TISSUE TO AIR PARTITION COEFFICIENTS FOR NONANE AND ITS ISOMERS

by

GHANASHYAM JOSHI

(Under the Direction of Jeff Fisher)

ABSTRACT

JP-8, a major fuel source used by US and NATO forces, is a complex mixture of aliphatic and aromatic isomers of hydrocarbons. Occupational exposure to JP-8 occurs through inhalation and dermal contact. Tissue/blood partition coefficients (PC) values are chemical specific parameters used in modeling. The partition coefficient values for n-alkanes tend to increase with the increasing carbon number but less is known about the trend for isomers of n-alkanes. PCs were first determined by vial equilibration methods developed by Sato and Nakajima (1979) and later modified by Gargas et al. (1989). PC values were obtained for five isomers of n-alkane nonane (C9), namely 3-methyloctane, 4-ethylheptane, 2,3-dimethylheptane, 2,2,4-trimethylhexane and 2,2,4,4-tetramethylpentane and n-nonane. The PC values tend to follow the published log octanol/water (O:W) PC values for n-nonane and its isomers. Experimentally determined PC values for n-nonane with the highest O:W ratio were greatest and the isomer 2,2,4,4-tetramethylpentane with the lowest O:W was the lowest. As expected fat tissue had the highest PC values for n-nonane and the isomers and muscle tissue the least. These reported PCs support the development of a jet fuel Physiologically Based Pharmacokinetic (PBPK) model.

KEY WORDS: JP-8, Partition coefficient, Alkane, Isomer, Octanol water, Physiologically-based Pharmocokinetic Modelling, Lipophilic

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DEDICATION

I dedicate this to my Parents and all my family members back in Nepal. Thank you for all the support that you people have given to me.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1. Background of JP-8

Jet Propellant – 8 (JP-8) specified by the U.S government in 1990 is a substance used as aircraft fuel by the military and U.S Air Force and other branches of the military. It was first introduced by NATO bases in 1978. In order to use less flammable and less hazardous fuel for better safety, the U.S Air Force replaced JP-4 with JP-8 completely by the fall of 1996.

The primary ingredient in JP-8 is kerosene, usually about 99.8% by weight. In addition to kerosene, JP-8 contains small amounts of many other substances, such as benzene, and various additives to inhibit icing, prevent static charge buildup and avoid oxidation. These additives make JP-8 more resistant to explosion, and enhances performance at high altitudes better than other commercial jet fuels (Ritchie et al., 2003). JP-8 also has a higher flash point than JP-4 and is less likely to ignite accidentally (McDougal and Rogers, 2004). Estimates suggest that more than 2 million people worldwide are exposed to 60 billion gallons of military and/or commercial jet fuel annually (Ramos et al., 2007).

1.2. JP-8 Composition

Jet fuels are composed of hundreds and perhaps thousands of individual hydrocarbon chemicals and their isomers. The composition of JP-8 varies continuously with each manufactured batch because the specification is based primarily on the performance characteristics rather than chemical composition (McDougal et al., 2000). JP-8 is a kerosene-based fuel consisting of

approximately 81% aliphatic hydrocarbons and 19% aromatic hydrocarbons (Pleil et al., 2000, Serdar et al., 2003, Zeiger and Smith, 1998). The aliphatic hydrocarbon compound JP-8 is comprised of aliphatic hydrocarbons in the chain length from 6 (C_6) to 18 (C_{18}) carbons; the C_9 to C_{14} n-alkanes constitute approximately 28% of the bulk fuel (Pleil et al., 2000).

1.3. JP-8 Exposure

All branches of the United States military use JP-8, whether it is used to fuel jet aircraft, tanks, fighting vehicles, ships, helicopters, portable heating, or air conditioning units. It is essentially, the universal fuel of the US military (Ritchie et al., 2003).

Human exposure to JP-8 occurs in vapor, aerosol and liquid forms (McDougal et al., 2000). Inhalation and dermal contacts are the primary route of exposure (Drake et al., 2003, McDougal and Garrett, 2007). Military personnel may be exposed to JP-8 from incomplete combustion, handling, and transport of JP-8 during normal activities (Edwards et al., 2005). Exposure of military personnel to JP-8 can also occur through fuel applications unrelated to aircraft fueling, including fueling of land vehicles, equipment, heaters, and lighting sources, use as a coolant (heat sink) in aircraft, aerosolization/combustion of JP-8 for use as a combat obscurant, use of JP-8 to suppress environmental sand or dust, decontamination of military vehicles and equipment with JP-8, or use of JP-8 as a carrier for herbicide application. Exposure of individuals not involved occupationally with jet fuel occurs through atmospheric, soil, or groundwater contamination with jet fuels or their combustion products, or through off-gassing from the skin and clothing of fuel exposed personnel (Ritchie et al., 2003). However, because jet fuel vapors and aerosols are mainly an inhalation hazard, JP-8 effects pose major risks to the respiratory system (Wong et al., 2009).

1.4. JP-8 Toxicity

Inhalation is the primary route of exposure to JP-8 although recently dermal exposure has also been recognized as a common route. JP-8 is less volatile than its predecessor, JP-4 (Drake et al., 2003, Mattie et al., 1991). Less volatility may reduce the inhalation exposure, where as absorption from dermal exposure may increase. JP-8's physical characteristics reduce vaporization and result in increased bioavailability and human exposure (Drake et al., 2003).

After the replacement of JP-4 with JP-8, there has been an increase in the number of self reported and/or medically diagnosed complaints from exposed personnel (Ritchie et al., 2003). Among the common self reported symptoms are dizziness, walking difficulties, weakness, skin rashes, and allergic reactions (Ritchie et al., 2003).

In an animal developmental toxicity study with orally administered JP-8, the number and type of fetal malformations and variations observed did not differ significantly between dose groups. The dams in the high concentration exposed group gained significantly less body weight than did the control dams (Cooper and Mattie, 1996). The pups in pregnant female rats exposed to 1000 ppm of n-hexane at 6h/d for 9 day, showed reduced postnatal growth (Bus et al., 1979). Women of child-bearing age constitute approximately 11% of Air Force active-duty personnel. Of these, an estimated 6000 women are assigned to jobs in which they could be exposed to JP-8 (Cooper and Mattie, 1996).

Male Sprague Dawley rats were exposed to inhalation of n-C9 to n-C13 alkanes (4438, 1369, 442, 142 and 41 ppm respectively) for 8 hours and observed for the following 14 days. These studies demonstrated that the concentration of alkanes in the brain exceeded that of blood for the lower carbon numbered alkanes, whereas the higher carbon numbered alkanes possessed a

brain/blood ratio equal to or less than unity (Nilsen et al., 1988). The n-C9 alkane nonane caused clinical signs of cerebellar dysfunction and damage to cerebellar neurons suggesting that the CNS is a target organ for toxic effects (Nilsen et al., 1988).

Mattie et al. (1991) exposed male F344 and C57B1/6 mice on a continuous basis for 90 days, to JP-8 vapor at 0, 500, 1000 mg/m^3, followed by a recovery period to 24 months of age. Though no pulmonary lesions and other histopathology difference were observed between the exposed and control rats, the male rat kidney developed a reversible ultra-structural increase in size and propensity. JP-8 vapor and aerosol exposure for 7, 28 and 56 day exposures at concentrations of 469-520 mg/m^3 and 814-1263 mg/m^3 caused lung epithelial cell apoptosis (Pfaff et al., 1996).

In addition, significant reduction in the organ weights (spleen and thymus) and total cell numbers recovered from each of the major immune system organs (spleen, thymus, lymph nodes, bone marrow, and peripheral blood were observed in short term (7 days) exposure to JP-8 (Harris et al., 1997), and also resulted in profound and significant alterations in the immune system (Harris et al., 2008).

Tumor bearing animals were exposed to JP-8 for 1h/day. This resulted in secretion of two immunosuppressive agents, interleukin -10 (IL-10) and prostaglandin E2 (PGE2) but showed no affect on the growth of the tumor. However, JP-8 exposed tumor bearing animals died at an accelerated rate as compared with air exposed tumor-bearing mice (Harris et al., 2007).

Recent studies have been done on the transient and persistent effects of JP-8 jet fuel exposure on auditory function in rats which showed exposure to JP-8 and noise produced an additive disruption in outer hair cell function (Fechter et al., 2007).

JP-8 has been shown to be irritating and causes molecular changes in the skin of laboratory animals. Hydrocarbons have also been shown to cause skin cancer with repeated application to the skin (McDougal and Rogers, 2004). Studies performed with kerosene based fuel, similar to JP-8 without performance additives increased the incidence of skin cancer in mice (Ritchie et al., 2003). While JP-8 has been characterized as a non-carcinogen, studies have shown it to be a possible tumor promoter (Ritchie et al., 2003).

JP-8, 8 aliphatic HC (nonane, decane, undecane, dodecane, tridecane, tetradecane, pentadecane, hexadecane) and 6 aromatic HC (ethyl benzene, *o*-xylene, trimethyl benzene, cyclohexyl benzene, naphthalene, dimethyl naphthalene) soaked cotton fabrics were topically exposed to pigs for 1 day and with repeated daily exposures for 4 days. Erythema (redness of the skin, caused by hyperemia of the capillaries in the lower layers of the skin), epidermal thickness, and epidermal cell layers were evaluated. No erythema was noted in 1-day *invivo* HC exposures but significant erythema was observed in 4-day tridecane, tetradecane, pentadecane, and JP-8 exposed sites. The aromatic HCs did not produce any macroscopic lesions in 1 or 4 days of *invivo* exposures. Morphological observations revealed slight intercellular and intracellular epidermal edema in 4-day exposures with the aliphatic HCs (Muhammad et al., 2005).

1.5. Partition Coefficients Introduction

Partition coefficients (PCs), also referred as solubility or distribution coefficient are defined as the ratio of chemical concentrations between two phases at equilibrium (Gargas et al., 1989). PCs are commonly reported as blood:air and tissue:blood. The tissue:air PC when divided by blood:air determined by head space vial equilibrium methods yields tissue:blood PC (Gargas et al., 1989, Sato and Nakajima, 1979, Smith et al., 2005).

PCs are important parameters in physiologically based pharmacokinetic (PBPK) models (Basak et al., 2006, Poulin and Krishnan, 1995). For many volatile chemicals blood:air partition coefficient values are essential for successful modeling efforts. Blood:air partition coefficient values describe steady state equilibrium ratios between blood and inhaled air concentrations of a chemical (Basak et al., 2004, Poulin and Krishnan, 1995). For many chemicals values of human or rat blood:air, tissue:air, or tissue:blood partition coefficients are not available and this may limit the development of PBPK models (Payne and Kenny, 2002). PCs values are used in PBPK models focused on the kinetic or dynamic behavior of occupational or environmental chemicals (Fisher et al., 1989, Ramsey and Andersen, 1984). In the case of the unavailability of the experimentally determined PC values, the lipophilicity of the chemical or (log P) is used to describe equilibrium partitioning of chemicals between two phases (Benfenati et al., 2003). PCs are commonly assumed to be independent of concentration, and measurements are frequently conducted at single concentration (Payne and Kenny, 2002).

1.6. Methods of Determining Partition Coefficient Values

PC values can be determined by two major methodologies, experimental (Beliveau and Krishnan, 2000, Gargas et al., 1989, Sato and Nakajima, 1979, Smith et al., 2005) and computational (Abraham et al., 2007, Abraham et al., 2008, Basak et al., 2004, Basak et al., 2006, Poulin and Krishnan, 1995). Experimental methods are subdivided into two types, *invivo* and *invitro*. PCs can be measured *invivo* through steady-state exposure (inhalation or continuous intra venous injection) followed by analysis of the chemical in each organ. This method works best for chemicals that are not metabolized in the body (Sterner et al., 2006). *Invivo* methods avoid disruption of normal tissue architecture and cellular structure, and may therefore provide a better representation of the biology (Dallas et al., 1995), but they are labor and animal intensive,

and virtually impossible to conduct in humans (Thrall et al., 2002). More frequently, PCs are determined *invitro* and vial equilibration is the most common *invitro* method for volatile chemicals (Sterner et al., 2006).

Experimental methods for measuring PC values can vary, ranging from the simple ‘‘shake flask’’ technique (Edelbach and Lodge, 2000) to popular chromatographic methods (Edelbach and Lodge, 2000, Gargas et al., 1989, Sato and Nakajima, 1979, Smith et al., 2005). However, experimental determination of PCs is time and material consuming and can only be carried out if the compound is available. When the chemical is unavailable or laboratory experiments are otherwise important the use of computational methods are preferred to predict PCs by using the structure of the chemical and physical:chemical databases (Benfenati et al., 2003).

The shake flask method is still popular and a widely applicable method for PC determinations. The shake flask method is literally based on shaking a solute with two immiscible solvents, l-octanol and water, in a flask (Sanemasa et al., 1994). Once the partition equilibrium is attained, the concentration in one or both phases is measured. However the disadvantage of this method is in the formation of emulsions during shaking, which affects the observed octanol:water (Ko:w) values. The aqueous phase often needs to be centrifuged to remove most of the small 1-octanol droplets (Sanemasa et al., 1994).

For volatile chemicals (VOCs) the vial equilibration method developed by Sato and Nakajima (1979), and later modified by Gargas et al. (1989), is the most common method for the determination of PCs. Vial equilibration method relies on chemical analysis of the headspace above a tissue, blood or tissue homogenate after the chemical has achieved equilibrium between the air and tissue phases (Jepson et al., 1994). A gas phase vial equilibration method used

1iquid:air and tissue:air partition coefficients for low molecular weight volatile chemicals (Gargas et al., 1989). Gargas et al. (1989) determined partition coefficients with 0.9% saline, olive oil, blood, liver, muscle, and fat tissues from rats for 55 compounds using the gas phase vial equilibration method.

Even though PCs are easier to determine for volatile chemicals, laboratory accessibility and costs can prohibit their measurement and thus PBPK modelers are often forced to look for alternatives to experimental PCs (Sweeney et al., 1996). Theoretical molecular descriptors have also been used for predicting PC's based upon molecular structural properties (Basak et al., 2004, Basak et al., 2006, Basak et al., 2003). Some authors have developed algorithms to predict partition coefficients from physical properties. In most cases the solubility of a chemical in n-octanol is assumed to correspond to its solubility in neutral body lipids and its solubility in water is assumed to correspond to the solubility in the fraction of tissue and blood that is water. Its solubility in phospholipids corresponds to both the solubility in water and n-octanol (Poulin and Krishnan, 1995). Although some PC algorithms are designed to directly calculate tissue:blood PCs, the majority result in tissue:air values (Sterner et al., 2006). PBPK modelers prefer measured blood:air and tissue:blood PC values to construct their model compartments and hence the experimental head-space chromatographic method is often preferred over computational methods .

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CHAPTER 2

TISSUE TO AIR PARTITION COEFFICIENTS FOR NONANE AND ITS ISOMERS

2.1. Introduction

Physiologically based pharmacokinetic (PBPK) modeling is widely used to describe the biological fate of chemicals (Andersen, 1991), dosimetry of chemicals (Payne and Kenny, 2002, Teo et al., 1994) and for risk assessments (Andersen, 1995, Filser et al., 1995). PBPK models incorporate partition coefficients (PCs) along with other physiochemical, biochemical and physiological parameter to predict the pharmacokinetics of the chemical (Béliveau and Krishnan, 2000, Gargas et al., 1989). PC is the ratio of chemical concentration between two dissimilar media at equilibrium and is the measure of tissue solubility (Andersen, 1991, Gargas et al., 1989, Smith et al., 2005). Blood to air and tissue to blood PCs are used for a PBPK model development. Unavailability of PC's for many chemicals limit the development of PBPK models (Payne and Kenny, 2002).

Partition coefficient can be measured by several methods from shake flask technique (Edelbach and Lodge, 2000) to popular chromatographic methods (Edelbach and Lodge, 2000, Finizio et al., 1997, Gargas et al., 1989, Kumarathasan et al., 1998, Sato and Nakajima, 1979, Smith et al., 2005). The headspace vial equilibration method is commonly used for experimental determination of PC (Gargas et al., 1989, Kumarathasan et al., 1998, Sato and Nakajima, 1979, Smith et al., 2005). Because the experimental determination of partition coefficient is time consuming in many cases the estimate of the lipophilicity of the chemical (log P) is predicted based on the chemical structure (Benfenati et al., 2003, Connell et al., 1993). Use of software for

predicting log P is also a common practice for determining the fate of the chemical (Benfenati et al., 2003, Mannhold and Dross, 1996, Mannhold and van de Waterbeemd, 2001, Tetko et al., 2001). Thus, estimation of the log P results in different values for the same chemical in different literatures (Benfenati et al., 2003).

Jet fuel (JP 8) is a complex mixture consisting of hundreds of aliphatic (ALI) and aromatic (ARO) hydrocarbons, of which less than 20% are aromatics and the remainder consist of most of the possible structural isomers for aliphatic hydrocarbons in the C_6 to C_{18} range (Pleil et al., 2000, Yang et al., 2006). C_9 to C_{14} n-alkanes constitute approximately 28% of the bulk fuel (Pleil et al., 2000). However the exact composition varies from batch to batch and supplier to supplier (Zeiger and Smith, 1998). Due to the chemical complexity of JP 8 it is difficult to conduct a toxicity study of all fuel components, and thus, studies on the representative chemical based on the composition are carried out (Dahl et al., 1988, Singh and Singh, 2004). Aliphatic hydrocarbons in the C9 to C13 range have become increasingly important as workplace contaminants, due to the increased use of low aromatic solvents and mineral oils (Nilsen et al., 1988) and nonane is a surrogate marker of alkanes and iso-alkanes found in JP-8 (Edwards et al., 2005).

Aromatic and aliphatic hydrocarbon components of JP-8 show different rates of absorption (into the skin) and permeability (McDougal and Garrett, 2007). Aliphatic compounds ranging from C9 to C14 were identified in rat skin at the end of 3.5 hour static diffusion cell studies (McDougal et al., 2000).

In this paper we report the partition coefficient of nonane and five isomers of nonane namely 2,2,4-trimethylhexane, 3-methyloctane, 4-ethylheptane, 2,3-dimethylheptane, 2,2,4-

trimethylhexane and 2,2,4,4-tetramethylpentane selected based on the octanol water partition coefficient value calculated using online Marvin calculator (http://www.chemaxon.com/marvin/sketch/index.jsp) using the vial equilibration head space method. These values will then be used for the development of the jet fuel mixture model.

2.2. Methods Partition coefficient

1) Chemicals

All chemicals were reported to be ≥ 99% purity except 2,2,4-trimethylhexane (≥ 98%). Nonane was purchased from Sigma-Aldrich (St. Louis, MO), and all others [3-methyloctane (CAS no: 2216-33-3), 4-ethylheptane (CAS no: 2216-32-2), 2,3-dimethylheptane (CAS no: 3074-71-3), 2,2,4-trimethylhexane (CAS no: 16747-26-5) and 2,2,4,4-tetramethylpentane (CAS no: 1070-87-7)] were purchased from TCE America (Portland, OR).

2) Animals

Twenty male F344/CD strain rats of approximately 300 gm were obtained from Charles River Inc (Raleigh, NC). Rats were housed two per shoe box style cage and were fed with standard laboratory rat chow and tap water. Animals were kept under controlled humidity and climate, and litters were changed weekly. Rats were kept in the storage facility for a minimum of seven days after their arrival untill they were sacrificed. Rats were anesthetized by carbon dioxide prior to the sacrifice. Blood samples were collected from the portal vein and was collected in a heparinized (180ul heparin) 15 ml tube. Liver, lung, renal fat, brain and muscle were collected and stored in scintillation vials at -80^{o}C until used.

3) Method development

To reduce the number of animals used and because of the time consuming analysis process, PC for two different tissues for a single chemical was determined in one day. Preliminary studies were performed to obtain the shortest run time possible while maintaining proper chromatographic separation for each chemical. Studies were also done to ensure time to equilibrium was sufficient with the most lipophilic tissues (fat and brain) for the chemical with the highest octanol:water coefficient value (n-nonane). Results indicated that fat and brain reached the equilibrium time after 4 hr, and the other tissues had an equilibrium time set to three hours.

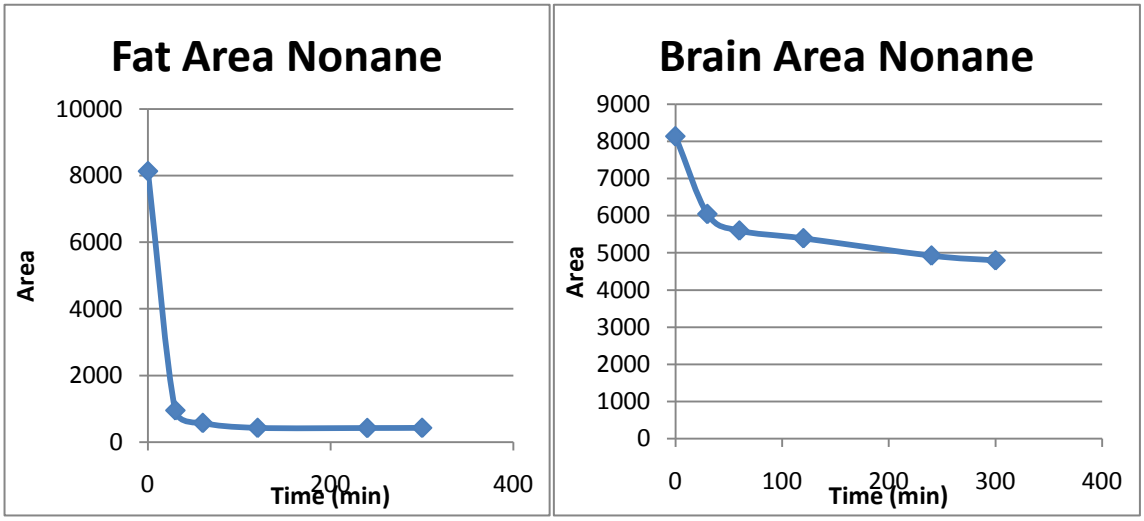

Fig 1: Graphs depicting equilibrium time for fat and brain

Partition coefficient (PC) was determined to be independent of concentration. Five samples and four references were made for blood and each tissue. Two different gas tight syringes (Hamilton Reno, NV) were used for the sample and reference. After each analysis the syringes were

cleaned by acetone then dried in a Hamilton syringe cleaner. Before each analysis the syringes were checked to see if any residual alkane or acetone remained by injecting the room air to the GC.

4) Preparation of the standard

SKC sampling tedlar bags (Cat no: 231-03) of 3 L capacity were used to prepare standards of known concentrations of the chemicals in air. Bags were filled to 80% capacity with filtered room air (Whatman carbon cap, cat no: 6704-1500). For each chemical, the amount needed to obtain a 3000 parts per million (ppm) was calculated using the following equation:

$$\text{Vol (ml)} = \frac{\text{ppm} \times \text{volume of air in bag} \times \text{molecular weight}}{\text{Specific Gravity of the chemical} \times \text{Molar Volume} \times 10^6}$$

The bags were then heated using an air dryer to obtain complete vaporization of the chemical. The bags were allowed to cool to room temperature prior to use.

5) Tissue preparation and equilibration

Glass scintillation vials containing frozen tissues were allowed to defrost in warm water for 20-30 minutes. Tissues were trimmed to remove connective tissues, minced in a crucible with the help of scissors then smeared into 10 mL headspace gas vials. About 0.5 g of liver, 1 g of muscle, 0.4 g of lung, 0.1 g of brain, 0.05 g of fat and 0.75 ml of the whole blood were used for the preparation of headspace samples for all the chemicals. Reference vials with no tissue were

also prepared for each set of samples. Each vial was then sealed with an aluminum cap with TEF/BUTYL septum (20mm; National Scientific). Samples and controls were vortexed at 37^{o}C for 20-30 minutes, then 1 ml of gas from the standard bag was added to each vial after removing 1 ml of air from the vials with the help of gas tight syringe. Prior to removing and adding 1 ml of air the pressure of the vials was released by the syringe without the plunger in it. Vials (sample and control) were then allowed to incubate in the vortexer with moderate shaking either 3 hours (blood, liver, lung and muscle) or 4 hours (fat and brain) before 0.5 ml of the headspace vapor was injected into the gas chromatograph for analysis.

6) Apparatus

Analyses were carried out with an Agilent Technology 6890 N series II gas chromatograph equipped with a DB5 megabore capillary column (15.0 m x 530 µm x 1.5 µm). The front inlet and the Flame Ionization Detector (FID) temperatures were both 200^{o}C. Oven temperature was 90^{o}C (isothermal).

7) Partition coefficient calculation

PCs were calculated using the equation found in Gargas *et al*. (1989),

$$P_i = \frac{C_{ref}\,(V_{vial}) - C_i\,(V_{vial} - V_i)}{C_i\,V_i}$$

Where, P_i = partition coefficient. C_i = concentration (area count) of n-alkane and isomers vapor contained in headspace of reference vial, V_{vial} = volume of reference vial (11.3 ml headspace

vial). *Ci* = concentration (area count) of n-alkane and isomers in headspace of test vial, and V*i* =: volume of tissue/blood in test vial. The volume of the reference vial was determined by weighing the vial filled with water.

8) Statistical analysis

Scheffe's test for the significant difference in the means for tissue to air, and blood to air was at 95% significance level. The computed octanol:water PC (logP) for n-nonane and its five isomers were each fit to the mean measured partition coefficient using simple linear regression which follows the equation y = mx + c where m is the slope and c the intercept (Table 4) by Systat Software Inc SigmaPlot (version 9).

2.3. Result

Mean (CV) blood:air and tissue:air partition coefficient values for n-nonane and its isomers (3-methyloctane, 4-ethylheptane, 2,3-dimethylheptane, 2,2,4-trimethylhexane, 2,2,4,4-tetramethylpentane) are listed in Table 1.

Table 1

n-Alkane tissue:air and blood:air partition coefficients (mean (CV)):

Chemical/PC (CV)	Liver:air	Blood:air	Fat:air	Brain:air	Lung:air	Muscle:air
Nonane	15.83 (0.10)	10.5 (0.05)	2206.14(0.04)	32.05(0.07)	13.01(0.05)	7.24 (0.08)
3-methyloctane	13.18 (0.10)	9.35 (0.11)	1755.95(0.03)	27.99(0.09)	12.81(0.06)	7.98 (0.05)
4-ethylheptane	11.73 (0.05)	13.56(0.05)	1941.69(0.03)	26.30(0.07)	12.37(0.09)	7.93 (0.07)
2,3-dimethylheptane	10.89 (0.10)	9.14 (0.08)	1663 (0.01)	24.07(0.06)	14.87(0.03)	5.46 (0.07)
2,2,4-trimethylhexane	4.48 (0.03)	5.79 (0.06)	790.77 (0.06)	17.52(0.09)	4.83 (0.02)	4.11 (0.10)
2,2,4,4-tetramethylpentane	4.92 (0.09)	4.24 (0.08)	698.55 (0.05)	11.53(0.05)	3.14 (0.15)	2.64 (0.15)

As predicted, these n-alkanes had the highest solubility in fat followed by brain and muscle. PC of liver and lung was found to be of almost the same value. Blood to air PC of 4-ethylheptane was found to be the highest of the entire set of test chemicals. The test was repeated twice with the same result. The lung to air PC was found to be highest for 2,3-dimethylheptane. The test could not be repeated due to inavailability of tissues. The coefficient of variation for all the analysis ranged from 1 to 15 percent.

The high blood to air PC for 4-ethylheptane caused the reduction in the tissue to blood PC as shown in Table 2. The trend of reduction in the tissue to blood PC for the chemicals as per the octanol:water PC (table 3) was seen in most lipophilic tissue (fat and brain) except for 4-ethylheptane because of its higher blood to air PC. The octanol:water PC used was from available literature and calculated using online Marvin software (http://www.chemaxon.com/marvin/sketch/index.jsp).

Generally speaking, the PC of the chemical in all the tissues decreased with the increased branching. That is to say the isomer with the highest branch 2,2,4,4-tetramethylpentane had the lowest PC in all the tissues followed by 2,2,4- trimethylhexane, with the exception of blood to air PC for 4-ethyheptane as mentioned above. The partition coefficient of the alkane with the highest octanol:water value had the highest value.

Table 2

n-Alkane tissue:blood partition coefficients (mean):

Chemical/PC (SE)	Liver/Blood	Fat/Blood	Brain/Blood	Lung/Blood	Muscle/Blood
Nonane	1.5	209.81	3.08	1.23	0.7
3-methyloctane	1.41	188.19	2.99	1.37	0.86
4-ethylheptane	0.87	143.1	1.94	0.91	0.59
2,3-dimethylheptane	1.19	181.92	2.63	1.63	0.6
2,2,4-trimethylhexane	0.78	136.41	2.76	0.83	0.71
2,2,4,4-tetramethylpentane	1.16	164.6	2.71	0.74	0.62

Table 3

Octanol:water PC from literature and calculated online

Chemical/PC (SE)	Octanol/Water PC Clogp (software program)[1]	Octanol/Water PC Chemaxon (Online computation)[2]
Nonane	5.65	4.46
3-methyloctane	5.32	4.31
4-ethylheptane	5.32	4.31
2,3-dimethylheptane	5.20	4.15
2,2,4-trimethylhexane	5.06	4.01
2,2,4,4-tetramethylpentane	Not available	3.86

[1] Gustafson, J.B., Tell, J.G., Orem, D. (1997) Selection of Representative TPH Fractions Based on Fate and Transport Considerations. Total Petroleum Hydrocarbon Criteria Working Group Series. Volume 3.

[2] http://www.chemaxon.com/marvin/sketch/index.jsp

Scheffe's test was used to see if the PC of the n-alkane and its isomers with the same carbon number were statistically different. The blood to air and tissue to air PC were tested at 95% significance level. The significance test, in general showed that the straight chain and the isomer with a single branch were significantly different as compared to highly branched isomers in both the tissues and blood (Table 4 – Table 9).

Table 4

Blood:Air PC significance test for test chemicals:

Chemical	Nonane	3-methyloctane	4-ethylheptane	2,3-dimethylheptane	2,2,4-trimethylhexane	2,2,4,4-tetramethylpentane
Nonane		0.5205	0.0026	0.0675	<.0001	<.0001
3-methyloctane	0.5205		<.0001	0.8299	0.0027	<.0001
4-ethylheptane	0.0026	<.0001		<.0001	<.0001	<.0001
2,3-dimethylheptane	0.0675	0.8299	<.0001		0.0419	<.0001
2,2,4-trimethylhexane	<.0001	0.0027	<.0001	0.0419		0.0931
2,2,4,4-tetramethylpentane	<.0001	<.0001	<.0001	<.0001	0.0931	

Table 5

Liver:Air PC significance test for test chemicals:

Chemical	Nonane	3-methyloctane	4-ethylheptane	2,3-dimethylheptane	2,2,4-trimethylhexane	2,2,4,4-tetramethylpentane
Nonane		0.1408	0.0017	0.0009	<.0001	<.0001
3-methyloctane	0.1408		0.4774	0.2606	<0.0001	<.0001
4-ethylheptane	0.0017	0.4774		0.9943	<.0001	<.0001
2,3-dimethylheptane	0.0009	0.2606	0.9943		<.0001	<.0001
2,2,4-trimethylhexane	<.0001	<0.0001	<.0001	<.0001		0.9977
2,2,4,4-tetramethylpentane	<.0001	<.0001	<.0001	<.0001	0.9977	

Table 6

Fat:Air PC significance test for test chemicals:

Chemical	Nonane	3-methyloctane	4-ethylheptane	2,3-dimethylheptane	2,2,4-trimethylhexane	2,2,4,4-tetramethylpentane
Nonane		<.0001	<.0001	<.0001	<.0001	<.0001
3-methyloctane	<.0001		0.0258	0.8632	<.0001	<.0001
4-ethylheptane	<.0001	0.8632		0.0029	<.0001	<.0001
2,3-dimethylheptane	<.0001	0.8632	0.0029		<.0001	<.0001
2,2,4-trimethylhexane	<.0001	<0.0001	<.0001	<.0001		0.5658
2,2,4,4-tetramethylpentane	<.0001	<.0001	<.0001	<.0001	0.5658	

Table 7

Brain:Air PC significance test for test chemicals:

Chemical	Nonane	3-methyloctane	4-ethylheptane	2,3-dimethylheptane	2,2,4-trimethylhexane	2,2,4,4-tetramethylpentane
Nonane		0.0904	0.0108	0.0005	<.0001	<.0001
3-methyloctane	0.0904		0.9153	0.2031	<.0001	<.0001
4-ethylheptane	0.0108	0.9153		0.7355	0.0003	<.0001
2,3-dimethylheptane	0.0005	0.2031	0.7355		0.0061	<.0001
2,2,4-trimethylhexane	<.0001	<0.0001	0.0003	0.0061		0.0133
2,2,4,4-tetramethylpentane	<.0001	<.0001	<.0001	<.0001	0.0133	

Table 8

Lung:Air PC significance test for test chemicals:

Chemical	Nonane	3-methyloctane	4-ethylheptane	2,3-dimethylheptane	2,2,4-trimethylhexane	2,2,4,4-tetramethylpentane
Nonane		0.9998	0.9318	0.1398	<.0001	<.0001
3-methyloctane	0.9998		0.9870	0.0813	<.0001	<.0001
4-ethylheptane	0.9318	0.9870		0.0087	<.0001	<.0001
2,3-dimethylheptane	0.1398	0.0813	0.0087		<.0001	<.0001
2,2,4-trimethylhexane	<.0001	<.0001	<.0001	<0.0001		0.2116
2,2,4,4-tetramethylpentane	<.0001	<.0001	<.0001	<.0001	0.2116	

Table 9

Muscle:Air PC significance test for test chemicals:

Chemical	Nonane	3-methyloctane	4-ethylheptane	2,3-dimethylheptane	2,2,4-trimethylhexane	2,2,4,4-tetramethylpentane
Nonane		0.8376	0.8835	0.0105	<.0001	<.0001
3-methyloctane	0.8367		1.00	0.0007	<.0001	<.0001
4-ethylheptane	0.8835	1.00		0.0009	<.0001	<.0001
2,3-dimethylheptane	0.0105	0.0007	0.0009		0.0145	<.0001
2,2,4-trimethylhexane	<.0001	<.0001	<.0001	0.0145		0.0398
2,2,4,4-tetramethylpentane	<.0001	<.0001	<.0001	<.0001	0.0398	

The computed octanol:water PC (logP) for n-nonane and its five isomers were each fit to the mean measured partition coefficient using simple linear regression (Fig 1) which follows the equation $y = mx + c$ where m is the slope and c the intercept (Table 10) by Sigmaplot.

Table 10

Regression parameter estimates and goodness-of-fit:

PC	Parameters		Goodness of fit (R^2)
	Intercept	Slope	
Blood:Air	0.0003	3.2456	0.9349
Brain:Air	0.0009	8.0440	0.9887
Fat:Air	0.0732	273.1030	0.9756
Liver:Air	0.0005	0.9793	0.9767
Lung:Air	0.0005	2.540	0.8866
Muscle:Air	0.0002	1.840	0.9598

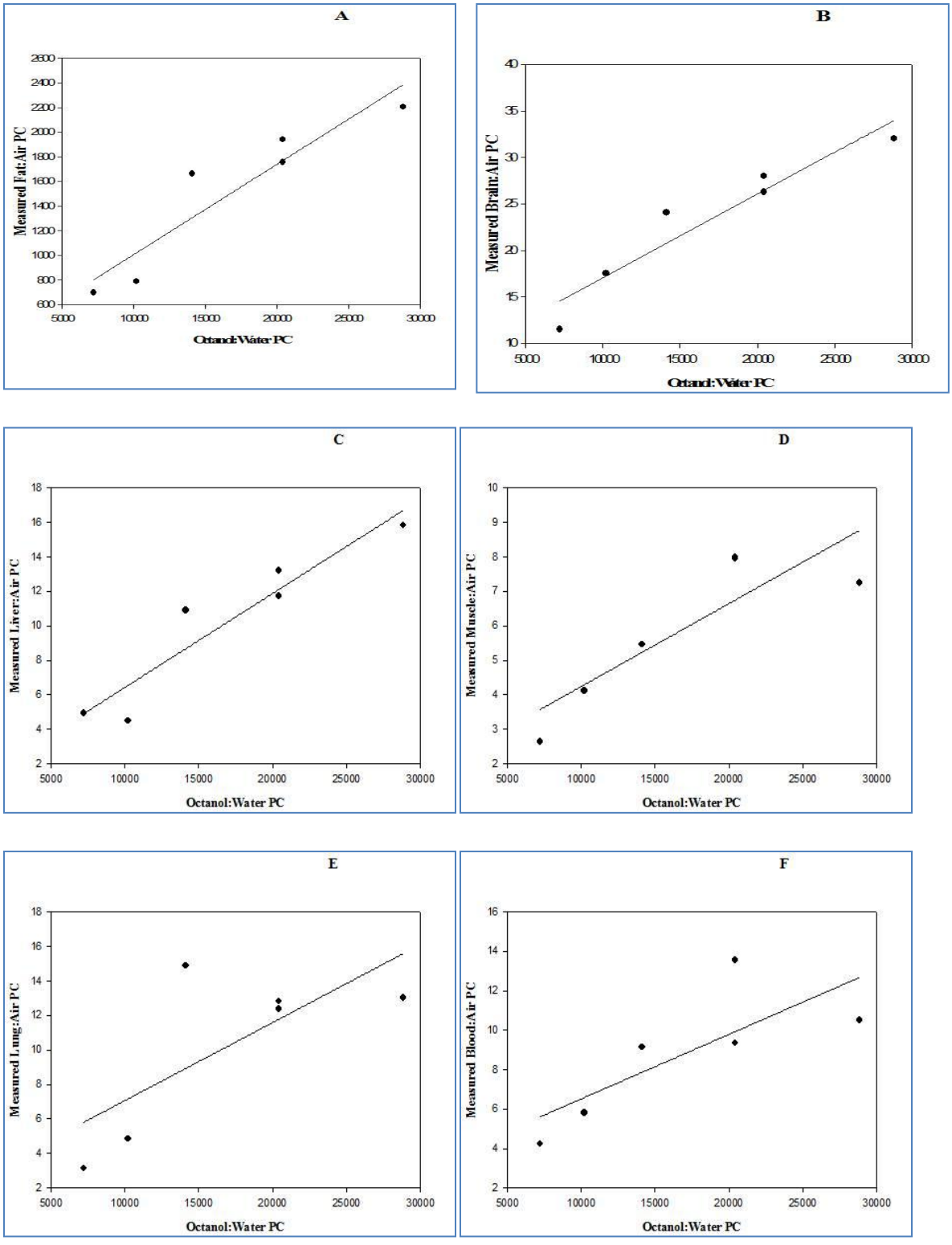

FIGURE 2: Graphs depicting regression used to predict (A) fat:air. (B) brain:air. (C) liver:air (D) muscle:air (E) lung:air and (F) blood:air PC values for n-nonane and five isomers using measured PC and computed Octanol:Water PC (table 3).

2.4. Discussion

Vial equilibration technique described by Sato and Nakajima (Sato and Nakajima, 1979) and later modified by Gargas (Gargas et al., 1989) was successfully used for the determination of partition coefficient for n-nonane and its isomers. While few other methods exist (Basak et al., 2006, Basak et al., 2003, Poulin and Krishnan, 1995) vial equilibration head space method has been the most widely used for volatile organic compounds (Gargas et al., 1989, Sato and Nakajima, 1979, Smith et al., 2005).

The only reported experimental value from head space vial method present in the literature is for nonane (Robinson, 2000; Smith *et al.,* 2005). No reported studies on the isomers tissue:air and blood:airPC have been performed. Our n-nonane PC values for tissue: air was consistent with one of either Smith et al. 2005 or Robinson, 2000 except for blood: air and fat: air. Smith et al., (2005) reported nonane PC for blood: air, liver: air, fat: air, muscle: air and brain: air as 5.8, 11.3, 1588, 4.7, and 22.3, respectively using thawed frozen tissue and blood. Robinson, 2000 reported these values as 5.1, 6.6, 1254, 7.1, and 25.9, respectively using fresh tissue and blood from rats. Our experimentally derived PC values for the above mentioned tissues were 10.5, 15.83, 2206.14, 7.24 and 32.05 respectively. The fat tissue: air PC determined experimentally by us was higher than reported by both Robinson and Smith. This could be because the sampled fat may not have been representative of the fat compartment of the animal as a whole, because the fat compartment is highly heterogeneous in terms of its partition coefficient (Robinson, 2000). The blood to air PC for 4-ethylheptane was found to be the highest of all the chemicals in the test. The test was repeated with the same result. The highest blood to air PC caused the reduction in the tissue to blood value for all the tissues. The PC values for both blood and tissues were found to follow the octanol:water coefficient value for most of the chemicals, which is a requisite

to determine the toxicity of any chemical and understanding the pharmacokinetic and pharmaco-distributive properties of highly toxic compounds (Czerwinski et al., 2006).

Gargas et al, (1988) found that based on the lipohilicity and hydrophilicity of the tissue the relative rank of increasing lipid character and decreasing aqueous character based on coefficients is Pblood < Pmuscle < Pliver < Pfat. Our results showed the same trend except for muscle and blood where we had the muscle value a bit lower than the blood, consistent with Robinson PC. We also had brain and lung coefficient values where the lung behaved similar to liver and brain PC, far below fat but more lipophilic than liver. The straight chain alkane n-nonane had the highest PC in most cases and the trend was clearly observed in most lipophilic tissue fat and brain.

Previous studies of the metabolism of n-alkane based on carbon number (Anand et al., 2007) showed decrease in metabolism with increase in carbon number, but no studies have been reported on the isomers of the same alkane. However a bio-concentration study conducted on n-dodecane and one of its isomer 2,2,4,6,6-pentamethylheptane on fat head minnows showed the n-alkane concentration to be higher than the isomer (Tolls and van Dijk, 2002). Smith et al. (2005) reported PC to be dependent on the number of carbon atoms, i.e. the lipophilicity of the chemical increases as it goes from chemical with lower to higher carbon, but the present study shows the lipophilicity to depend not only on the number of the carbon atom but also the structure of the chemical. The partition coefficient decreases as we go from straight chain alkane to the highly branched isomers and so may the metabolism and bio-concentration.

A mixture PBPK model for JP-8 is under development incorporating the n-alkane, aromatic and its isomers to better understand the toxicity effect of the jet fuel. Thus far PBPK models have

been developed for n-alkane and aromatics and no model has incorporated isomers. Incorporation of isomers in the model development will better help to understand the dosimetry toxicity of JP-8.

2.5. Conclusion

PCs for nonane and its five isomers selected based on octanol:water value for five tissues (liver, brain, fat, muscle and lungs) and whole blood were produced from the study. The n-alkanes octanol:water PC (logP) ranged from 4.46 for n-nonane, a straight chain alkane to 3.86 for 2,2,4,4-tetramethylpentane, the most branched alkane. The findings show that the PC value decreases with the increasing branching of the chemical. That is, the partition coefficient of the alkane with the highest octanol:water value had the highest value. As predicted, these n-alkanes had the highest solubility in fat, followed by brain and muscle the least. PC of liver and lung was found to be of almost the same value. Blood to air PC of 4-ethylheptane was found to be the highest of the entire set of test chemicals. The coefficient of variation for all the analysis ranged from 1 to 15 percent.

Experimental values from the head space vial method present in the literature is limited to nonane (Robinson, 2000; Smith *et al.,* 2005). No reported studies on the isomers tissue: air and blood: air partition coefficient has been done. Our n-nonane PC values for tissue: air was quite consistent with one of either smith et al. 2005 or Robinson, 2000 except for blood: air and fat: air. Smith et al., 2005 reported nonane PC for blood: air, liver: air, fat: air, muscle: air and brain: air as 5.8, 11.3, 1588, 4.7, and 22.3, respectively using thawed frozen tissue and blood. Robinson, 2000 reported these values as 5.1, 6.6, 1254, 7.1, and 25.9, respectively using fresh tissue and blood from rats. Our experimentally derived PC values for the above mentioned tissues were 10.5, 15.83, 2206.14, 7.24 and 32.05 respectively.

Scheffe's test was used to see if the PC of the n-alkane and its isomers with the same carbon number were statistically different. The blood to air and tissue to air PC were tested at 95% significance level. The significance test, in general showed that the straight chain and the isomer with a single branch were significantly different as compared to highly branched isomers in both the tissues and blood.

The vial equilibration technique was successfully used to determine the partition coefficient. As an alternative to the vial equilibrium method one possible way would be to derive the regression equation based on the finding, which could latter be used to determine the PC of the remaining chemical. We used the Sigma plot to fit the mean measured partition coefficient using simple linear regression which follows the equation $y = mx + c$ where m is the slope and c the intercept. The regression equation derived from on the basis of octanol:water PC and the experimentally derived PC for tissues and blood can be used for determining the PC of other isomers of nonane.

This study is an important step in understanding the pharmacokinetic behavior of n-alkane and its isomers. These PCs provide valuable insight into the future development of PBPK models for JP-8, which constitute several hundred alipathic and aromatic chemicals along with their isomers. Development of this PBPK model will then ultimately help in the risk assessment of JP-8 exposure.

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APPENDIX A

PARTITION COEFFICIENT DETAIL METHODS

Bag preparation

1) 80 % of 3 L tedlar bag is filled with a filtered room air using a whatman filter (charcoal filter)
2) Known volume of the chemical is added to the sampling bag using the following equation:

$$\text{Vol (ml)} = \frac{\text{ppm x Volume of air in bag x molecular weight}}{\text{Specific gravity x Molar Volume x } 10^6}$$

For e.g. for Nonane to make a bag of 3000 ppm the volume of the chemical to be added is calculated as:

PPM = 3000 ppm, Sp. Gravity of the nonane = 0.72, Molecular weight of noanane = 128,

Volume of air in bag = 2.4 L, Molar volume = 24.45 L at 25^{o}C and 760 mm Hg.

Thus Vol (ml) = (3000*2.4*128)/(24.45*10^6) = 0.052 ml = 0.052*1000 = 52 ul.

For the isomers all the other parameters except the specific gravity is same.

3) Heat the bag gently using the heat gun until the chemical is completely vaporized.
4) Let the bag cool before adding the vapor to the test vials.

Tissue Preparation

1) Take out the tissue from the -80^{o}C freezer and allow it to thaw in water bath for about 15-20 minutes
2) Remove the connecting tissues using the scalpel and scissors.
3) Mince the tissue into small pieces
4) Place tissue in the pre weighted vials (volume of tissue are: liver = 0.5g, brain = 0.1g, fat = 0.05g, lung = 0.4g, muscle = 0.75g and whole blood = 0.75ml)
5) Cap the vials firmly using the aluminum caps and Teflon lined butyl rubber septa

Vial Treatment

1) Vials were placed on the vortex evaporator at 37^{o}C for 15-20 minutes
2) Syringes were checked to see if there were no impurities by injecting the room air to the Gas Chromatograph (GC).
3) After 15-20 minutes the pressure in each vials were released using the syringe with no plunger in it.
4) After the pressure is released in each vial, 1 ml of air is removed from all the vials in the vortex evaporator.
5) 1 ml of air from the sampling bag is then added to the vials.
6) Vials are then allowed to stand in the vortex evaporator under moderate shaking until it has reached the equilibrium time (equilibrium time for fat and brain was found to be 4 hours and for other tissues liver, muscle, lung and whole blood as 3 hours)

GC Analysis

1) 0.5 ml of the headspace air from each tissue and reference vials was injected into Agilent 6890 GC for analysis.
2) The area for the tissue and reference vials is noted down in order to calculate the Partition coefficient using the following equation:

$$P_i = \frac{C_{ref}\ (V_{vial}) - C_i\ (V_{vial} - V_i)}{C_i\ V_i}$$

Where, P_i = partition coefficient. C_i = concentration of n-alkane vapor contained in headspace of reference vial, V_{vial} = volume of reference vial (11.3 ml headspace vial). C_i = concentration of n-alkane in headspace of test vial, and V_i =: volume of tissue/blood in test vial.

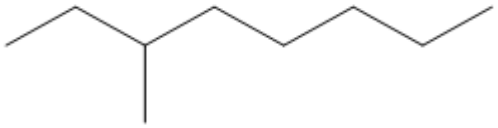
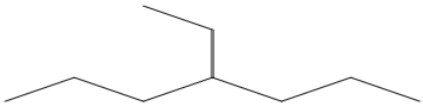
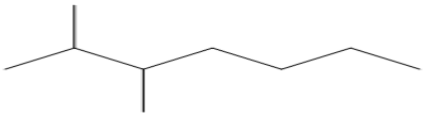
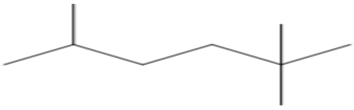
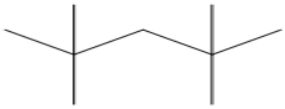
APPENDIX B

PREDICTED BLOOD TO AIR AND TISSUE TO AIR PARTITION COEFFICIENT FOR N-NONANE AND ITS ISOMERS

ISOMERS NONANE	Blood:Air	Fat:Air	Brain:Air	Liver:Air	Lung:Air	Muscle:Air
Nonane	11.90	2384.21	34.00	15.40	16.96	7.61
2-methyloctane	9.37	1767.66	26.42	11.19	12.75	5.92
3-methyloctane	9.37	1767.66	26.42	11.19	12.75	5.92
4-methyloctane	9.37	1767.66	26.42	11.19	12.75	5.92
2,2-dimethylheptane	7.58	1331.16	21.05	8.21	9.77	4.73
2,3-dimethylheptane	7.48	1307.08	20.76	8.04	9.60	4.67
2,4-dimethylheptane	7.48	1307.08	20.76	8.04	9.60	4.67
2,5-dimethylheptane	7.48	1307.08	20.76	8.04	9.60	4.67
2,6-dimethylheptane	7.48	1307.08	20.76	8.04	9.60	4.67
3,3-dimethylheptane	7.58	1331.16	21.05	8.21	9.77	4.73
3,4-dimethylheptane	7.48	1307.08	20.76	8.04	9.60	4.67
3,5-dimethylheptane	7.48	1307.08	20.76	8.04	9.60	4.67
4,4-dimethylheptane	7.58	1331.16	21.05	8.21	9.77	4.73
3-ethylheptane	9.37	1767.66	26.42	11.19	12.75	5.92
4-ethylheptane	9.37	1767.66	26.42	11.19	12.75	5.92
2,2,3-trimethylhexane	6.32	1022.15	17.25	6.10	7.66	3.89
2,2,4-trimethylhexane	6.32	1022.15	17.25	6.10	7.66	3.89
2,2,5-trimethylhexane	6.32	1022.15	17.25	6.10	7.66	3.89
2,3,3-trimethylhexane	6.32	1022.15	17.25	6.10	7.66	3.89
2,3,4-trimethylhexane	6.32	1022.15	17.25	6.10	7.66	3.89
2,3,5-trimethylhexane	6.18	988.44	16.84	5.87	7.43	3.79
2,4,4-trimethylhexane	6.32	1022.15	17.25	6.10	7.66	3.89
3,3,4-trimethylhexane	6.32	1022.15	17.25	6.10	7.66	3.89
2-methyl-3-ethylhexane	7.48	1307.08	20.76	8.04	9.60	4.67
2-methyl-4-ethylhexane	7.48	1307.08	20.76	8.04	9.60	4.67
3-methyl-3-ethylhexane	7.58	1331.16	21.05	8.21	9.77	4.73
3-methyl-4-ethylhexane	7.48	1307.08	20.76	8.04	9.60	4.67
2,2,3,3-tetramethylpentane	5.42	803.39	14.56	4.60	6.16	3.29
2,2,3,4-tetramethylpentane	5.37	791.32	14.42	4.52	6.08	3.26
2,2,4,4-tetramethylpentane	5.42	803.39	14.56	4.60	6.16	3.29
2,3,3,4-tetramethylpentane	5.37	791.32	14.42	4.52	6.08	3.26
2,2-dimethyl-3-hylpentane	6.32	1022.15	17.25	6.10	7.66	3.89
2,3-dimethyl-3-hylpentane	6.32	1022.15	17.25	6.10	7.66	3.89
2,4-dimethyl-3-ethylpentane	6.16	984.78	16.79	5.84	7.40	3.78
3,3-diethylpentane	7.58	1331.16	21.05	8.21	9.77	4.73

APPENDIX C

STRUCTURE OF TEST CHEMICALS

Chemical	Structure
nonane	
3-methyloctane	
4-ethylheptane	
2,3-dimethylheptane	
2,2,4-trimethylhexane	
2,2,4,4-tetramethylpentane	

APPENDIX D

LogP of NONANE AND ITS ISOMERS

ISOMERS NONANE	LogP
Nonane	4.46
2-methyloctane	4.31
3-methyloctane	4.31
4-methyloctane	4.31
2,2-dimethylheptane	4.16
2,3-dimethylheptane	4.15
2,4-dimethylheptane	4.15
2,5-dimethylheptane	4.15
2,6-dimethylheptane	4.15
3,3-dimethylheptane	4.16
3,4-dimethylheptane	4.15
3,5-dimethylheptane	4.15
4,4-dimethylheptane	4.16
3-ethylheptane	4.31
4-ethylheptane	4.31
2,2,3-trimethylhexane	4.01
2,2,4-trimethylhexane	4.01
2,2,5-trimethylhexane	4.01
2,3,3-trimethylhexane	4.01
2,3,4-trimethylhexane	4.01
2,3,5-trimethylhexane	3.99
2,4,4-trimethylhexane	4.01
3,3,4-trimethylhexane	4.01
2-methyl-3-ethylhexane	4.15
2-methyl-4-ethylhexane	4.15
3-methyl-3-ethylhexane	4.16
3-methyl-4-ethylhexane	4.15
2,2,3,3-tetramethylpentane	3.86
2,2,3,4-tetramethylpentane	3.85
2,2,4,4-tetramethylpentane	3.86
2,3,3,4-tetramethylpentane	3.85
2,2-dimethyl-3-ethylpentane	4.01
2,3-dimethyl-3-ethylpentane	4.01
2,4-dimethyl-3-ethylpentane	3.99
3,3-diethylpentane	4.16