DETERMINATION OF PARTITION COEFFICIENTS FOR SELECTED N-ALKANES AND OTHER KEY CONSTITUENTS OF JP-8

by

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(Under the Direction of Jeff Fisher)

ABSTRACT

JP-8, the primary aviation fuel source used by the U.S. and NATO forces, is comprised of a complex mixture of aliphatic and aromatic hydrocarbons. Exposure occurs primarily via inhalation and dermal contact. A complex mixture PBPK model for JP-8 is in development to aid in understanding target tissue dosimetry. N-alkanes, n-octane through n-dodecane (C_8-C_{12}) comprise a large majority of neat JP-8 (by weight). As part of model development efforts, tissue:air and blood:air partition coefficients for C_8-C_{12} n-alkanes, and other key constituents of JP-8 were determined. Rat tissue:air partition coefficients for liver, muscle, brain, fat, and whole blood were determined using the vial equilibration method modified by Gargas et al. (1989). The results indicate increasing partitioning into tissues with increasing carbon chain length of n-alkanes. These reported tissue solubility results represent an important step in the future development of a PBPK model for JP-8.

INDEX WORDS: JP-8, Partition Coefficients, n-alkanes, vial-equilibration method

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B.S., University of Georgia, 1994

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2004

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DEDICATION

I dedicate this work to my wife and children. Allyson thanks for six great years of marriage and two wonderful children. Thank you for your daily sacrifices, support and love you bring to our lives. My children, Emma Kate and Quin, thank you for being my never-ending inspiration. Thanks to the Lord for blessing my life, my family and giving me the strength to achieve my goals.

ACKNOWLEDGEMENTS

This research was funded by the Air Force Office of Scientific Research, grant # F49620-03-1-0157. I would like to express my sincere thanks to my major professor, Dr. Jeff Fisher for his continuous insight and guidance during the past two years. I would also like to thank, my graduate committee members, Dr. Mary Alice Smith and Dr. Hisham El-Masri for there input and expertise during this process. Additional thanks to Jerry Campbell for his constant assistance and troubleshooting in the lab. Finally I would like to thank Reiko Perleberg, Tara Almekinder, and John Swint for their assistance in the laboratory.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Background of JP-8

Jet Propellant –8 (JP-8), is essentially commercial aviation fuel (Jet A), with a military additives package for increased performance. Developed in the early 1960's JP-8 is the primary aviation fuel used by the U.S. military today. The U.S. Air Force began the transition from JP-4 to JP-8 at its bases in Great Britain in 1979 and completed the transition at all its U.S. bases in 1995. JP-8 is the current aviation fuel of choice of the United States and other North Atlantic Treaty Organization (NATO) countries. It is a kerosene-based fuel with a military specifications additives package. JP-8 differs from commercial jet fuel, Jet A, by the addition of a fuel system icing inhibitor, a corrosion inhibitor, static dissipators and a lubricity additive (Ritchie, 2003, and Maurice et al., 2000). JP-8 represents the largest single chemical exposure in the U.S. military (Ritchie et al. 2003). JP-8 replaced its predecessor JP-4, due to enhanced chemicophysical properties that increase aircraft survivability, imparted added safety, and simplified battlefield logistics (Keil et al., 2003 and Smith et al., 1997).

JP-8 Composition

JP-8 is a mixture of literally thousands of hydrocarbons, divided into three broad classes – aromatics (20%) branched and straight chained alkanes (60%), and cycloalkanes (20%) (Maurice et al., 2001). JP-8 is produced under performance specifications and is not based on

chemical composition; therefore each batch of JP-8 contains slightly different concentrations of individual components. As a kerosene-based fuel JP-8 is comprised primarily of hydrocarbon compounds with chain lengths ranging from 9 to 16 carbons. The C_9-C_{14} n-alkanes constitute approximately 28% of the bulk fuel (Pleil et al., 2000). More specifically, the C_9-C_{12} n-alkanes are known to exist in higher concentrations in breath samples than other well-known constituents of JP-8 such as benzene, toluene, naphthalene, and xylenes (Carlton and Smith, 2000, Pleil et al., 2000).

JP-8 Exposure

JP-8 exposure occurs primarily from dermal contact and inhalation of aerosols and vapors (Drake et al., 2003). Military personnel are most likely to be exposed via job related activities. Personnel most likely to be exposed include aircraft fuel system workers, bulk fuel handlers, mechanics, avionics personnel and aircrew. Additional exposure can occur to civilians and military personnel not working in close proximity, via exhaust emitted during aircraft taxiing, take-offs and landings.

The current interim 8-hr time weighted average (TWA), set by the Department of Defense is 350 mg/m^3 and the 15-minute short-term exposure limit is 1800 mg/m^3. JP-8 vapor and aerosol have been measured at concentrations well above these levels (Carlton and Smith, 2000). Recently the National Research Council's, Committee on Toxicology, Subcommittee on Jet-Propulsion Fuel-8 has determined the interim PEL of 350 mg/m^3 may not be protective of human health (NRC, 2003). The U.S. Air Force has recently reduced the 8-hr TWA of JP-8 to 200 mg/m^3 based largely upon the findings of the National Research Council.

Two recent inhalation studies examined the level of JP-8 exposure at U.S. Air Force bases. In the first study, fuel tank repair work at U.S. Air Force bases was examined. Fuel tank studies were conducted comparing ambient air concentrations of JP-8 from tanks with and without explosion-suppression foam insulation. It was found that purging of the non-foam fuel tanks was an effective means of reducing exposures, where as, tanks containing foam actually had increased levels of JP-8 vapor due to volatilization of fuel retained in the foam (Carlton and Smith, 2000). The highest eight-hour TWA fuel exposure measured from tanks containing foam was 1304 mg/m^3; and the highest 15 minute short-term exposure was 10,295 mg/m^3 (Carlton and Smith, 2000). These values are roughly 4 times the 8-hr TWA, and 5 times the 15-minute short-term exposure limit. These values were taken inside the fuel tanks by workers wearing respiratory protection. The fuel tank attendant personnel outside the tank, assisting personnel inside tanks, did not wear respiratory protection, however no breath measurements were taken from this group.

The second study by Pleil and Smith (2000) found that Air Force personnel, especially fuel system workers are chronically exposed to JP-8 performing their daily work activities. C_9-C_{12}, n-alkanes were identified as fingerprint compounds for JP-8 inhalation exposure in this study. Ambient air and breath samples taken from fuel tank attendant personnel where found to contain 65, 1824, 612, 159, 70 and 5, 73, 84, 17, and 6 ppb octane, nonane, decane, undecane, and dodecane respectively. Breath samples are much more useful than ambient air concentrations because there is a direct relationship with blood levels (Pleil et al., 2000).

Limited research is available on JP-8 dermal exposure in humans. The most significant source of dermal exposure occurs during fuel tank entry by personnel wearing cotton-cloth coverall garments. The only human dermal exposure study located was with female workers at a

ball-bearing factory exposed to kerosene daily. Symptoms include itching or burning of skin, skin redness or rash, skin dryness or dermatitis or skin sensitization (Ritchie et al., 2003). Animal studies have produced symptoms including mild skin irritation, inflammation, necrosis, and tumorogenisis (Kinkead et al.,1992, McDougal et al., 2000, NRC, 2003) and have shown evidence of systemic effects to the immune system (Ramos et al., 2002).

JP-8 Toxicity

Inhalation is often identified as the primary means of exposure to JP-8. Dermal absorption has recently gained attention due to a lack of dermal exposure studies and the fact that, exposure guidelines currently do not exist for this route of exposure. JP-8 is less volatile than its predecessor JP-4, which may reduce the inhalation exposure, while dermal exposure may actually increase. Among the most common health complaints received from fuel workers exposed to JP-8 are itching or burning of skin, skin redness or rash, skin dryness or dermatitis, skin lesions or weeping, or skin sensitization (Ritchie et al., 2003).

Few JP-8 dermal toxicity studies in animals have been conducted in recent years. Rabbits exposed to a single dermal application of 0.5 ml of neat JP-5, similar aviation fuel currently used aboard U.S. Navy ships, showed no signs of skin irritation (NRC, 2003, Ritchie et al., 2003). However, Kinkead et al. (1992) found mild skin irritation in rabbits, which received a single dermal application of JP-8. Ritchie et al. (2003) found that studies with kerosene based fuels, similar to JP-8, without performance additives, increased the incidence of skin cancer in mice treated dermally for $\leq$ 24 months. While JP-8 is characterized as a non-carcinogen, studies suggest it as a possible skin tumor promoter. (Ritchie et al., 2003) .

The respiratory system and the lung are most susceptible to inhaled JP-8 aerosol and vapor. Mattie et al. (1991) exposed male F344 rats and C57Bl/6 mice of both sexes to JP-8 vapor at 0, 500, or 1000 mg/m^3 on a continuous basis for 90 d followed by a 24-month recovery period. No pulmonary lesions or other significant histopathology were reported in this study. Also, Mattie et al. (1995) reported no significant histopathological changes in the lungs or nasal turbinates of male rats administered up to 3g/kg/d JP-8 by oral gavage once daily for 90 days. Pfaff et al. (1995) found that rats exposed to JP-8 vapor and aerosol had decreased body weights as compared to controls and increased pulmonary clearance rates suggesting possible disruption of epithelial cell integrity, which may permit increased access of inhaled toxins, such as JP-8 to the lung interstitium. Significant pulmonary toxicity in mice was demonstrated in rodents exposed to JP-8 vapor/aerosol concentrations as low as 50 mg/m^3 for as little as 1h/d for 7 d (Robledo and Witten, 1998). Lung epithelial cell apoptosis has been identified as a result of 1-h/day nose-only 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 (Pfaff et al., 1996). Morphological lung injury has been identified at doses of 50 mg/m^3 (Robledo et al., 2000). Robledo et al. (2000) found that exposure to levels greater than 26 mg/m^3 resulted in alterations to type II epithelium cells as well as the appearance of pulmonary edema and/or interalveolar hemorrhaging in B6.A.D. mice. A 250 mg/m^3 JP-8 exposure level resulted in the alteration of 41 proteins in whole lung samples (Drake et al., 2003). One of these protein deficiencies, α1-anti-trypsin (AAT), is one of the primary risk factors that contribute to chronic obstructive pulmonary disorder (COPD) and pulmonary emphysema (Drake et al., 2003).

Studies also identify the immune system as susceptible to insult from JP-8 exposure. Mattie et al. (1995) reported significant increases spleen/body weight ratio in male Sprague-

Dawley rats administered 3 g/kg/d net JP-8 by oral gavage for 90 d. Exposure to JP-8 vapor and aerosol concentrations of 1000 mg/m^3 for one-hour a day for seven days were found to cause decreases in thymus and spleen weights and a reduction in viable cell numbers that continued to decline with additional days of exposure (Harris et al., 2002). These short-term exposures resulted in long-lasting effects, which did not return to normal baseline levels until one month post-exposure. Dudley et al. (2001) reported that oral gavage exposure of mice to 2g/kg/d JP-8 for 7 d resulted in significant decreases in thymus weight and cellularity. Harris et al. (2000) found that female mice exposed to 1 g/m^3 JP-8 aerosol and vapor for 1h/d for 7 d had significantly deficiencies in Natural killer (NK) and lymphokine activated killer (LAK) cell activity. NK cells are known to be involved in immune surveillance against newly developed malignancies, in defense against viral infections, and in control of immune B cell function (Ritchie et al., 2003).

Carlton and Smith (2000) describe the CNS as the major target for toxicity of JP-8**.** Smith et al. (1997) report the CNS is the primary target of toxicity after acute inhalation. Symptoms include headaches, dizziness, nausea, fatigue, staggered gait, postural balance, and mental confusion (Smith et al., 1997, Carlton and Smith, 2000, ATSDR, 2003, Ritchie et al, 2003). One actual case is documented in which Navy pilots exposed to an in-flight fuel leak of JP-5 into the cock-pit were reported to have experienced nausea, vomiting, incoordination/impairment of hand-eye coordination, anorexia, euphoria and laughing, and memory impairment (Ritchie et al., 2003). Repeated JP-8 exposure to 1 g/m^3 reduced the capacity of rats to learn highly difficult operant tasks, compared to lower dose 500 mg/m^3 JP-8 or control exposures (Ritchie et al., 2003).

N-alkanes

JP-8 is comprised of hundreds or even thousands of individual chemical isomers. It is impractical and expensive to test and understand the toxicity of each individual component. Therefore, a more practical means of understanding JP-8 toxicity, specifically inhalation toxicity, is to attempt to understand classes or groups of chemicals that make up significant amounts of a complex mixture such as JP-8 and are present in large quantities within breath samples. These classes, for potential toxicity, are the only practical short-term approach for understanding JP-8 toxicity.

N-alkanes represent the largest percentage of any single class of chemicals in JP-8. The C_7-C_{18} n-alkanes account for ~20 % by weight of total JP-8 (Potter and Simmons, 1998). The C_8-C_{12} n-alkanes account for ~10 % by weight of total JP-8, and were summed to provide a simple indicator of JP-8 vapor exposures (Pleil et al., 2000, Potter and Simmons, 1998). Due to their relative volatility C_9-C_{12} n-alkanes have been found in high concentrations both in ambient air samples and breath measurements. The presence of C_9-C_{12} n-alkanes in vapor samples taken during inhalation studies suggests that these n-alkanes dominate the vapor phase of JP-8 (Pleil et al., 2000).

Limited but increasing research has been conducted on individual or groups of n-alkanes. N-alkanes, especially nonane through tridecane (C_9-C_{13}) have been chosen for recent dermal penetration and absorption studies. McDougal et al. (2000) identified C_9-C_{13} as n-alkanes capable of passing through F344 rat skin samples. However, the concentrations were so small that contact of the hands with JP-8 for 8 hours would be expected to give a body burden of about 4 orders of magnitude less than the body burden at the inhalation exposure limit (McDougal et al. 2000). Singh and Singh (2003) found that tridecane exhibited greater permeability through

pig ear skin among n-alkanes, suggesting it as a possible systemic toxicant. Allen et al. (2001) found that undecane, dodecane, tridecane and hexadecane induced a proinflamatory cytokine, interleukin-8 (IL-8) release, and thus may be the inciting agent for irritation. Chou et al. (2002) also reported that dodecane caused an increased release in IL-8,- compared to the C_8-C_{16} n-alkane range tested. Chou et al. (2002) reported that acute exposures to C_6-C_{16} n-alkanes significantly increased human epidermal keratinocytes (HEK) mortality, such that the increase in cytotoxicity corresponded with the decrease in carbon chain length.

Additional Constituents

Additional constituents of JP-8 were chosen for this study based upon our inability to determine partition coefficients for n-alkanes above dodecane due to limitations associated with extremely low vapor pressures. However, additional constituents of JP-8 were of interest. N-propylcyclohexane, o-ethyltoluene, 1,2,4-Trimethylbenzene (1,2,4-TMB) and 1,3,5-Trimethylbenzene (1,3,5-TMB) were chosen as additional chemicals of interest based upon their significant presence in JP-8 vapor samples. These samples were obtained from ongoing rat inhalation studies at the University of Arizona. These compounds represent 1.6,1.1,2.9 and 1.8 % of total JP-8 peak area from gas chromatography (J. Campbell,personal communication, September, 15,2003).

Toxicity studies for these four constituents are limited. Rats exposed to 1,2,4-TMB at 123-1230 mg/m^3 showed low systemic toxicity with no changes in body weight gain or organ/body weight ratio when compared to controls, however at the highest concentration, a decrease in red blood cell and an increase in white blood cells was noted (Korsak et al., 2000). Like other hydrocarbon exposures, excessive exposure to TMB's produces neurological and

behavioral effects (Ritchie et al, 2003). No studies on toxicity were located for n-propylcyclohexane or o-ethyltoluene.

Partition Coefficients

Partition coefficients (PCs), also referred to as solubility or distribution coefficients, are a measure of the concentration of a chemical between two phases under equilibrium conditions. PC's are commonly reported as tissue:air, blood:air or tissue:blood PC values. Blood:air PC values are important determinants of the pulmonary uptake of volatile organic chemicals (Poulin and Krishnan, 1996a), such as this group of n-alkanes. Tissue:blood PC values represent an important set of input parameters for physiological based pharmacokinetic (PBPK) models (Poulin and Krishnan, 1996b). PC's are commonly assumed to be independent of concentration, and measurements are frequently conducted at a single concentration (Payne and Kenny, 2002). PC's are key chemicophysical parameters required to describe the uptake, distribution, biotransformation, and excretion of organic chemicals in biological systems (Jepson et al., 1994).

The objective of this research was to determine the PC's for n-alkanes C_8-C_{12}, and four other key constituents, which may be used for future development of a PBPK total hydrocarbon model for JP-8.

Methods of Determining Partition Coefficients

PC methods can be divided into two main groups, experimental and computational methods. Historically experimental methods have been the most popular. Experimental methods are subdivided into two types, in vivo and in vitro. In vivo methods avoid disruption of normal tissue architecture and cellular structure and may therefore provide a better representation of the

biology (Dallas et al., 1995). However, in vivo determinations are also more labor and animal intensive (Thrall et al., 2002), thus in vitro methods are more common.

For volatile organic chemicals (VOC's) the most commonly used experimental method is the vial equilibration method developed by Sato and Nakijima (1979), and later modified by Gargas et al. (1989). The vial equilibration method measures the concentration of chemical in the "headspace", area not occupied by tissue, homogenate or blood, of the test and reference vials. The difference between these concentrations is determined to be the concentration of chemical within the tissue or blood. Thrall et. al (2002) in a review of 50 published PBPK models revealed that roughly 60% of all PC values referenced were reported by Gargas et al. (1989). This method was chosen for our work based upon the success experienced with other volatile chemicals and its wide use throughout the literature. For relatively non-volatile chemicals with vapor pressures below 1 mmHg, Jepson et al. (1993) used an ultrafiltration method with homogenized tissue and blood in saline solution followed by an extraction procedure. The ultrafiltration method was applied to chemicals with a wide range of chemical structures and tissue solubilities (Jepson et al, 1994). This method may assist future n-alkane determinations above C_{12}, where the vial equilibration method is inappropriate, due to extremely low vapor pressures.

Most recently computational methods of determining PC's have become popular, primarily as a more practical and cost effective way to estimate PC's of individual components of mixtures, such as, JP-8. Computational methods for predicting PC's are largely based upon a chemical's existing n-octanol:water, vegetable oil:water, olive oil:air, saline:air, or water:air partition coefficients. Sources of computational methods for predicting PC's of VOC's are described in the works of Poulin and Krishnan (1995,1996a,1996b), DeJongh et al., (1997),

Meulenberg and Vijverberg (2000), and Basak et al., (2003). Here tissue solubility is the sum of its solubilities in neutral lipids, phospholipids, and water, while blood solubility is described as the sum of its solubilities in neutral lipids, phospholipids, and water contained within plasma and erythrocytes. Meulenberg and Vijverberg (2000) predicted PC's for VOC's as an additive function of their olive oil:air and saline:air PC's. Basak et al. (2003) developed methods of predicting PC's based upon molecular structural properties.

Previous Research

PC information for these five n-alkanes, octane through dodecane and the four additional chemicals, is extremely limited. Only blood:air PC values for octane and decane (Liu et al. 1994) and brain:air PC values for 1,2,4-TMB and 1,3,5-TMB (Meulenberg and Vijverberg, 2003) have been experimentally reported in the literature. Meulenberg and Vijverberg (2000) have reported predicted tissue:air and blood:air PC values for octane, decane, 1,2,4-TMB and 1,3,5-TMB. This limited set of data reinforces the need for experimentally determined tissue and blood:air PC's for these chemicals. PC's for the n-alkanes should prove invaluable in the development of a PBPK total hydrocarbon model for assessing potential human health risk associated with JP-8.

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CHAPTER 2

DETERMINATION OF PARTITION COEFFICIENTS FOR SELECTED N-ALKANES AND OTHER KEY CONSTITUENTS OF JP-8.[1]

[1]Smith A.Q., J.L. Campbell and J.W. Fisher. Submitted to the Journal of Toxicology and Environmental Health, Part A, 2/13/04.

Abstract

JP-8 is the primary aviation fuel source used by U.S. and NATO forces. JP-8 is comprised of a complex mixture of aliphatic and aromatic hydrocarbons. Exposure occurs primarily via inhalation and dermal contact. A complex mixture PBPK model for JP-8 is in development to aid in understanding target tissue dosimetry. N-alkanes, particularly n-octane through n-dodecane (C_8-C_{12}) comprise a large majority of neat JP-8 (by weight). As part of model development efforts, tissue:air and blood:air partition coefficients for C_8-C_{12} n-alkanes were determined. Rat tissue:air partition coefficients for liver, muscle, brain, fat, and whole blood were determined using a modified version of the vial equilibration method developed by Sato and Nakajima (1979), and later modified by Gargas et al. (1989). A tissue smear technique was used verses the traditional tissue homogenate technique. Resulting tissue:air partition coefficient values increased with number of carbon atoms for C_8-C_{12} hydrocarbons with few noted exceptions. Also, partition coefficients increased across tissues with increasing tissue lipophilicity from muscle <blood <liver <brain <fat. Tissue:air partition coefficients were determined for four additional chemicals (o-ethyltoluene, n-propylcyclohexane, 1,2,4-Trimethylbenzene, and 1,3,5-Trimethylbenzene) found in JP-8 aerosol samples. These partition coefficient values were generally higher than n-alkane values. These reported tissue solubility results represent an important step in the future development of a PBPK model for JP-8. Further research and method development may be necessary to determine partition coefficients for hydrocarbons above C_{12}.

Introduction

Jet Propellant-8 (JP-8) represents the largest single chemical exposure for U.S. military personnel (Ritchie et al. 2003), and the primary aviation fuel used by U.S. and NATO Forces. JP-8 replaced its predecessor JP-4, due to enhanced chemicophysical properties that increase aircraft survivability, imparted added safety, and simplified battlefield logistics (Keil et al., 2003 and Smith et al., 1997). JP-8 differs from commercial jet fuel, Jet A-1, by the addition of a fuel system icing inhibitor, a corrosion inhibitor, static dissipators and a lubricity additive (Ritchie, 2003, Maurice et al., 2001, ATSDR, 1998). JP-8, like JP-4, is a kerosene-based mixture of hundreds of aliphatic and aromatic hydrocarbons, with chain lengths ranging primarily from C_7-C_{18} (Potter and Simmons, 1998). Straight-chained alkanes (n-alkanes), C_9-C_{14} comprise ~ 28 % of neat JP-8 (Pleil, 2000). JP-8's physical characteristics reduce vaporization and result in increased bioavailability and human exposure (Drake et al., 2003). Inhalation studies by Pliel et al. (2000) found that breath samples of Air Force personnel contained several hydrocarbons found in JP-8, specifically n-alkanes; nonane through dodecane and aromatics; benzene, toluene, ethylbenzene, and xylenes. JP-8 exposure occurs primarily from inhalation and dermal contact. Military personnel most likely to be affected include fuel tank workers, bulk fuel handlers, aircraft refuelers, pilots, and mechanics.

Currently little human toxicological information is available for JP-8. What is known about JP-8 toxicity is from self-reported health complaints of exposed personnel. Pulmonary effects include tightness of the chest and difficult breathing. Dermal effects observed include dry skin, rashes, inflammation and dermatitis. Additionally, the central nervous system is noted as the primary target of toxicity after acute inhalation of JP-8, with reported symptoms including headaches, nausea, staggered gait, slurring of speech, and mental confusion (Smith et al., 1997).

Numerous studies in rodents have shown JP-8 vapor and aerosols cause increased respiratory edema, mild cellular necrosis, and other histopathological changes (Ritchie et al., 2003). Lung epithelial cell apoptosis and edema has been reported in rodent studies at JP-8 concentrations as low as 50 mg/m^3, which is well below the inhalation standard of 350 mg/m^3, based on an 8-hr time weighted average (Robledo et al., 2000). Other rodent studies have shown that JP-8 affects the immune, lymphatic, and hepatic systems. Results from these studies have shown altered red and white blood cell counts, decreases in spleen, thymus, and liver weights, and a significant reduction in cell recovery from spleen, thymus, lymph nodes, bone marrow and peripheral blood (Harris et al., 2002).

Our laboratory is developing a physiologically based pharmacokinetic (PBPK) model to describe the pharmacokinetics and interactions of JP-8 hydrocarbon fractions in rodents. The PBPK JP-8 hydrocarbon model will be used to evaluate the dose response characteristics of several published JP-8 toxicity studies. Many of the prominent hydrocarbons in JP-8, namely the n-alkanes (C_9-C_{14}) have not been evaluated for their tissue solubility properties. Therefore, tissue/air partition coefficient (PC) values are needed for development of the PBPK JP-8 hydrocarbon model that would include these specific hydrocarbons. In this paper we report the PC tissue/air values for several n-alkanes found in JP-8 and four aromatic hydrocarbon constituents found in JP-8 vapor from an inhalation exposure chamber. The only reported rodent PC values found in the peer reviewed literature were blood:air PC values for n-octane (7.53) and n-decane (7.3) (Liu et al., 1994).

Human and rat blood: air and tissue: air PC's are usually measured using the vial equilibration method (Poulin and Krishnan, 1996). It is estimated that 60% of all PBPK models have used PC's determined by Gargas et al. (Thrall et al., 2002). This study will expand the

partition coefficient database for future PBPK modeling efforts. A modified-version of the vial-equilibration method by Sato, A. and Nakijima (1979) and later by Gargas et al. (1986) was developed which employs the use of tissue smears rather than tissue homogenates.

Methods and Materials

Animals

Male Sprague-Dawley rats were obtained from Charles River Breeding Laboratories (Wilmington, MA). Animals were housed two per "shoe-box" style cage. Litter was changed weekly and animals had unrestricted access to PMI #5001 rodent chow (PMI feeds, St. Louis, MO) and water. Animals were kept in a humidity/climate-controlled facility with a 12-hour light/dark cycle for a minimum of 14 days prior to euthanasia. Male rats (300-500g) were euthanzied by CO_2 asphyxiation. Heparinized blood was collected via portal vein. Tissues (liver, perirenal fat, thigh muscle, whole brain) and blood were removed and stored at -20°C. Tissues were chosen based upon the development of the PBPK JP-8 hydrocarbon model.

Chemicals

Octane, nonane, decane, undecane, and dodecane (CAS #'s 111-65-9, 111-84-2, 124-18-5, 1120-21-4, and 112-40-3) respectively, were 99 %+ pure (Sigma-Aldrich, St. Louis, MO). N-Propylcyclohexane (CAS # 1678-92-8) was 97% pure (Fisher, Pittsburg, PA) and 1,2,4-Trimethylbenzene (1,2,4-TMB) (CAS # 95-63-6), and 1,3,5-Trimethylbenzene (1,3,5-TMB) (CAS # 108-67-8) were both 98% pure (Sigma-Aldrich, St. Louis, MO). O-ethyltoluene was 99% pure (CAS # 611-14-3) (Fisher, Pittsburg, PA).

Procedure

Tissues were minced and smeared upon the inside of vials, versus preparing tissue homogenates. Each experiment consisted of five reference vials and seven sample vials. Vials used were 10-ml round-bottomed headspace vials (Kimble Glass Co., Vineland, NJ). Reference vials contained 1 ml of test chemical vapor, while sample vials contained pre-determined amounts of tissue (about 1.0g muscle, 0.75ml of whole blood, 0.5g of liver, 0.1g of brain and .05g of perirenal fat) and 1 ml of test chemical vapor. Tissue and blood were allowed to thaw in a water bath at room temperature for 30 minutes prior to mincing or pipetting. Tissues were minced and then smeared along inside wall of pre-weighed vials with a stainless steel spatula. Blood was pippetted into pre-weighed vials by weight. Vials were crimp-capped with aluminum caps containing teflon-lined butyl rubber septa (National Scientific Co., Duluth, GA). Reference and sample vials were then placed on a vortex evaporator (Labconco, Kansas City, MO) and heated for 15 minutes at 37°C. Each vial was then vented to room air with a gas tight syringe (Hamilton Co., Reno, NV) and 26-gauge side port needle (without the plunger assembly). One ml of air was removed from each sealed reference and sample vial prior to adding 1 ml of air from the gas sampling bag.

Test chemical was added to each vial from a 3.0 L Tedlar gas-sampling bag (SKC, Eighty Four, PA). Sampling bags were filled at 80% capacity with filtered room air. The test chemical of interest was then injected using a gas-tight syringe with a sideport needle creating bag concentrations between 500-5000 ppm. The bag was then uniformly and gently warmed with a heat gun to assist with evaporation. Analyses of bag concentrations were made pre and post filling of vials. Vials were allowed to incubate at 37° C in a vortex evaporator with moderate shaking until at equilibrium. Vials containing blood, liver, and muscle were allowed to

incubate for 3 hours, while vials containing brain and fat tissue were incubated for 4 hours. A time to equilibrium of 6 hours was determined for fat in n-undecane and n-dodecane.

After the incubation period, 0.5 ml of headspace vapor from each reference and sample vial (all chemicals except nonane) was injected via hand-injection for analysis on the Agilent 6890 Series II gas chromatograph (GC). GC conditions were as follows: The column was a HP-5 15M x .53mm x .0015mm column with nitrogen as a carrier gas (40.0 ml/min). Hydrogen flow was 37.5 ml/min and air flow was 375 ml/min. The injector temperature was 200ºC, flame ionized detector (FID) temperature was 260ºC and isothermal oven temperatures ranged from 110° to 140°C with column retention time of two to five minutes. Nonane analysis was performed using an HP 5890 Series II GC. A HP-5 10m x .53mm x 2.65um column was used with a helium carrier gas flow of 2.27 ml/min. The hydrogen flow was 23 ml/min and airflow was 210 ml/min with a split @ 2.15 ml/min. The injector temperature was 230ºC, the FID temperature was 270ºC and the oven temperature was 140ºC.

Partition Coefficients

Partition coefficients were determined for whole blood, muscle, liver, brain, and perirenal fat. Partition coefficients were calculated according to Equation 1 found in Gargas et al. (1989).

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

Where; P_i=partition coefficient, and C_{ref}=concentration of chemical contained in headspace of reference vial, V_{vial}= volume of reference vial (10 ml headspace vial), C_i= concentration of chemical in headspace of test vial, and V_i=volume of tissue/blood in test vial.

Results

Blood:air and tissue:air partition coefficient values for octane, nonane, decane, undecane and dodecane are listed in Table 1. These n-alkanes were the least soluble in muscle and blood and the most soluble in fat. Generally speaking, the solubility of these n-alkanes in muscle, blood, liver, brain and fat increased as the number of carbon atoms increased from eight to twelve. There was an exception, the brain:air PC value for undecane (35.3) was less than expected when compared with decane (38.7) and dodecane (485.6) and the dodecane brain:air PC value was greater than expected compared to the brain:air PC values for the other n-alkanes. The reason for this is unknown. Experiments were repeated to verify brain:air PC values for undecane and dodecane, with similar results.

To gain an understanding for the physical/chemical properties of these alkanes, SPARC (SPARC Performs Automated Reasoning in Chemistry) a computational program developed by the USEPA and the University of Georgia, was used to estimate their vapor pressures at 20°C, solubilities in water and the log octanol:water PC values (Table 3). The computed log octanol:water PC values for these n-alkanes increased with the number of carbons (Table 3) which is consistent with results obtained for our fat:blood PC values of 246.6, 273.8, 385.5, 529.0, and 544.6 for octane, nonane, decane, undecane and dodecane, respectively. The n-alkanes blood:air PC values also increased with number of carbon atoms suggesting that lipophilicity of the blood was more important than the water solubility of these alkanes. The water solubility of the n-alkanes decreased with increasing number of carbon atoms (Table 3).

The blood:air and tissue:air PC values for four aromatic compounds present in JP-8 aerosol samples are listed in Table 2. Generally, the solubility's of n-propylcyclohexane, o-ethyltoluene, 1,2,4-TMB and 1,3,5-TMB were greater in muscle, blood, liver, brain and fat than

the n-alkanes used in this study. The fat:air PC values were the highest (Table 2). Interestingly, the muscle:air PC value for 1,2,4-TMB was substantially greater (178) than for 1,3,5-TMB (98). The reason for this is unknown. The differences between the blood:air and tissue:air PC values for 1,2,4- and 1,3,5-TMB were not as great in other tissues. SPARC predicted the log octanol:water for the TMB isomers would be the same, while there would be slight differences in water solubility. Water solubility may play a more prominent role in tissue and blood solubility for these aromatic compounds compared to the n-alkanes because of increased water solubility.

Discussion

This study describes the further modification of the vial-equilibration method developed by Sato and Nakijima (1979) and later modified by Gargas et al. (1989). We elected to use tissue "smears" versus preparing tissue homogenates to determine partition coefficients because the method is simple and the tissues remain intact. The use of small minced tissue smears represents a slight modification to headspace analysis using the vial-equilibration method. Other investigators, including Fiserova-Bergerova and Diaz (1986) and Gearhart et al. (1993) used smeared homogenates for PC determinations. Kumarathasan et al. (1998) reports that they spiked "unhomogenized" tissue samples. The preparation of tissues as homogenates remains the most common method for measuring solubility of chemicals in tissues reported in the literature (Sato and Nakijima, 1979, Gargas et al., 1989, Thrall et al., 2002, and Meulenberg and Vijverberg, 2003).

N-alkane PC values were determined for C_8-C_{12}, despite the tremendous drop in vapor pressure for undecane and dodecane (Table 3). Difficulty was encountered trying to determine PC values for tridecane and tetradecane (C_{13} –C_{14}) using vial equilibration because of low vapor

pressures. Thus these chemicals were not evaluated. Special consideration was required when handling C_9-C_{12} alkanes which included care in cleaning syringes, cleaning tedlar bags and determining the atmospheric saturation for these compounds. Our data on PC values was consistent with the computed log K_{ow} properties as would be expected for high lipid content tissues such as fat and brain.

Our PC values with octane compared favorably to PC values reported by Gargas et al. (1989) for heptane. Gargas et al. (1989) reported a mean heptane blood:air partition coefficient of 4.8 (SE ± .15) which is similar to our octane blood:air partition coefficient of 3.1 (± .15). Heptane muscle:air, liver:air and fat:air partition coefficients reported by Gargas et al. (1989) were 4.2 ± .8, 15.0 ± 0.7, and 379.0 ± 6. compared to our results for octane of 3.0 ± .5, 6.0 ± .4, and 772 ± 32.7, respectively. Our in vitro blood:air PC values for octane and dodecane (3.1 ± 0.4 and 8.1 ± 1.3) were about one-half of the in vivo PC values reported by Liu et al. (1994) (7.5 ± 0.5 and 17.3 ± 1.8). It is interesting to note that Meulenberg and Vijverberg (2000) used an algorithm to predict the fat:air partition coefficients for 1,2,4- and 1,3,5-TMB (5878 and 6068) which were similar to our experimental fat:air partition coefficients of 5557and 5745, respectively.

The brain:air PC values for undecane and dodecane appear inconsistent with the other data sets for the brain and other tissues. That is, the undecane brain:air PC value (35.25) was lower than in decane (38.73) and the brain:air partition coefficient in dodecane (485.9) was fourteen times larger than undecane. The reason is not known, however we did repeat the experiments and obtained similar findings to our first experiments.

The second set of chemicals analyzed for tissue solubility were collected as vapor samples from an inhalation chamber containing JP-8 vapor and aerosol droplets at the University

of Arizona. These four chemicals represented about 1-3 % of the mass of vapor in the chamber with JP-8. Only human, blood:air PC values for 1,2,4 and 1,3,5-TMB determined empirically were found in the literature for these chemicals, therefore we measured PC values in this study. We did not expect to find differences in the solubility of 1,2,4 TMB and 1,3,5 TMB in any of the tissues or blood. However, this was not the case, the muscle:air PC values for 1,2,4-TMB and 1,3,5-TMB were (178) and (98), respectively. Water solubility may be one of the discriminating physical/chemical factors that may explain this difference.

The vial equilibration method using tissue smears was successful. This study is an important first step in understanding the pharmacokinetics of selected JP-8 hydrocarbon constituents at equilibrium. These PC values provide a first step in the future development of PBPK models that will provide risk assessment insights into JP-8 exposure. Subsequent research and method development are required to determine PC values for n-alkanes above C_{12} because of low vapor pressures and limits of detection with our analytical methods. One possible alternative is described by Jepson et al.(1993) involving partition coefficient determination of nonvolatile chemicals with vapor pressures well below 1 mmHg.

Acknowledgements

I would like to take this time to thank Reiko Perleberg, Tara Almekinder, and John Swint for their contributions in the laboratory. This research was funded by AFOSR [grant # F49620-03-1-0157]. The animal use described in this study was conducted in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals", National Research Council, 1996, and the Animal Welfare Act of 1996, as amended.

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TABLE 1: n-Alkane Tissue:Air and Blood:Air Partition Coefficients (Mean ± SE) n=7.

Chemical	Muscle	Blood	Liver	Brain	Fat
Octane	2.96 ± .46	3.13 ± .15	6.01 ± .38	4.38 ± 1.5	771.91 ±32.7
Nonane	4.72 ± .44	5.80 ±.41*	11.32 ± 1.3	22.30 ± 1.3	1588.16 ± 140*
Decane	6.92 ± .45*	8.13 ± .50*	15.98 ± .76*	38.73 ± 4.1*	2667.58 ± 198*
Undecane	14.68 ± 1.4	20.41 ± .69	31.07 ± 1.7	35.25 ± 4.2*	10797.14 ± 867
Dodecane	30.27 ± 5.2	24.57 ± 1.5	45.55 ± 4.3*	485.58 ± 31.9*	16484.70 ± 3058

* n=14

TABLE 2: Key Constituent Tissue:Air and Blood:Air Partition Coefficients (Mean ± SE) n=7.

Chemical	Muscle	Blood	Liver	Brain	Fat
n-Propylcyclohexane	15.7 ± 3.2	16.6 ± .09	25.95 ± 1.3	48.0 ± 2.5	2684 ± 105
o-Ethyltoluene	87.8 ± 16.1	58.8 ± .76	116.00 ± 4.1	148.0 ± 3.8	4891 ± 226
1,2,4 Trimethylbenzene	178.0 ± 37.1*	39.0 ± 1.6*	97.60 ± 2.7	166.0 ± 10.7	5557 ± 284
1,3,5 Trimethylbenzene	98.0 ± 14.3*	49.5 ± .57	67.60 ± 4.2	205.0 ± 23.8	5745 ± 375

* n=14

TABLE 3: Important Chemical Properties of JP-8 Key Constituents

Chemical	Molecular Weight	Boiling Point °C	Vapor Pressure (mmHg) 20°C	Solubility mg/l (water)	Log P O:W
n-octane	114.23	126.59	12.16	0.632	5.34
n-nonane	128.26	153.15	3.54	0.152	5.95
n-decane	142.28	176.98	1.06	0.035	6.56
n-undecane	156.31	199.12	.319	0.013	7.19
n-dodecane	170.34	219.53	.074	0.009	7.81
o-ethyltoluene	120.19	167.6	2.246	84.52	3.44
1,2,4 TMB	120.19	169.0	1.649	77.70	3.49
1,3,5 TMB	120.19	164.0	2.188	70.59	3.54
n-propylcyclohexane	126.24	151.6	2.356	0.7391	5.24

All chemical property values referenced here were generated using SPARC, an on-line calculator at http://ibmlc2.chem.uga.edu/sparc/smiles/Smiles.cfm. Calculations were conducted for 20°C and 760 mmHg. SPARC was developed jointly by the U.S. EPA and the University of Georgia.

CHAPTER 3

CONCLUSIONS

This study produced PC's for select n-alkanes and other chemical constituents, which are found in high concentrations in JP-8 vapor samples. The results show that n-alkanes (C_8-C_{12}) produce PC values that increase with a corresponding increase in carbon chain length of the constituent. Our PC values were consistent with the computed log octanol:water PC properties as would be expected for high lipid content tissues such as fat and brain. The PC values ranged from a low muscle:air value of 2.96 with octane to a high fat:air PC value of 16485 with dodecane. Brain and fat PC values were higher than values observed in muscle, liver, and blood, indicating an affinity of these chemicals for tissues rich in lipids. Additionally PC values for four key constituents of JP-8 were also determined. Overall, these chemicals produced PC values that were generally higher than observed in the n-alkanes.

Experimental PC values for these chemicals are limited to blood:air values in octane and dodecane (Liu et al., 1994) and brain:air values in 1,2,4-TMB and 1,3,5-TMB (Meulenberg and Vijverberg, 2003). The blood:air PC's of Liu et al. (1994) of 7.53 and 17.3 for octane and decane are about twice our values of 3.13 and 8.13, respectively. The brain:air values for 1,2,4-TMB and 1,3,5-TMB of 224 and 160 compare relatively well with our values of 166 and 205, respectively.

With exception to the aforementioned experimental values, only predicted tissue:air and blood:air PC values for octane, decane, 1,2,4-TMB and 1,3,5-TMB exist (Meulenberg and Vijverberg, 2000). The rat blood, fat, brain, liver, and muscle PC values were 7.53, 844, 75.9, 38.9, 14.3 and 17.3, 8563, 778, 377, and 144 for octane and decane, respectively. These values

do not compare well with the octane and decane tissue:air PC values we report in this paper of 2.96, 3.13, 6.01, 4.38, 771.91 and 6.92, 8.13, 15.98, 38.73, and 2667.58 respectively. There was much better agreement between our experimental values in 1,2,4-TMB and 1,3,5-TMB compared to the predicted values presented by Meulenberg and Vijverberg (2000). The predicted fat:air and muscle:air PC values of 5878 and 100 for 1,3,5-TMB, were very similar to our values of 5745 and 98, respectively. For 1,2,4-TMB the predicted fat:air value of 6068 was similar to our value of 5557, however the predicted muscle:air value of 104 was relatively small compared to our value of 178.

N-alkanes water solubilities (mg/L) ranged from 0.63 in octane to 0.01 in dodecane compared to .74 in n-propylcyclohexane to 84.52 in o-ethyltoluene. We did not expect to find differences in the tissue solubility of 1,2,4 TMB and 1,3,5 TMB whose corresponding water solubilities were 77.7 mg/L and 70.59 mg/L, respectively. However, this was not the case, the muscle:air PC values for 1,2,4-TMB and 1,3,5-TMB were 178 and 98, respectively.

The vial equilibration method using tissue smears was successful. Our results compare favorably to those reported by Gargas et al. (1989) using vial equilibration of lower-chained n-alkanes. Our results did not compare as favorably with computationally predicted PC's, such as those of Meulenberg and Vijverberg (2000). Subsequent research and method development are required to determine PC's for n-alkanes above C_{12} because of low vapor pressures and limits of detection with our analytical methods. Alternatives to the vial-equilibration method are currently being researched. One possible alternative is described by Jepson et al. (1993) involving PC determination of nonvolatile chemicals with vapor pressures well below 1 mmHg. This study is an important step in understanding the pharmacokinetics of selected JP-8 hydrocarbon

constituents at equilibrium. These PC's provide valuable insight into the future development of PBPK models that will provide risk assessment for JP-8 exposure.

APPENDIX

Partition Coefficient Protocols:

Each experiment consisted of five reference vials and seven test vials. Reference vials contained one milliliter of "chemical of interest vapor" from a gas-sampling bag. Reference vials contained one milliliter of chemical of interest plus a known amount of tissue or blood. Each vial was treated in succession throughout the entire process, five reference vials followed by seven test vials.

Bag Sample Preparation:

1. Fill a 3L Tedlar gas sampling bag with 2.4 L of room air filtered through a charcoal filter.
2. Add known (ul) volume of neat chemical of interest into bag. Prepare bag concentration as desired between (500-5000ppm), for a 10 ml headspace vial, one ml of chemical vaporfrom a bag = ~50-500 ppm concentration inside each vial.
3. Gently heat the bag with a heat-gun until liquid chemical inside bag has entered the vapor phase. Wait 30 minutes prior to adding vapor to vials.

Tissue Preparation:

1. Remove tissue from -20°C freezer and thaw in water bath for 30 minutes.
2. Mince tissue into small pieces (~.25x.25) inches using a surgical scalpel and blade.
3. Place tissue into a pre-weighed vial using a stainless-steel spatula.
   Tissue volumes were as follows: (liver-0.5g, muscle-0.75g, fat-.05g, brain-0.1g, and blood-.75 ml)
4. Vials where then capped with aluminum caps and teflon-lined butyl rubber septa.
5. Vials were crimped closed.

Vial Treatment:

1. Vials are placed on vortex-evaporator for 15 minutes to allow vial and tissue to warm to 37°C.
2. Vent each vial in succession for 10 seconds each with gas-tight syringe open to room air to relieve pressure build-up.
3. Withdraw one ml of headspace air from each reference and test vial.
4. Add one ml of chemical vapor from the freshly prepared gas-sampling bag. Shake moderately on vortex-evaporator for required incubation (equilibration) time (liver, muscle, blood-all chemicals 3 hrs), (brain- all chemicals, 4 hours), (fat-undecane and dodecane 6 hours, otherwise 4 hours).

GC Analysis:

1. Withdraw 0.5 ml of headspace air from each vial and hand-inject into Agilent 6890 Plus GC for analysis.

   Conditions as follow:

Column:	HP-5
Length:	15M x .53mm x .0015mm
N2 Flow:	40 ml/min
Air Flow:	375 ml/min
H2 Flow:	37.5 ml/min
Injector Temp:	200°C
FID Temp:	260°C
Oven Temp:	110-140°C
Retention time:	2-5 minutes

   Oven Temperatures were adjusted to maintain retention times between 2-5 minutes.

Equipment:

Gas Sampling Bags- SKC, Eighty-four, PA

Syringes- gas tight syringe, Hamilton Co., Reno, NV

Vials-10-ml round-bottomed headspace vials, Kimble Glass Co., Vineland, NJ

Caps/Septa- aluminum caps containing teflon-lined butyl rubber septa, National Scientific Co., Duluth, GA.

Vortex-Evaporator, Labconco, Kansas City, MO

Chemicals

Octane(111-65-9), nonane (111-84-2), decane (124-18-5), undecane (1120-21-4) and dodecane (112-40-3) were 99% + pure (Sigma-Aldrich, St. Louis, MO). N-Propylcyclohexane (1678-92-8) was 97% pure (Fisher, Pittsburg, PA) and 1,2,4-Trimethylbenzene (1,2,4-TMB) (95-63-6), and 1,3,5-Trimethylbenzene (1,3,5-TMB) (108-67-8) were both 98% pure (Sigma-Aldrich, St. Louis, MO). O-ethyltoluene (611-14-3) was 99% pure (Fisher, Pittsburg, PA).