help in the laboratory and Dr. Melvin E. Andersen for his support and suggestions on how to develop the refined PBPK model for decane. Thank you Deborah Keys for her guidance and expertise in the development of the PBPK model. Thank you to my major professor, Jeff Fisher, to my committee members, Michael Bartlett and Luke Naeher, for their support and input that went into this project. Finally, I would like to thank all those who made this possible, as well as those who helped me get to this point in my life and career.
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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Background of Jet Propellant-8

Jet Propellant-8 (JP-8) is a jet fuel used by North Atlantic Treaty Organization (NATO) countries for military aircraft and ground vehicles, with an annual use of approximately 5 billion gallons (TIEHH, 2001). JP-8 is very similar in composition to Jet A. That is, JP-8 is derived from the same fraction of crude oil as Jet A, although Jet A has anti-icing, anti-corrosion, and anti-static agents (Zeiger and Smith, 1998). JP-8 components are also found in other industries, including organic solvents in degreasers, paints, and glues.

JP-8, a kerosene-based jet fuel, has replaced JP-4 due to JP-8’s higher flash point and comparatively lower vapor pressure; which reduces the evaporative losses and resists crash-induced fires and explosion (Mattie et al., 1991). Like other petroleum distillate fuels, JP-8 is a complex mixture of aromatic and aliphatic hydrocarbons (Mattie et al., 1991). JP-8 consists mostly of C\(_{8}\)-C\(_{17}\) branched and straight-chained alkanes (~81% by mass) (Zeiger and Smith, 1998).

Ground crews have reported highly visible JP-8 aerosol emissions during jet aircraft “cold starts,” which results in some crewmembers (i.e., those working behind the aircraft) to become “drenched” in fuel (Bruckner and Warren, 2001). Since JP-8 is a common chemical mixture to which military personnel are exposed, occupational exposure by military personnel to JP-8 has now become a health concern to the military (Liu and Pleil, 1999).
**JP-8 Toxicology**

Military personnel have reported objectionable odors, skin irritation, dizziness and the persistent taste of JP-8 long after exposure (TIEHH, 2001). The breath of U. S. Air Force personnel has been reported to contain multiple hydrocarbons including: \(n\)-octane, nonane, decane, undecane and dodecane, benzene, toluene, methylbenzene, and xylenes (Pliel et al., 2000). In animal studies, JP-8 is reported to affect the immune system (Harris et al., 2002), lung protein expression (Drake et al., 2003), testicular protein expression (Witzmann et al., 2003), pulmonary function (Pfaff et al., 1995), skin tumor genesis (middle distillates) (Nessel et al., 1998), and embryo growth (Cooper and Mattie, 1996). An investigation conducted on aircraft maintenance personnel with chronic low-level JP-8 exposure reported modest changes in postural balance (Smith et al., 1997).

**Decane Toxicology**

The main focus for this paper is decane (\(n\)-decane), a semi-volatile component of JP-8. In addition to being found in jet fuel, decane is a common indoor air pollutant originating from building materials (Molhave, 1982). Decane was one of the most frequently found compounds in air around 42 building materials with an average concentration of 1.49 \(\mu g/m^3\) (Molhave, 1982). Few toxicology studies are reported in the literature with decane alone or as a component in a mixture of semi volatile hydrocarbons. \(n\)-Alkanes, except for hexane, have been reported to be nontoxic or at least of low toxicity (Kjaergaard et al., 1989).

One decane inhalation study reports no toxic effects when rats were exposed to 540 ppm decane for 91 days (Nau et al., 1966). In another study, van Duuren and Goldschmidt (1976) reported that decane was a tumor-promoting agent, as well as increasing carcinogenic activity of benzo(a)pyrene in female ICR/Ha Swiss mice skin. Decane more than doubled the number of
mice with papillomas and carcinomas (van Duuren and Goldschmidt, 1976). Compounds were applied three times weekly to the mouse’s skin with a 5 µg/application of benzo(a)pyrene and a 25 µg/application of decane for 440 days. Out of 50 mice, 38 mice were reported to develop a total of 73 papillomas. Papillomas were only reported if greater than 1 mm in diameter and lasting longer than 30 days. Decane was also found to be a tumor-promoting agent (van Duuren and Goldschmidt, 1976 and Sice, 1966) when applied solely to the mouse’s skin.

Decane exposure was shown to cause sensory irritation effects in CF-1 male mice (Kristiansen and Nielsen, 1988). Respiratory patterns were monitored for each mouse by attaching a pressure transducer to each plethysnograph, which was attached to the exposure chamber. Average respiratory rates of each group of mice were monitored individually and then recorded as mean values for 1 minute periods. Body movements (escape activities) were used as an indicator of central nervous system effects in this study. Movements were observed over the entire exposure period (Kristiansen and Nielsen, 1988). Non-cannulated mice showed sensory irritation patterns for decane at and above 300 ppm. Kristiansen and Nielsen (1988) suggests that the sensory irritation potency of n-alkanes increases with number of carbon atoms (n-heptane through n-undecane), because lipid solubility increases with increasing number of carbon atoms. The estimate of the upper limit for sensory irritation acceptable in an industrial working environment for decane is 22 ppm (Kristiansen and Nielsen, 1988).

A dose-response study of human reactions to decane vapor exposure, as an indoor air pollutant, was performed in a climate chamber (Kjaergaard et al., 1989). Sixty-three subjects, in a controlled, double blinded study (Latin square exposure design) were exposed to pure n-decane concentrations of 0, 10, 35, or 100 ppm in air. Questionnaires and a linear potentiometer were used to measure subjective reactions to decane exposures. Physiological reactions were
measured on the external eye (tear film stability test), changes in eye redness (photographic test), and tear secretion (cytological evaluation). The study found a dose dependent relationship in irritation of mucous membranes, increased sensation of odor intensity, increased conjunctival polymorphonuclear leucocytes, and reduced air quality. All exposure concentrations showed decreased tear film stability. The study concluded that even low-level exposure to decane resulted in symptoms similar to that of sick building syndrome (Kjaergaard et al., 1989).

**Decane Inhalation and Metabolism Studies**

The semi-volatile fraction of JP-8 consists of C_9-C_{14} hydrocarbons, including decane. Decane constitutes 1.3% average weight of JP-8 (Potter and Simmons, 1998). In studies conducted at the University of Arizona conducted by Dr. Mark Witten (no data shown), we found that decane represented the highest percentage of any component in vaporized JP-8 (5.6%). Decane was selected as a representative compound within the semi-volatile fraction of JP-8. Previous research on decane consisted of inhalation exposures by Zahlsen et al. (1992) and Lof et al. (1999), as well as some metabolic studies (Ichihara et al., 1969, and Rabovsky et al., 1986).

Single component inhalation studies with C_6-C_{10} aliphatic, aromatic and naphthenic hydrocarbon exposures at 100 ppm were conducted in Sprague-Dawley rats for 12 hr/day for 3 days (Zahlsen et al., 1992). Brain, liver, perirenal fat and blood were collected directly after exposure each day and analyzed by head-space gas chromatography. From this study, decane showed the highest tissue concentrations and the slowest clearance rate from perirenal fat than any of the other alkanes studied (n-hexane, n-heptane, n-octane, and n-nonane).

Lof et al. (1999) conducted a kinetic inhalation study in Wistar rats of dearomatised White Spirit in blood, brain, and perirenal fat. Male rats were exposed to dearomatised White Spirit vapor for 6 hr/day, 5 days/week for 1, 2, or 3 weeks to 0, 400, or 800 ppm. After the third
week, 2, 4, 6, 24 hr time points were also collected. Lof et al. (1999) reported that decane was nearly the same concentration after 1, 2, and 3 weeks of exposure to White Spirit for 6 hrs per day (blood, brain). However, once exposures ceased, decane cleared rapidly from the blood (75% drop in concentration after 2 hrs) and brain (50% drop in concentration after 2 hrs), while perirenal fat clearance was very slow (7% drop in concentration after 2 hrs) (Lof et al., 1999).

Few metabolic studies with decane have been reported. Ichihara et al. (1969) carried out a microsomal hydroxylation study in mouse liver that showed a dependence on NADPH and \( \text{O}_2 \) for the oxidation of decane. Decanol, decanoic acid, and decamethyleneglycol were identified as the major oxidation products. This suggests that the oxidation of decane is initiated by hydroxylation to decanol. The \( k_m \) was approximately 71.14 mg/L with a rate constant of 1.64e\(^{-4}\) mg/hr/g rat for decane.

Rabovsky et al. (1986) studied the effect of straight chain alkanes (C\(_6\)-C\(_{12}\)) on rat liver and lung cytochrome P-450 \textit{in vitro}. Microsomes were prepared by differential centrifugation of the homogenates and stored at \(-80^\circ \text{C}\). The two P-450 enzyme activities studied were benzo[a]pyrene hydroxylase (BaPOHase) and 7-ethoxycoumarin deethylase (Ecase) in both control and \( \beta \)-naphthoflavone-treated rats. Results for the BaPOHase enzymatic activity showed that the conversion from BaP to 3HOBaP by rat liver microsomes decreased in the presence of \( n \)-alkanes, while the lung showed no significant change. Results for Ecase were similar to BaPOHase with reduced recovery of the product (7HOC) in liver microsomes, and to a lesser degree in lung microsomes. Therefore, alkanes may affect health by their own metabolic paths, as well as by interfering with normal metabolic function.
Physiologically-Based Pharmacokinetic (PBPK) Modeling

Physiologically-based pharmacokinetic (PBPK) models have been developed to predict distribution, absorption, metabolism, and excretion of chemicals (e.g. Robinson, 1999; Willems et al., 2001; Andersen et al., 2001; Dennison et al., 2003). PBPK models have been useful tools in dose-response assessment and risk assessment. There is no published PBPK model for decane. Only a few PBPK models have been developed for the less volatile hydrocarbons found in JP-8. Robinson (1999) reports on the preliminary development of a PBPK model for n-nonane. The first PBPK model for naphthalene was reported by Sweeney et al. (1996). Later, pharmacokinetic studies completed with naphthalene in conjunction with National Toxicology Profile (NTP) toxicology studies on naphthalene were used to develop PBPK models for naphthalene (Quick and Shuler, 1999, and Willems et al., 2001).

The new PBPK models for mixtures expand on previous PBPK models by creating sub-models for two or more chemicals, which individually describe each chemical in the mixture, while creating an overall kinetic exposure matrix. Primary focus has been on constructing mixture models for the volatile fraction of fuel components such as benzene, toluene, ethyl benzene, xylenes, and dichloromethane (Haddad et al., 2001, Krishnan, et al., 2002, Tardif et al., 1997). PBPK models for other solvent mixtures such as trichloroethylene, tetrachloroethylene, 1,1,1-trichloroethane, toluene, perchloroethylene, and carbon tetrachloride (Dobrev et al., 2001, 2002, Thrall et al., 2000, Fisher et al., 2003) have been evaluated for metabolic interactions. Liao et al. (2002) developed a reaction network model to predict metabolic products from combinations of chemicals. Dennison et al. (2003) developed a gasoline PBPK model in which they used a lumped compartment to represent the bulk of the gasoline fraction and used published PBPK models for specific chemicals found in gasoline (n-hexane, benzene, xylene,
toluene, ethylbenzene and o-xylene). Competitive metabolic inhibitions between these specific chemicals were implemented in the PBPK model compartments (Dennison et al., 2003).

The objective of this project was to develop a PBPK model for decane, as an initial step in the development of a suite of PBPK hydrocarbon models to represent JP-8, a complex mixture of hundreds of hydrocarbons. We collected pharmacokinetic information for decane from several organs and tissues to gain insights for the kinetic behavior of decane in the rat and to develop a PBPK that can simulate the kinetic behavior of decane.
References for Chapter 1


11. Harris, D. T., Sakiestewa, D., Titone, D., Young, R. S., and Witten, M. (2002). JP-8 jet fuel exposure results in immediate immunotoxicity, which is cumulative over time. Toxicology and Industrial Health 18, 77-83.


16. Liao, K., Dobrev, I., Dennison, J., Andersen, M., Reisfeld, B., Reardon, K., Campain, J., Wei, W., Klein, M., Quann, R. J., and Yang, R. (2002). Application of biologically based computer modeling to simple or complex mixtures. Environmental Health Perspectives 110 Suppl. 6, 957-63.


CHAPTER 2

DEVELOPMENT OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL
FOR DECANE, A CONSTITUENT OF JET PROPELLENT-8

Abstract

Decane, a 10-carbon n-alkane and one of the highest vapor phase constituents of jet propellant-8 (JP-8), was selected to represent the semi-volatile fraction for the initial development of a physiologically-based pharmacokinetic (PBPK) model for JP-8. Rats were exposed to decane vapors at time-weighted average concentrations of 1200, 781, or 273 ppm in a 32 L leach chamber for 4 hrs. Time course samples for 1200 ppm and end of exposure samples for 781 and 273 ppm decane exposures were collected from blood, brain, liver, fat, bone marrow, lung, skin, and spleen. The pharmacokinetics of decane could not be described by flow-limited assumptions and measured in vitro tissue/air partition coefficients. A refined PBPK model for decane was then developed using flow-limited (liver and lung) and diffusion-limited (brain, bone marrow, fat, skin, and spleen) equations to describe the uptake and clearance of decane in the blood and tissues. Partition coefficient values for blood/air and tissue/blood were estimated by fitting end of exposure pharmacokinetic data and assumed to reflect the available decane for rapid exchange with blood. PBPK model predictions were adequate in describing the tissues and blood kinetics for decane. For model validation, the refined PBPK model for decane had mixed successes in predicting tissue and blood concentrations for lower concentrations of decane vapor, suggesting that further improvements in the model may be necessary to extrapolate to concentrations less than 100 ppm.
Introduction

Jet Propellant-8 (JP-8) is a jet fuel used by North Atlantic Treaty Organization (NATO) countries for military aircraft and ground vehicles, with an annual use of approximately 5 billion gallons (TIEHH, 2001). JP-8, a kerosene-based jet fuel, has replaced JP-4 due to JP-8’s higher flash point and comparatively lower vapor pressure; this reduces the evaporative losses and resists crash-induced fires and explosion (Mattie et al, 1991). Like other petroleum distillate fuels, JP-8 is a complex mixture of aromatic and aliphatic hydrocarbons (Mattie et al, 1991). JP-8 is primarily composed of branched and straight chain alkanes (C_8-C_{17}), which constitute approximately 81% of JP-8 by mass (Zeiger and Smith, 1998).

Since JP-8 is a common chemical to which military personnel are exposed, occupational exposure to JP-8 has now become a health concern to the military (Liu and Pleil, 1999). Military personnel have reported objectionable odors, skin irritation, dizziness and the persistent taste of JP-8 long after exposure (TIEHH, 2001). The breath of U.S. Air Force personnel exposed to JP-8 have been reported to contain hydrocarbons including octane, nonane, decane, undecane and dodecane; and benzene, toluene, methylbenzene, and xylenes (Pliel et al., 2000). An investigation conducted on aircraft maintenance personnel with chronic, low-level JP-8 exposure reported modest changes in postural balance (Smith et al, 1997). In animal studies, JP-8 is reported to affect the immune system (Harris et al., 2002), lung protein expression (Drake et al., 2003), testicular protein expression (Witzmann et al., 2003), pulmonary function (Pfaff et al, 1995), kidney function (Mattie D. R. et al., 1991), skin tumor genesis (middle distillates) (Nessel et al., 1998), and embryo growth (Cooper and Mattie, 1996).

The semi-volatile fraction of JP-8, which consists primarily of C_9-C_{14} n-alkanes, includes decane. Decane represents about 1.3% of JP-8 by weight (Potter and Simmons, 1998).
Atmospheric samples collected from an animal inhalation chamber containing JP-8 aerosol and vapor at the University of Arizona (Dr. Mark Witten, no data shown), suggest that decane represents the greatest fraction of the many hydrocarbons found in JP-8 vapor (about 5.6%). Therefore, decane was selected for PBPK model development as a representative compound for the semi-volatile n-alkanes fraction of JP-8.

In addition to being found in jet fuel, decane is a common indoor air pollutant originating from building materials (Molhave, 1982). Only a few toxicology studies are reported in the literature with decane alone or as a component in a mixture of semi volatile hydrocarbons. n-Alkanes, except for hexane, have been reported to be nontoxic or at least of low toxicity (Kjaergaard et al., 1989).

One decane inhalation study reports no toxic effects when rats were exposed to 540 ppm decane for 91 days (Nau et al., 1966). In another study, van Duuren and Goldschmidt (1976) reported that decane was a tumor-promoting agent, as well as increasing carcinogenic activity of benzo(a)pyrene in female ICR/Ha Swiss mice skin. Decane has been shown to cause sensory irritation effects in CF-1 male mice (Kristiansen and Nielsen, 1988). Kristiansen and Nielsen suggest that the sensory irritation potency of n-alkanes increases with number of carbon atoms (n-heptane through n-undecane) because lipid solubility increases with increasing number of carbon atoms. The estimate of the upper limit for sensory irritation acceptable in an industrial working environment for decane was reported to be 22 ppm (Kristiansen and Nielsen, 1988). In a controlled human inhalation study Kjaergaard et al. (1989) exposed humans to decane vapors ranging from 58.2-582 mg/m$^3$ for 6 hours. These authors reported that decane caused irritation of mucous membranes in the eye and skin and increased sensation of odor intensity. The authors
concluded that decane caused symptoms similar to that of sick building syndrome (Kjaergaard et al., 1989).

A few pharmacokinetic studies with decane are reported in the literature (Zahlsen et al. (1992) and Lof et al. (1999)) and a few metabolism studies (Ichihara et al., 1969, and Rabovsky et al., 1986). Zahlsen et al. (1992) reported that in Sprague-Dawley rats exposed to decane vapors, the decane concentrations were the highest in fat and cleared the slowest from the fat compared to other smaller \( n \)-alkanes (\( n \)-hexane, \( n \)-heptane, \( n \)-octane, and \( n \)-nonane). In another pharmacokinetic study, Lof et al. (1999) reported that decane was nearly the same concentration in the blood and brain after 1, 2, and 3 weeks of exposure to White Spirit for 6 hrs per day. However, once exposures ceased, decane cleared rapidly from the blood (75% drop in concentration after 2 hrs) and brain (50% drop in concentration after 2 hrs), while perirenal fat clearance was very slow (7% drop in concentration after 2 hrs) (Lof et al., 1999).

Ichihara et al. (1969) reported that decane is metabolized to decanol, decanoic acid, and decamethyleneglycol using microsomal preparations from mice livers. The author suggested that the oxidation of decane is initiated by hydroxylation of decane to form decanol. Using the limited data provided in the report, we estimated the metabolic rate for oxidation to be very low (well below 0.01 mg/hr/g of rat). The \( k_m \) was reported to be relatively high (~ 70 mg/L). Based on these estimates of metabolic rate, we assumed that metabolism was negligible for modeling purposes. Physiologically-based pharmacokinetic (PBPK) models have been developed to predict distribution, absorption, metabolism, and excretion of chemicals (e.g. Robinson, 1999; Willems et al., 2001; Andersen et al., 2001; Dennison et al., 2003).

Physiologically based pharmacokinetic (PBPK) models have been useful tools in dose-response assessment and risk assessment. There is no published PBPK model for decane. Only
a few PBPK models have been developed for the less volatile hydrocarbons found in JP-8.

Robinson (1999) reports on the preliminary development of a PBPK model for \textit{n}-nonane. The first PBPK model for naphthalene was reported by Sweeney et al. (1996). Later, pharmacokinetic studies completed with naphthalene in conjunction with National Toxicology Profile (NTP) toxicology studies on naphthalene were used to develop PBPK models for naphthalene (Quick and Shuler, 1999, and Willems et al., 2001). Dennison et al. (2003) developed a gasoline PBPK model in which they used a lumped compartment to represent the bulk of the gasoline fraction and used published PBPK models for specific chemicals found in gasoline (\textit{n}-hexane, benzene, xylene, toluene, ethylbenzene and \textit{o}-xylene).

The objective of this project was to develop a PBPK model for decane, as an initial step in the development of a suite of PBPK hydrocarbon models to represent JP-8, a complex mixture of hundreds of hydrocarbons. We collected pharmacokinetic information for decane from several organs and tissues to gain insights for the kinetic behavior of decane in toxicologically relevant organs.

**Method**

**Animals**

Forty-eight male Fischer 344 rats were purchased from Charles Rivers (Raleigh, NC), weighing between 186 g to 240 g (mean BW=211g). All animals were housed in a controlled environment with a 12 hr light/dark cycle at 21 °C. Purina food and water was available at liberty, except during exposures. Rats were given a minimum acclimation period of 2 weeks before experiments were begun. All inhalation exposures commenced between 7-8 a.m.
Chemicals

Decane (n-decane) (99+% purity, Alfa Aesar, Ward Hill, MA), chloroform (99.9% purity, ECD tested Acros Organics, Morris Plains, NJ), and trichloroacetic acid (A.C.S., Fisher Scientific, Pittsburgh, PA) were obtained from Fisher Scientific Company.

Exposure System

Gas Uptake Chamber. Initially, decane was evaluated in a newly built gas uptake chamber, without rats to determine if gas uptake could be utilized to obtain information on the metabolism of decane. Slight modifications were made to previously described gas uptake exposure systems (Gargas et al., 1986a, 1986b; Gargas et al., 1988). Decane was injected into the system through an injection port (10 µL and 5 µL) at the incoming air stream to the 7 L closed system chamber. However, this method was deemed unsuccessful for the analysis of decane metabolism because the atmospheric loss rate of decane to the glass chamber (without an animal) was too great (Fig. 1). Pharmacokinetic studies were then carried out in a Leach chamber.

Leach Chamber. A schematic of the leach chamber system is shown in Fig. 2. The exposure chamber consisted of a 32 L battery jar (Greenberg et al., 1999). Two metal bellows pumps (MB-41, Senior Operations Inc. Sharon, MA) were used to create inhalation exposures. The atmospheric pressure within the battery jar was monitored by a Magnehelic pressure gauge (Dwyer Instruments Inc., Michigan City, Ind. 46360) and maintained between 0 and –1 inches of water by adjusting the flow through the chamber. A 40/50 Pyrex bubbler containing decane was mixed with ambient room air to achieve a specified vapor concentration of decane over a four-hour exposure period. Aerosols generated by the bubbler were removed via glass wool. All tubing was a ¼ inch throughout system, except for the ¼ inch tubing to the gas chromatograph. Tubing was stainless steel. The flow rates of the bubbler and exhaust from the leach chamber
were monitored by Gilmont Instruments flow meters. Exhaust was monitored to determine chamber concentration by splitting the exhaust flow so a portion of the exhaust was routed to the gas chromatograph. The sample loop for the gas chromatograph was set to a flow rate of ~100 ml/min. The flow rate of the split from the exhaust to the gas chromatograph was monitored by a Matheson 600 HA1 flow meter. Flow rates through the bubbler to obtain decane concentrations of 273 to 1200 ppm ranged from 1 L/min to 4 L/min. Ambient air flow was mixed with the bubbler flow rate to give a total flow rate of 8 L/min to the chamber. Chamber atmospheric concentrations were monitored in 10-minute intervals over the entire exposure duration using an auto-sampling valve mounted on the gas chromatograph. Exposure concentrations were calculated as a Time Weighted Average (TWA) over the 4 hr exposure period.

**Experimental**

Fischer 344 rats were exposed for 4 hrs to concentrations of 1200 ppm (n=12 per exposure), 781 ppm (n=8 per exposure), and 273 ppm (n=8 per exposure). Tissue samples were collected for the 1200 ppm time course from the end of exposure and up to 24 hr, while only end of exposure tissue samples were collected for 781 and 273 ppm. Rats were placed in a box and sacrificed by CO₂. After rats were sacrificed, tissue samples were quickly collected and placed into 2 ml pre-weighed screw cap vials (National Scientific Co., Scottsdale, AZ) to be weighed. All samples collected were approximately 0.2 g. Blood, brain, bone marrow, spleen, liver, lung, perirenal fat, and skin tissues were collected from 5 minutes up to 24 hours post exposure for the 1200 ppm concentration. Bone marrow was extracted by scrapping from the inside of both femurs. Skin samples were collected from the abdomen after clipping the hair. For the 1200 ppm exposure
decane was not detected in blood at 8 hr and skin and bone marrow at 28 hr because the samples were below the limit of detection of our analytical method.

**Analytical**

Table 1 provides gas chromatography (Hewlett-Packard, Palo Alto, CA) conditions for analysis of decane in Leach exposure chamber and in rat tissues. Decane was extracted from tissues by placing a 600 µL aliquot of chloroform and a 10 µL aliquot of 2 M Trichloroacetic acid (TCA) into 2 mL vials containing 0.2 g of tissue (except bone marrow, ~0.02 g). The vials were shaken for an hour at 125 rpm using a Vortemp shaker (Labnet International, Inc.). After shaking, approximately 150 µL of the chloroform was removed with a pipette and placed into a 200 µL glass insert (Target Polyspring) housed in a 2 mL crimp vial (National Scientific, Scottsdale, AZ) for analysis by Gas Chromatography (GC) equipped with a flame ionization detector (FID).

Extraction efficiencies for removing decane from tissues with chloroform and 2 M TCA were determined for each individual tissue using spiked homogenates (6 to 6000 µg/mL or g) except for bone marrow. The small mass of bone marrow was too small for our experimental methods. The saline diluted homogenates were prepared in a 2:1 ratio. A known amount of decane was spiked into the homogenates contained in 2 mL vials and shaken for an hour to allow the decane to partition into the tissues and analyzed by GC/FID. Brain, fat, liver, lung, skin, and spleen extraction efficiencies were 97.9%, 111.1%, 89.4%, 93.0%, 103.9%, and 93.4% respectively. Heparinized and non-heparinized whole blood average extraction efficiencies were 55.6% and 41.5%. Plasma had an extraction efficiency of 41.9%. Only blood sample concentrations reported in this study are corrected for extraction efficiency. For these analytical methods, the limit of quantification was 0.06 µg/mL and the detection limit was 0.02 µg/mL.
PBPK Model Development

AsclXtreme v1.3.2 (Aegis Technologies Group, Inc., Huntsville, AL) simulation software was used to implement the PBPK model for decane.

Preliminary PBPK Model. A preliminary or exploratory inhalation model was constructed with nine flow-limited compartments (Fig. 3A) to describe the kinetics of decane. The PBPK model compartments consisted of organs (lung, brain, bone marrow, fat, liver, skin, and spleen) and lumped tissues (rapidly and slowly perfused tissues) plus an algebraic description of alveolar exchange of decane with lung blood. The decane in venous blood leaving the spleen compartment was described as flowing directly into the liver compartment. Compartments were selected based on JP-8 toxicology (National Research Council, 2003) and previously developed PBPK modeling approaches for inhaled gases (Ramsey and Andersen, 1984).

A preliminary PBPK model for decane was created and exercised by simulating the 4 hr 1200 ppm decane inhalation exposure and comparing the pharmacokinetic data collected following this exposure with model predictions. Visual inspection of the data indicated that clearance of decane from some tissues was slower than clearance from blood (e.g., fat) indicating that venous equilibration (flow-limited) equations (Ramsey and Andersen, 1984) may not be appropriate. A series of attempts to fit the pharmacokinetic data in each tissue was undertaken using flow-limited equations and tissue:air partition coefficient values for decane reported by Smith et al. (2004) (Table 2). Chemical specific parameters for the preliminary model can be found in Table 2 and physiological model parameters in Table 3. There was a systematic failure to simultaneously predict tissues levels of decane (see Fig. 4).

Evidence for Metabolism of Decane. In vitro metabolism studies with rat liver microsomes revealed decanol, decanoic acid, and decamethyleneglycol as metabolites (Ichihara et al., 1969).
A low affinity $k_m$ value of 71.14 mg/L was estimated in this study and we estimated a very low rate of oxidation with a first order rate constant of $1.65 \times 10^{-4}$ mg/hr per rat. Therefore, metabolism of decane was disregarded in the PBPK model for decane.

**Refined PBPK Model.** A revised PBPK model (Fig. 3B) for decane was constructed. Several tissues (brain, bone marrow, fat, skin, and spleen) were described with diffusion-limited equations (Vinegar et al., 1992). The remaining compartments (blood, liver, lung, and rapidly and slowly perfused tissues) were described as flow-limited compartments. Partition coefficients were fitted (estimated) to end of exposure tissue concentration data following the 1200 ppm exposure (see below for details).

**PBPK Model Parameters.** Table 3 displays the physiological model parameters used in the refined PBPK model for decane. Tissue volumes, blood flows, and blood volumes, were taken from the literature (Andersen et al., 1993; Brown et al., 1997; Delp et al., 1991; Schoeffner et al., 1999; Travis et al., 1990).

Because of the number of adjustable parameters (9 partition coefficients (PCs), 5 permeability-area cross products (PAs)), initial trial and error estimates for these 14 parameters were made by visually fitting the 1200 ppm data sets for each individual tissue in an iterative fashion. Time courses for each compartment described as diffusion-limited were fit visually by adjusting both the partition coefficient (PC) and permeability-area cross-product (PA) term, while the flow-limited compartments were fit visually by adjusting only the partition coefficient. Then, maximum likelihood estimation, as implemented in ACSL Math/Optimize V 2.5.4 (Aegis Technologies Group, Inc., Huntsville, AL) software was used in an iterative fashion to estimate the values of the 14 chemical specific parameters. The Nelder-Mead algorithm was used for likelihood estimation. The error model was set to relative. For each iteration, three different
starting values resulting in successful conclusion of the Nelder-Mead algorithm were used for each adjustable parameter.

First the blood/air PC was estimated. Then, tissue-specific parameters (PC and PA terms) were fit sequentially by tissue in order of volume (largest to smallest). For diffusion-limited compartments, both the PC and PA terms were fit simultaneously. Therefore, once the blood/air PC was estimated, skin, slowly perfused, fat, liver, bone marrow, brain, lung, and spleen time courses were fit in this order and fit repeatedly until there was less than 5% change for all parameters between iterations. Global parameter estimation of all 14 parameters simultaneously was not achievable in this study due to the large number of adjustable parameters.

Model Validation. End of exposure tissue and blood decane concentrations from rats exposed for 4 hours to 781 or 273 ppm decane were used to compare model predictions with observations. Also, previously published decane pharmacokinetic data in male Sprague-Dawley rats was used for model validation purposes. Zahlsen et al. (1992) conducted repeated decane exposures for 12 hrs/day over 3 days at 100 ppm. These authors collected several tissues for analysis of decane.

Sensitivity Analysis. An analysis of the sensitivity of model predicted blood \((C_A)\), brain \((C_B)\), and lung \((C_{LU})\) decane concentrations to selected model parameters was performed. The brain and lung represent target organs for potential toxicity and the blood is an important indicator of internal dose. Sensitivity analysis was performed using ACSL Math/Optimize V 2.5.4 (Aegis Technologies Group, Inc., Huntsville, AL) software. The method of central differences was selected for calculation with delta set to 0.01 or 1%. Sensitivity coefficients were log-normalized and then multiplied by their respective parameter value. The resulting sensitivity
coefficient can be interpreted as the % change in model prediction per % change in parameter value. The decane PBPK model was exercised with a concentration of 200 ppm and sensitivity coefficients were calculated for 3, 5, and 7 hr time points for blood and lung and 3, 5, 15, 25 hr time points for brain.

Results
The initial and final (refined) PBPK model simulations for decane kinetics in the rat are presented in Figure 4. Even with adjusting the blood concentration to account for the low extraction efficiencies, the initial PBPK model over predicted the blood time course kinetics for decane after exposure to 1200 ppm decane for 4 hrs (Fig. 4A). The end of exposure time points for lung, spleen, skin and fat concentrations were over predicted, while bone marrow concentrations were under predicted, and the brain and liver concentration predictions compared favorably with observation. The shape of the clearance kinetics of decane for some tissues agreed favorably with model prediction, despite poor agreement between the predicted and observed tissue concentrations. Clearance of decane was over-predicted in the blood, liver, lung, and fat over all time points. Other clearance predictions for the initial model are provided in Figure 4. In an effort to better understand the kinetics of decane in the rat other formulations of the initial PBPK model for decane were attempted (simulations not shown) to obtain agreement between observation and prediction, including the incorporation of metabolism, protein binding in blood, and adjusting breathing rates. These attempts were ineffective at obtaining general agreement between model prediction and observation.

The refined PBPK model for decane represents an iterative fitting process to describe the diffusion-limited behavior observed in some tissues and abandoning the measured PC values for
decane (Smith et al., 2004) and fitting the tissue/blood and blood/air PC values to the 1200 ppm tissue concentration time courses. This rather unusual approach for estimating tissue solubility stems from recent research with octamethylcyclotetrasiloxane (D4) in which Andersen and colleagues (2001) describe the complex kinetics of D4, a volatile, lipophilic compound by assuming that D4 is sequestered into ‘deep’ pools of the body and unavailable for rapid exchange with blood or tissues. In this case, we assume that the measured PC values for decane (Table 2) do not give a good approximation of the available decane for exchange between tissues and blood. The fitted PC values represent apparent or effective tissue solubility for decane (Table 2). PC values for blood/air and fat/blood PC were lowered from 8.1 to 5.3 and from 328 to 26, respectively, and the bone marrow/blood PC value, which was initially set to the measured brain/blood PC value, was increased from 4.8 to 10.0. Other changes in the tissue/blood PC values are provided in Table 2.

Generally speaking, the agreement between model predictions and measured concentrations of decane in tissues was improved with the refined model (Fig 4). The use of flow-limited assumptions for lung, liver and blood was successful for predicting the kinetics of decane (Fig. 4 A, B, C). Other tissues were individually fit assuming diffusion limitations. The addition of diffusion-limited assumptions for these tissues (brain, bone marrow, spleen, skin, and fat) improved the agreement between the model predicted uptake and clearance of decane with the measured decane concentrations for the 4 hr 1200 ppm decane exposure. Figure 4 (A, B, C) shows the significant improvement in model performance for predicting the later time points in the flow-limited compartments (blood, liver, lung). Blood and perirenal fat (Fig. 4 A and H) decane predictions were also improved (Fig. 4 A and O) and the refined model captured the slow release of decane from the brain and bone marrow (Fig. 4 D and E).
**Model Validation.** The refined PBPK model was then used to simulate two lower decane inhalation exposures conducted in our laboratory (781 or 273 ppm). Rats were exposed to decane for 4 hrs and sacrificed immediately after the 4 hr exposure. Fig. 5 provides a series of model predicted and measured decane concentrations at the end of exposure for the same tissues collected for model development. Blood, lung, and liver decane concentrations were slightly lower than predicted for both exposure groups. The model under predicted the spleen (781 ppm concentration) and over predicted the skin (273 ppm concentration). Better agreement between prediction and observation was found for spleen (273 ppm concentration), brain, fat and bone marrow.

Zahlsen et al. (1992) conducted pharmacokinetic studies with rats and several hydrocarbons, including decane. Rats were exposed to 100 ppm decane exposures for 12 hrs per day for 3 days. Fig. 6 depicts the refined model predictions of decane in blood, liver, brain and perirenal fat. Excellent agreement was obtained between model prediction for blood, liver and brain at the end of exposure. The refined PBPK model for decane under predicted the perirenal fat concentrations.

**Sensitivity Analysis.** A sensitivity analysis performed at 200 ppm for blood, brain, and lung decane concentrations revealed several of the model parameters to be sensitive to these endpoints (Table 4). The blood partition coefficient value was sensitive for predicting blood, brain and lung decane concentrations. The blood and lung concentrations were sensitive to the fat, bone marrow, and skin permeability-area cross products, ventilation rate, body weight, as well as fat and liver partitions. The model predicted brain concentrations of decane were sensitive to the body weight, partition coefficient value for the brain, the permeability-area cross product for decane transport into and out of the brain, and the volume of the brain.
Discussion and Conclusions

The PBPK model for decane consists of seven compartments representing organs plus two lumped compartments representing the slowly perfused tissues such as muscle and the remaining rapidly perfused tissues. For one decane inhalation exposure (1200 ppm) experimental time course data was collected for the seven organs plus the mixed venous blood supply. This represents a wealth of pharmacokinetic data to gain insights about the kinetic behavior of decane. The initial decane model developed using flow-limited assumptions and measured partition coefficient values failed to simulate the pharmacokinetic behavior of decane, while the refined PBPK model improved the agreement between predictions and observations. These improvements in the model predictions occurred because several organs were described as diffusion-limited instead of flow-limited to account for the slower clearance of decane from these tissues. In addition, partition coefficients were fitted to provide a better description of the availability of decane for exchange between tissues and blood. Our findings suggest that decane may be sequestered into unidentified ‘deep’ pools of the body where exchange between the tissue and blood is unusually slow.

The refined model for decane had mixed successes at predicting tissue and blood concentrations for lower concentrations of decane vapor. This suggests that further improvements in the decane PBPK model may be necessary to use this PBPK model for extrapolating to low inhaled decane concentrations (less than 100 ppm).

Decane was found to be difficult to extract from blood, while all other tissues in this study had extraction efficiencies greater than 90%. Liu and Pleil (2001) reported the blood extraction efficiency of decane (while a component of JP-8) to be 35%, which is similar to our calculated extraction efficiency for decane of 41.5%. In this study, we found little difference in
extraction efficiencies for decane in whole blood and plasma. We also added 2 M TCA to assist
in increasing our extraction efficiency for decane in blood or plasma. In Liu and Pleil (2001)
study, with human whole blood, solutions were mixed with 3 mL of bovine plasma followed by
the addition of 3 mL of sodium-chloride-saturated PBS solution to achieve mock sample
concentrations of 0.16%, 0.016%, 0.0032% of JP-8 in a 6 mL volume. Two drops of sulfuric
acid were added to precipitate proteins. Samples were shaken for a minute, after 6ml of pentane
were added; then centrifuged for 30 min. These authors report that repeated extractions did not
improve their extraction efficiency and that evaporation effected recovery.

In summary, a PBPK model for inhaled vapors of decane was constructed for the rat.
The model is a hybrid model using both flow-limited and diffusion-limited differential equations
to describe tissue kinetics. Decane was assumed to be cleared from the body by exhalation.
Metabolism was deemed insignificant for this model. The decane PBPK model is robust, in that
the kinetics of several organs were evaluated over a wide range of concentrations (273-1200
ppm). The decane PBPK model is our first hydrocarbon marker to represent the semi-volatile
fraction of JP-8. The ultimate goal is to develop a PBPK model for JP-8 using a similar
approach as described for gasoline (Dennison et al., 2003). Decane is an important constituent in
neat JP-8 (1.3%) and has been found in human breath samples. The irritating effects of decane
on mucous membranes in humans (Kjaegaard et al., 1989) may provide clues for ascertaining
which hydrocarbon fractions of JP-8 may be responsible for portal of entry toxicity in animal
studies (National Research Counsel, 2003).
Acknowledgments

This research was supported with funding from the Air Force Office of Science Research contract No. F49620-0300157. The animal used described in this research was conducted in accordance with the National Institutes of Health guidelines for the care of laboratory animals. A special thanks goes to Jerry Campbell, Andy Smith, Tara Almekinder, Wilson Everett, John Swint, Deidre Mahle and Kathy Frank for their help in the laboratory and Dr. Melvin E. Andersen for his support and suggestions on how to develop the refined PBPK model for decane.
References for Chapter 2


Table 1. Gas chromatograph parameters for leach chamber, tissue analysis, and extraction efficiency.

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<th>Leach Chamber</th>
<th>Tissue Analysis/ Extraction Efficiency</th>
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</thead>
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<td>Agilent</td>
</tr>
<tr>
<td>HP6890plus</td>
<td>HP5890 II</td>
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<tr>
<td>Column</td>
<td>Column</td>
</tr>
<tr>
<td>DB5</td>
<td>Volcol</td>
</tr>
<tr>
<td>Length (m)</td>
<td>Length (m)</td>
</tr>
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<td>15</td>
<td>30</td>
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<tr>
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<td>Diameter (mm)</td>
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<td>0.53</td>
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<td>Film thickness (µm)</td>
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<td>Run time (min)</td>
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<td>N₂ (mL/min)</td>
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<td>He (mL/min) split</td>
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<td>5.3</td>
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Table 2. Chemical Specific parameters for PBPK models for decane in rats.

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<th>Parameter</th>
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<tr>
<td><strong>Initial Model Partition Coefficients (Measured)</strong></td>
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<tr>
<td>Blood/Air (P_{WB})</td>
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</tr>
<tr>
<td>Liver/Blood (P_{L})</td>
<td>1.96</td>
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<td>328.12</td>
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<td>Brain/Blood (P_{B})</td>
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<td>Smith et al., 2004</td>
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<td>Slowly perfused/Blood (P_{S})</td>
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<td>Bone Marrow/Blood (P_{BM})</td>
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<td>Lung/Blood (P_{LU})</td>
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<td><strong>Refined Model Partition Coefficients (Fitted)</strong></td>
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<td><strong>Diffusion Limited Tissue Parameters</strong></td>
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<tr>
<td>Tissue Permeability Area-Cross Product (L/hr)</td>
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Table 3. Physiological Parameters for PBPK models for decane in rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Source</th>
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<tr>
<td>Alveolar ventilation rate (l/hr/kg^{0.75}) (Q_{PC})</td>
<td>15.6</td>
<td>Delp et al. 1991</td>
</tr>
<tr>
<td>Cardiac output (l/hr/kg^{0.75}) (Q_{CC})</td>
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<td>Body weight (kg) (BW)</td>
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<tr>
<td><strong>Blood flows (% of Cardiac output)</strong></td>
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<tr>
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<td>78.0-Q_{LC}-Q_{BMC}-Q_{BC}</td>
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<td>Skin (BVSK)</td>
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<td><strong>Tissue Volumes (% Body Weight)</strong></td>
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<tr>
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<td>10.0-V_{LC}V_{LUC}V_{SNC}V_{BMC}V_{BC}</td>
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Table 4. Sensitivity Analysis for selected decane model parameters at 200 ppm evaluated during exposure (3 hr) and post exposure (5-25 hr). All model parameters were analyzed, but only eleven are shown for these three targeted parameter outcomes. Targeted parameters are arterial blood concentration ($C_A$), lung tissue concentration ($C_{LU}$), and brain tissue concentration ($C_B$). Values bolded have a sensitivity coefficient greater than a $^*/0.50$.

<table>
<thead>
<tr>
<th>Model Parameter</th>
<th>Target Model Parameters</th>
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<th>5</th>
<th>7</th>
<th>3*</th>
<th>5</th>
<th>7</th>
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* during exposure
**Figures Legend**

Figure 1. Atmospheric clearance of decane (10 µL, 5 µL) from a gas uptake chamber without animals.

Figure 2. Schematic of a leach inhalation chamber for exposing rats to decane vapors.

Figure 3. (A) Schematic of the initial flow-limited 9 compartment PBPK model developed for decane in rats. (B) Schematic of the refined 9 compartment PBPK model developed for decane in rats. Delivery of chemical to lung, liver, rapidly perfused, and slowly perfused tissues are flow-limited while delivery of chemical to the remaining tissues are diffusion-limited.

Figure 4. Initial model predictions (thin line), refined model predictions (thick line), and the observed levels of decane in rats dosed via inhalation to 1200 ppm for 4 hrs (circles) in (A) blood (B) liver, (C) lung, (D) brain, (E) bone marrow, (F) spleen, (G) skin, (H) perirenal fat.

Figure 5. Refined model predictions of 781 ppm (thick line) and 273 ppm (thin line) with the observed decane levels in rats exposed to decane vapors of 781 ppm (black diamonds) and 273 ppm (white circles) for 4 hrs.

Figure 6. Refined model predictions (line) and observed decane levels in rats exposed by inhalation to 100 ppm of decane for 12 hrs a day over 3 days (circles) (Zahlsen et al., 1992) in (A) blood, (B) liver (C) brain and (D) perirenal fat.
32L Battery Jar
Ambient Air
Input
Bubbler Scrubber
Purge Output
G C
Alveolar blood

Brain

Fat

Skin

Bone Marrow

Rapidly

Slowly

Liver

Spleen

Inhale

Exhale

A

Alveolar space

Arterial blood

Venous blood

B

41
CHAPTER 3

SUMMARY

The physiologically-based pharmacokinetic (PBPK) model describes the kinetic behavior of decane in a rat. The PBPK model consists of nine compartments, which predict the concentration of inhaled decane in various rat tissues. The blood, liver, and lung compartments have flow-limited assumptions. Diffusion-limited assumptions were made for the brain, bone marrow, perirenal fat, skin, and spleen compartments. The decane PBPK model is a robust model that encompasses the overall kinetics of decane with some slight inconsistencies at lower concentrations.

This research has shown that decane has a complex kinetic behavior, leading to the implementation of diffusion-limitation in the model structure. Decane has shown to be a lipophilic compound that is primarily excreted by exhalation. With a negligible metabolic rate ($1.65 \times 10^{-4}$ mg/hr/g rat), metabolism was determined to be insignificant and not included in the model structure.

Previous studies have shown that decane plates or sticks to glassware. This was demonstrated in the gas uptake studies, which established that decane had a loss rate greater than the acceptable range ($< 10\%$ per hour). Validation with other more volatile chemicals (Trichloroethylene (TCE), $n$-hexane, and $n$-octane) showed that decane was not lost due to system leaks. Therefore, a leach chamber study was conducted with the use of a bubbler to generate a vapor concentration and a glass wool scrubber to eliminate the presence of aerosols. Inhalation runs were four hours long with time course data (5 min.-24 hrs) for a 1200 ppm
exposure along with two end of exposure concentrations (781 and 273 ppm) being collected for validation purposes.

Extraction efficiency studies showed that decane behaved differently in blood than other tissues studied. The extraction efficiency for whole blood was 41.5%, while all other tissues were above 89.4%. In dealing with this issue, the whole blood data was corrected up to 100% for the model. From this finding, decane may have more complex interactions with blood than other tissues.

Diffusion-limitation was implemented due to the physiological characteristics of decane. Decane is a lipophilic compound that is slowly released from brain and bone marrow and even more slowly from perirenal fat, skin, and spleen. Similar issues arose with octamethylcyclotetrasiloxane (D4) (Andersen et al., 2001) and naphthalene (Willems et al., 2001), which both implemented diffusion-limitation and were both lipophilic compounds.

Similar to the decane model, the naphthalene and D4 models had limitations with predicting blood time-course data. The post exposure blood time course concentrations, for these chemicals, would clear more slowly than the basic flow-limited model would predict. Like naphthalene, D4 is a highly lipophilic compound that sequesters in fat depots. The D4 model added a blood lipid compartment to describe the chemical’s kinetic behavior. Differing from the decane model, both of these models added arterial and venous blood compartments to describe the unusual behavior of these compounds in blood.

The results of this research and other similar research suggest further work is needed to better understand the kinetics of lipophilic blood compounds, as well as better physiological descriptions that account for their behavior in PBPK models. The decane PBPK model developed with this research can be utilized in further studies of decane, as well as a PBPK
model template for other semi-volatile \textit{n}-alkanes. In addition, the model can be a predictive tool in determining kinetic tissue doses for rats over a range of concentrations. Extrapolation of the model’s physiological parameters to human or other animals will allow the decane model to be used as a predictive tool in establishing exposure levels, as well as describing of the kinetic behavior within a particular species. Furthermore, similar to the lumped compartment gasoline PBPK model developed by Dennison et al. (2003), the model could become part of a larger PBPK model that describes a particular mixture (e.g. JP-8).
References for Chapter 3


APPENDIX A

GAS UPTAKE SYSTEM OPERATING PROCEDURES

Run Preparation
1. Check connections and injection port to make sure properly attached.
2. Check GC septa, should be changed every 100 injections
3. Injection port septa should be changed at least every 3-5 injections.
4. Turn on pump to warm-up (~5min).
5. Set flow rates to chamber (2L/min) and GC (90mL/min).
6. Open up Agilent software to run GC.
7. Load test samples
   a. Name samples
   b. Chose method
   c. Set interval for injection times (10min)
   d. Make sure on autosampler
8. If test sample show contaminants
9. Connect Purge hose from laboratory hood
10. Purge system until residuals are gone.
11. Retest the system by loading more test samples

Start Run
12. If test samples show no contaminants
13. Load real samples on to the GC
14. Prep the injection port by slightly heating injection port and pipe area until warm to almost hot
15. Inject chemical via glass syringe into injection port
16. Press start on GC software to start run (~4hr runs with 10 intervals)

End Run
17. Once runs have ceased, turn off the pump.
18. Connect the purge hose.
19. Restart the pump
20. Purge ~ 30min post run.
21. Syringe (Prep/Clean)
   a. Cleaned out the carbon disulfide
   b. Dried by blowing out carbon disulfide with a pump
   c. Then running nitrogen through 2-3 times.
   d. Check by Hand injection on GC to see if any residual is left on syringe
   e. Set up HI runs like Gas Uptake runs only not by auto-sampler it is a HI
APPENDIX B

LEACH CHAMBER SYSTEM OPERATING PROCEDURES

Start Run
1. Poor n-decane into bubbler approximately until half full.
2. Put bubbler and scrubber in line
3. Check all connections (pressure fluctuation on gauge when shutting chamber door)
4. Make sure flow to bubbler is completely closed.
5. Turn on both pumps to warm up and to purge system.
6. Once warmed up, set airflow of the makeup air to 8L/min (with bubbler completely off)
7. Set out flow to ~8L/min and GC flow to ~100ml/min
8. Make sure that the chamber pressure is slightly negative (between 0 and (-1) inH₂O)
9. Do a test run with auto-sampler on GC:
   a. to see if working properly
   b. to see if completely purged
10. Set up all runs for entire run time at intervals of 10min.
11. Then place rats into chamber (2 people)
12. Close chamber door
13. Same time (2 people)
14. Start the GC
15. Set the bubbler to set amount established to achieve concentration

Note: Bubbler and makeup airflow have to add up to 8L/min

During Run
1. Monitor air pressure for entire run
2. Adjust bubbler and makeup airflow to a desired concentration to give a desired TWA

End of run
1. Prepare cages for easy transfer
2. Put on respirators (2 people)
3. Put on metal mesh glove (2 people)
4. Turn off bubbler at end of run time
5. Immediately open chamber door
6. Immediately transfer rats to cages or to CO₂ chamber (~2min.)
7. Close chamber door and let system purge
8. At time points desired place rats into CO₂ chamber (~2min.)
9. Immediately exhume tissues desired by necropsy
10. Turn off pumps ~30min after run
11. Disassemble leach chamber system (chamber jar)
12. Clean out chamber jar in sink with soap and brushes
13. Allow chamber jar to dry over night
14. Reassemble system for next run
ACSLXTREME MODEL CODE

Decane Model (.CSL FILE)
PROGRAM: PBPK MODEL for n-Decane
! 9 compartments to model tissue data
! Lung (LU), Skin (SK), Brain (B), Fat (F), Slowly Perfused (S),
! Rapidly Perfused (R), Spleen (SN), Liver (L), Bone Marrow (BM),
! Whole Blood (WB)
! Last edited (1/22/04)
! by Reiko Perleberg

INITIAL
CONSTANT QPC = 15.6   !Alveolar ventilation rate (l/hr/kg)
   !QPC from Delp 1991
CONSTANT QCC = 15.6 !Cardiac output (l/hr/kg)
   !QCC from Delp 1991
CONSTANT BW  = 0.211 !Body weight (kg)

!Blood flows (%Cardiac Output)
CONSTANT QLC = 0.174  !Fractional blood flow to liver Brown et al, 1997
CONSTANT QSNC= 0.014  !Fractional blood flow to spleen Delp et al. 1991
CONSTANT QFC = 0.07   !Fractional blood flow to fat Brown et al, 1997
CONSTANT QSKC = 0.058 !Fractional blood flow to skin Brown et al, 1997
CONSTANT QBC = 0.02   !Fractional blood flow to brain Brown et al, 1997
CONSTANT QBMC = 0.039 !Fractional blood flow to bone marrow tissue Travis et al.1990
CONSTANT QLUC = 1     !Set to 1 (100% of blood passes through lung tissue Brown et al, 1997)

!Volumes (% Body Weight)
CONSTANT VLC  = 0.0397  !Fraction liver tissue Schoeffner et al, 1999
CONSTANT VFC = 0.0867   !Fraction fat tissue Schoeffner et al, 1999
CONSTANT VSKC = 0.19   !Fraction skin tissue Brown et al, 1997
CONSTANT VBC  = 0.0076  !Fraction brain tissue Schoeffner et al, 1999
CONSTANT VLUC = 0.0047  !Fraction lung tissue Schoeffner et al, 1999
CONSTANT VSNC = 0.0023  !Fraction spleen tissue Schoeffner et al, 1999
CONSTANT VBMC = 0.03   !Fraction bone marrow tissue Travis et al, 1990
!DECANE Partition Coefficients
CONSTANT  PWB = 5.28   !Blood/air partition coefficient DECANE
CONSTANT  PL  = 6.52   !Liver/blood partition coefficient DECANE
CONSTANT  PF  = 25.54   !Fat/blood partition coefficient DECANE
CONSTANT  PBM = 9.95   !Bone marrow/blood partition coefficient DECANE
CONSTANT  PB  = 3.41    !Brain tissue/blood partition coefficient DECANE
CONSTANT  PSN = 1.11   !Spleen tissue/blood partition coefficient DECANE
CONSTANT  PLU = 6.50    !Lung tissue/blood partition coefficient DECANE
CONSTANT  PSK = 1.20   !Skin tissue/blood partition coefficient DECANE
CONSTANT  PS  = 0.1    !Slowly perfused tissue/blood partition coefficient DECANE
PR = PL    !Rapidly perfused tissue/blood partition coefficient DECANE
CONSTANT  MW = 142.28  !DECANE Molecular weight (g/mol)

!DIFFUSION LIMITATION Parameters
!Permeation area cross products (L/hr)
CONSTANT PAF  = 0.0136    !Permeation area cross product for fat
CONSTANT PAB  = 0.00155   !Permeation area cross product for brain
CONSTANT PABM = 0.0165    !Permeation area cross product for bone marrow
CONSTANT PASN = 0.00002   !Permeation area cross product for spleen
CONSTANT PASK = 0.00586   !Permeation area cross product for skin

!Blood Volume Fractions (% Tissue)
CONSTANT BVF  = 0.05   !Blood volume fraction of fat (%)
CONSTANT BVBM = 0.04   !Blood volume fraction of bone marrow (%)
CONSTANT BVB  = 0.03   !Blood volume fraction of brain (%)
CONSTANT BVSN = 0.22   !Blood volume fraction of spleen (%)
CONSTANT BVSK = 0.02   !Blood volume fraction of skin (%)

!Inhalation Dosing Parameters
CONSTANT  CONC   = 0.    !Inhaled DECANE concentration (ppm)
CONSTANT  TCHNG  = 4.0   !Length of inhalation exposure (hrs)

!Timing commands
CONSTANT tstop    = 240   !Length of simulation (min)
constant DTIME    = 24.
CONSTANT EXPTIM   = 64.  !Length of simulation (hr) for validation
Constant Dosestop = 72

!Graphing Parameters
CONSTANT  POINTS = 330.  !Number of points in plot
CINT = TSTOP/POINTS    !Communication Interval for ACSL

!Scaled parameters
!Blood flows (L/hr)
QL1C = QLC-QSNC  !Remainder of blood flow of liver minus spleen
QC = QCC*BW**0.75
QP = QPC*BW**0.75
QL = QL1C*QC !Blood flow to liver minus spleen
QF = QFC*QC !Blood flow to fat
QBM = QBMC*QC !Blood flow to bone marrow
QSK = QSKC*QC !Blood flow to skin
QB = QBC*QC !Blood flow to brain
QSN = QSNC*QC !Blood flow to spleen
QU = QLUC*QC !Blood flow to lung
QS = 0.22*QC-QF-QSK !Blood flow to slowly perfused tissue
QR = 0.78*QC-QL-QSN-QBM-QB !Blood flow to rapidly perfused tissue

Volumes (L)
VL = VLC*BW !Liver volume
VF = VFC*BW !Fat volume
VBM = VBMC*BW !Bone Marrow volume
VB = VBC*BW !Brain volume
VSK = VSKC*BW !Skin volume
VSN = VSNC*BW !Spleen volume
VLU = VLUC*BW !Lung volume
VS = 0.82*BW-VF-VSK !Slowly perfused volume
VR = 0.10*BW-VL-VBM-VB-VSN-VLU !Rapidly perfused volume

END !END OF INITIAL

DYNAMIC

ALGORITHM IALG=2 !GEAR METHOD

DERIVATIVE

procedural
pflag = pulse(0.,exptim,tchng) !Validation dose timing function
pflag1 = pulse(0.,tstop,dosestop) !Validation clearance timing function
CI = conc*pflag*pflag1*mw/24450.
end

!CA1 = Concentration in venous blood supply perfusing lung at site of
gas exchange (mg/l)
CA1 = (QC*CV+QP*CI)/(QC+(QP/PWB))
AUCWB = INTEG(CA1,0.)

!CV = Mixed venous blood concentration (mg/l)
CV =
(QF*CVF+(QL+QSN)*CVL+QS*CVS+QR*CVR+QBM*CVBM+QB*CVB+QSK*CVSK)/QC
CVAUC = integ(cv,0.)
AX = Amount exhaled (mg)
CX = CA/PWB  !Concentration in Exhaled breath (mg/L)
CXPPM = (0.7*CX+0.3*CI)*24450./MW
RAX = QP*CX  !Rate of change exhalation
AX = INTEG(RAX, 0.)  !Amount exhaled (mg)
RAI = QP*CI  !Rate of change inhalation
AI = integ(RAI,0.)  !Amount inhaled (mg)
doseinh = ai-ax

Lung Tissue Compartment (Flow-Limited)
RALU = QLU*(CA1-CA)  !Rate of change in the lung tissue
ALU = INTEG(RALU,0.)  !Amount in lung tissue (mg)
CLU = ALU/VLU  !Concentration in lung tissue (mg/L)
CA = ALU/(VLU*PLU)  !Concentration in arterial blood (mg/L)

Slowly Perfused Tissue Compartment (Flow-Limited)
RAS = QS*(CA-CVS)  !Rate of change in the slowly perfused tissue compartment
AS = INTEG(RAS,0.)  !Amount in slowly perfused tissues (mg)
CVS = AS/(VS*PS)  !Concentration in slowly perfused tissues (mg/L)
CS = AS/VS

Rapidly Perfused Tissue Compartment (Flow-Limited)
RAR = QR*(CA-CVR)  !Rate of change in the rapidly perfused tissue compartment
AR = INTEG(RAR,0.)  !Amount in rapidly perfused tissues (mg)
CVR = AR/(VR*PR)  !Concentration in rapidly perfused tissues (mg/L)
CR = AR/VR

Fat Compartment
Fat Venous blood Compartment (Diffusion-Limited)
RAVF = QF*(CA-CVF)+(PAF*CF)/PF-PAF*CVF  !Rate of change in the venous blood of fat
AVF = INTEG(RAVF,0.)  !Amount in blood volume of fat (mg)
CVF = AVF/(BVF*VF)  !Concentration in venous blood of fat (mg/L)

Fat Tissue Compartment (Diffusion-Limited)
RAF = PAF*CVF-(PAF*CF)/PF  !Rate of change in fat tissue
AF = INTEG(RAF, 0)  !Amount in fat tissue (mg)
CF = AF/((1-BVF)*VF)  !Concentration in fat tissue (mg/L)
AUCF = INTEG(CF,0.)

Skin Compartment
Skin Venous blood Compartment (Diffusion-Limited)
RAVSK = QSK*(CA-CVSK)+(PASK*CSK)/PSK-PASK*CVSK  !Rate of change in the venous blood of skin
AVSK = INTEG(RAVSK,0.)  !Amount in blood volume of skin (mg)
CVSK = AVSK/(BVSK*VSK)  !Concentration in venous blood of skin (mg/L)
!Skin Tissue Compartment (Diffusion-Limited)
\[ RASK = PASK \times CVSK - (PASK \times CSK)/PSK \]
\[ ASK = \text{INTEG}(RASK, 0) \]
\[ CSK = ASK/((1-BVSK)\times VSK) \]
\[ AUCSK = \text{INTEG}(CSK,0.) \]

!Brain Compartment
!Brain Venous blood Compartment (Diffusion-Limited)
\[ RAVBN = QB \times (CA-CVB)+(PAB\times CB)/PB-PAB\times CVB \]
\[ AVBN = \text{INTEG}(RAVBN,0.) \]
\[ CVB = AVBN/(BVB\times VB) \]

!Brain Tissue Compartment (Diffusion-Limited)
\[ RAB = PAB\times CVB-(PAB\times CB)/PB \]
\[ AB = \text{INTEG}(RAB, 0) \]
\[ CB = AB/((1-BVB)\times VB) \]
\[ AUCBN = \text{INTEG}(CB,0.) \]

!Spleen Compartment
!Spleen Venous blood Compartment (Diffusion-Limited)
\[ RAVSN = QSN \times (CA-CVSN)+(PASN\times CSN)/PSN-PASN\times CVSN \]
\[ AVSN = \text{INTEG}(RAVSN,0.) \]
\[ CVSN = AVSN/(BVSN\times VSN) \]

!Spleen Tissue Compartment (Diffusion-Limited)
\[ RASN = PASN\times CVSN-(PASN\times CSN)/PSN \]
\[ ASN = \text{INTEG}(RASN, 0) \]
\[ CSN = ASN/((1-BVSN)\times VSN) \]
\[ AUCSN = \text{INTEG}(CSN,0.) \]

!Bone Marrow Compartment
!Bone Marrow Venous blood Compartment (Diffusion-Limited)
\[ RAVBM = QBM \times (CA-CVBM)+(PABM\times CBM)/PBM-PABM\times CVBM \]
\[ AVBM = \text{INTEG}(RAVBM,0.) \]
\[ CVBM = AVBM/(BVBM\times VBM) \]

!Bone Marrow Tissue Compartment (Diffusion-Limited)
\[ RABM = PABM\times CVBM-(PABM\times CBM)/PBM \]
\[ ABM = \text{INTEG}(RABM, 0) \]
CBM = ABM/((1-BVBM)*VBM)  !Concentration in bone marrow tissue (mg/L)
AUCBM = INTEG(CBM,0.)

!Liver Compartment  
!Liver Tissue Compartment (Flow-Limited)
RAL = QL*(CA-CVL) + QSN*(CVSN-CVL) !Rate of change in liver  
!Blood flow from liver and spleen
AL = INTEG(RAL,0.)  !Amount in liver tissue (mg)
CVL = AL/(VL*PL)
CL = (AL)/VL
AUCL = INTEG(CL,0.)

!MASS = mass balance (mg)
MASS = AF+AL+AS+AR+AX+ASK+AB+ABM+ASN+ALU
BAL=AI-MASS
!.....................................................................

TERMT(T.GE.TSTOP)

END  !'End of derivative'
END  !'End of dynamic'
END  !'End of program'
Example M.file for plotting both model simulations
%PBPK MODEL for Decane, Fischer Rat Blood.M datafile
%Fisher unpublished
%Created 7/8/2003 by Reiko Perleberg
%Last Modified: 7/8/2003
%Modified by: Reiko Perleberg

%****simulation prompt commands********

!!s bw=0.21 !Average body weight from study
!!s tstop=8.5, tchng=4
!!s conc=1200
!!Start /nc
t_run1=_t
cv_run1=_cv

%Blood (corrected values) Decane level predictions from flow-limited model'
%(t, ca)
run_wb = [Data exported from other model];

plot(t_run1,cv_run1,inh1200_wb(:,1),inh1200_wb(:,2), run_wb(:,1),run_wb(:,2),
'inh1200_wb1.aps')

Example M.file for plotting multiple concentrations
%PBPK MODEL for Decane, Fischer Rat Blood.M datafile
%Fisher unpublished
%Created 7/8/2003 by Reiko Perleberg
%Last Modified: 7/8/2003
%Modified by: Reiko Perleberg

%****simulation prompt commands********

!!s bw=0.21 !Average body weight from study
!!s tstop=8.5, tchng=4
!!s conc=1200
!!Start /nc
t_run1=_t
cv_run1=_cv

!!s bw=0.21 !Average body weight from study
!!s tstop=8.5, tchng=4
!!s conc=781
!!Start /nc
t_run2=_t
cv_run2=_cv
Average body weight from study
Average tstop=8.5, tchng=4
Average conc=273

Start /nc

t_run3=_t
cv_run3=_cv

plot(t_run1,cv_run1,t_run2,cv_run2,t_run3,cv_run3,inh1200_wb(:,1),inh1200_wb(:,2),
inh781_wb(:,1),inh781_wb(:,2),inh273_wb(:,1), inh273_wb(:,2), 'inh781_wb.aps')

Example CMD.file

Decane CMD file
Includes datasets for multiple tissues
Created: 11/05/02 by Deborah Keys
Last Modified: 07/10/03 by Reiko Perleberg

prepar t,cl,cr,cf,cs,csk,clu,csn,cb,mass,cbm,ca,ax,am,al,cv, cv

Data M.File

PBPK MODEL for Decane, Fischer Rat .M datafile
DECANE
Fisher unpublished
Created 7/8/2003 by Reiko Perleberg
Last Modified: 7/8/2003
Modified by: Reiko Perleberg

**** simulation prompt commands ********
**** data section ****************
blood Decane levels corrected values'
(t, ca)

inh1200_wb = [
4.0833  6.99
4.0833  4.99
4.0833  7.04
4.0833  8.44
4.5  0.23
4.5  1.58
4.5  1.34
4.5  0.84
5  1.42
5  0.80
5  0.39
5  1.08
6  0.66
6  0.42
6  0.36
%Blood 8hr point below detection limit

%Liver Decane levels'
%(t, cl)
inh1200_l = [
4.0833  31.80
4.0833  25.93
4.0833  31.50
4.0833  45.74
4.5   9.40
4.5   33.55
4.5   15.99
4.5   10.99
5    5.92
5    2.27
5    6.70
5    5.78
6    2.48
6    1.23
6    0.58
6    0.37
8    1.05
8    0.62
8    0.28
8    0.27];

%Fat Decane levels
%(t, cf)
inh1200_f = [
4.0833  151.50
4.0833  109.57
4.0833  67.44
4.0833  91.14
4.5   15.10
4.5   53.44
4.5   57.12
4.5   116.39
5    164.50
5    162.74
5    76.01
5    48.29
6    40.92
60
%Spleen Decane levels
%(t, csn)
inh1200_sn = [
4.0833  7.73
4.0833  11.22
4.0833  8.76
4.0833  16.65
4.5  2.76
4.5  11.37
4.5  15.67
4.5  14.28
5  10.28
5  5.14
5  2.59
5  5.34
6  8.66
6  3.36
6  2.61
6  5.24
8  2.35
8  4.50
8  3.70
8  2.38
10  5.62
10  7.54
10  1.61
]
%Lung Decane levels
%\( t, \text{clu} \)
\( \text{inh1200}_\text{lu} = [ \)
4.0833 11.44
4.0833 30.66
4.0833 11.54
4.0833 19.36
4.5 6.46
4.5 12.37
4.5 3.82
4.5 5.29
5 15.15
5 1.85
5 3.20
5 3.86
6 1.40
6 4.40
6 6.06
6 6.59
8 0.18
8 0.49
8 1.01
8 0.62];

%Brain Decane levels
%\( t, \text{cb} \)
\( \text{inh1200}_\text{b} = [ \)
4.0833 130.75
4.0833 88.15
4.0833 134.62
4.0833 127.99
4.5 117.26
4.5 104.97
4.5 102.53
4.5 54.64
5 72.32
%Bone Marrow Decane levels
%(t, cbm)
inh1200_bm= [
  4.0833   249.63
  4.0833   246.50
  4.0833   405.93
  4.0833   141.54
  4.5      75.40
  4.5      351.46
  4.5      50.81
  4.5      304.63
  6       33.29
  6       191.96
  6       189.88
  6       218.08
  10      65.19
  10      37.27
  10      80.07
  10      46.03
  16      4.44
  16      6.78
  16      5.39
];
<table>
<thead>
<tr>
<th></th>
<th>Skin Decane levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>inh1200_sk= [</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>7.95</td>
</tr>
<tr>
<td>28</td>
<td>1.71</td>
</tr>
<tr>
<td>28</td>
<td>1.37</td>
</tr>
<tr>
<td>28</td>
<td>1.88</td>
</tr>
</tbody>
</table>

%Bone Marrow 28 point below detection

%Skin Decane levels
%(t, csk)
inh1200_sk= [4.0833 10.56
4.0833 11.45
4.0833 5.69
4.0833 5.02
4.5  9.66
4.5  4.90
4.5  14.23
4.5  6.09
5    12.69
5    7.61
5    63.03
5    13.17
6    9.47
6    20.08
6    7.75
6    4.43
8    2.58
8    0.63
8    7.18
8    23.06
10   7.02
10   7.21
10   32.81
10   36.91
16   0.73
16   2.60
16   0.06
28   0.61
28   0.15
28   2.68
28   0.58];

%Below detection skin 16 point

%Spleen Decane levels
%(t, csn)
inh781_sn= [4.0833 12.52
64]
4.0833   7.54  
4.0833   20.69 
4.0833   17.38  
4.0833   10.72  
4.0833   10.46 
4.0833   8.25  
4.0833   9.04];

%iLiver Decane levels  
%\((t, cl)\)  
inh781_l=[  
4.0833   42.64 
4.0833   23.85 
4.0833   28.94 
4.0833   44.94 
4.0833   47.32  
4.0833   19.75 
4.0833   30.79 
4.0833   39.89];

%iWhole Blood Decane levels corrected 58.1% 
%\((t, ca)\)  
inh781_wb=[  
4.0833   2.91  
4.0833   3.13 
4.0833   1.47  
4.0833   3.14 
4.0833   1.32  
4.0833   2.34 
4.0833   3.22 
4.0833   3.89];

%iLung Decane levels 
%\((t, clu)\)  
inh781_lu=[  
4.0833   16.48 
4.0833   17.53 
4.0833   7.76  
4.0833   14.07 
4.0833   17.72 
4.0833   9.10  
4.0833   12.78 
4.0833   11.52];

%iFat Decane levels 
%\((t, cf)\)
inh781_f = [ 
4.0833   84.89 
4.0833   38.47 
4.0833   72.71 
4.0833   28.60 
4.0833   51.60 
4.0833   35.82 
4.0833   29.99 
4.0833   66.03];

%Brain Decane levels
%(t, cb)
inh781_b= [ 
4.0833   67.81 
4.0833   52.70 
4.0833   65.66 
4.0833   61.79 
4.0833   61.86 
4.0833   56.19 
4.0833   60.75 
4.0833   62.00];

%Bone Marrow Decane levels
%(t, cbm)
inh781_bm= [ 
4.0833   132.33 
4.0833   115.99 
4.0833   133.84 
4.0833   89.13 
4.0833   64.50 
4.0833   153.82 
4.0833   178.92];

%Spleen Decane levels
%(t, csn)
inh273_sn= [ 
4.0833   3.37 
4.0833   1.56 
4.0833   1.95 
4.0833   1.98 
4.0833   1.82 
4.0833   2.79 
4.0833   1.98 
4.0833   1.68];
%Liver Decane levels
%(t, cl)
inh273_l = [ 
4.0833 5.06
4.0833 3.21
4.0833 5.76
4.0833 5.31
4.0833 3.73
4.0833 4.38
4.0833 4.13
4.0833 3.58; 

%Whole Blood Decane levels corrected values 58.1%
%(t, ca)
inh273_wb = [ 
4.0833 0.42
4.0833 0.42
4.0833 0.39
4.0833 0.33
4.0833 0.61
4.0833 0.75
4.0833 0.64
4.0833 0.38; 

%Lung Decane levels
%(t, clu)
inh273_lu = [ 
4.0833 3.00
4.0833 1.62
4.0833 2.19
4.0833 2.14
4.0833 3.07
4.0833 2.50
4.0833 1.79
4.0833 2.93; 

%Fat Decane levels
%(t, cf)
inh273_f = [ 
4.0833 8.87
4.0833 6.82
4.0833 10.81
4.0833 9.88
4.0833 17.82
4.0833 11.55
4.0833 13.77

67
4.0833 34.93];

%S skin Decane levels
%(t, csk)
in273_sk = [
4.0833 2.38
4.0833 1.43
4.0833 0.88
4.0833 1.11]

%B brain Decane levels
%(t, cb)
in273_b = [
4.0833 18.88
4.0833 18.10
4.0833 17.73
4.0833 14.77
4.0833 18.95
4.0833 20.74
4.0833 16.56
4.0833 22.93]

%B bone Marrow Decane levels
%(t, cbm)
in273_bm = [
4.0833 36.94
4.0833 68.03
4.0833 45.58
4.0833 53.63
4.0833 65.53
4.0833 64.42
4.0833 30.11
4.0833 55.59]