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A Harmonized Physiologically-Based Pharmacokinetic Model for Nonane as a Component of Jet Fuel

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14. ABSTRACT

JP-8 is a complex mixture of hundreds of components including straight and branched chain alkanes, cycloalkanes, diaromatics and naphthalenes. Inhalation and dermal are the most prevalent routes of exposure. Occupations of interest include aircraft fuel tank and exhaust workers. To assess potential health effects of such exposures, it is useful to predict target site dosimetry of JP-8 components. A first step in this process is to develop a physiologically-based pharmacokinetic (PBPK) model for representative mixture components. Single-chemical models can then be "harmonized" (same physiological structure) and combined into a composite mixture model. A harmonized model structure should be complex enough to take into account all important physiological processes undergone by any component in an integrated fashion; the same physiological structure must underlie each of the individual models. The initial framework for such a model is developed here on the basis of observed kinetic behavior of nonane, an aliphatic JP-8 component. Nonane is highly lipophilic and distributes preferentially in brain tissue.

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PREFACE

This research was accomplished at the Applied Biotechnology Branch, Human Effectiveness Directorate of the Air Force Research Laboratory, Wright-Patterson AFB, OH, under John J. Schlager, Branch Chief. Work was performed under the In-House Contract # FA8650-05-2-6518. This technical report was written for AFRL Workunit 2312A215. Funding for this work was provided by the Air Force Office for Scientific Research, under the program management of Dr. Walter Kozumbo.

This report contains data generated by Melanie C. Caracci, Dr. James N. McDougal (GEOCENTERS) and Sgt James D. McCafferty (AL/OET) in 1995. As these data were not reported in a public forum, they have been included in order to provide a reference useful to government and outside researchers. This research was completed under the ManTech/GEOCENTERS Joint Venture contract (Contract #s F33615-90-C-0532 and F41624-96-C-9010). Lt Col Terry A. Childress served as Contract Officer Representative for the U.S. Air Force, Armstrong Laboratory, Toxicology Division (AL/OET). Dr. Darol E. Dodd served as Program Manager for the ManTech/GEO-CENTERS Joint Venture contract. The authors of this technical report would like to acknowledge the valuable contributions of all of these researchers and supporters.

The animals used in this study, performed in 1995, were handled in accordance with the principles in the *Guide for the Care and Use of Laboratory Animals*, prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animals Resources, National Research Council, DHHS, National Institute of Health Publication #86-23 (1985) and the Animal Welfare Act of 1966, as amended.

SUMMARY

JP-8 is a complex mixture of hundreds of components including straight and branched chain alkanes, cycloalkanes, diaromatics and naphthalenes. Inhalation and dermal are the most prevalent routes of exposure. Occupations of interest include aircraft fuel tank and exhaust workers.

To assess potential health effects of such exposures, it is useful to predict target site dosimetry of JP-8 components. A first step in this process is to develop a physiologically-based pharmacokinetic (PBPK) model for representative mixture components. Single-chemical models can then be "harmonized" (same physiological structure) and combined into a composite mixture model. A harmonized model structure should be complex enough to take into account all important physiological processes undergone by any component in an integrated fashion; the same physiological structure must underlie each of the individual models.

The initial framework for such a model is developed here on the basis of observed kinetic behavior of nonane, an aliphatic JP-8 component. Nonane is highly lipophilic and distributes preferentially in brain tissue. Its behavior in the body can be described in a PBPK model that includes the blood, lungs, liver, slowly perfused tissue, rapidly perfused tissue, brain, skin and fat. The model incorporates absorption via inhalation and dermal exposure. It predicts body burdens of nonane under occupational exposure conditions and is consistent with limited occupational body burden data. The implications of this model, in which the components of JP-8 interact via joint metabolic pathways or through mutual effects on tissue distribution, are explored.

INTRODUCTION

JP-8 is a complex mixture of hundreds of components. Component classes include: straight chain alkanes, branched chain alkanes, cycloalkanes, diaromatics and naphthalenes. The Total Petroleum Hydrocarbon Criteria Working Group (TPHCWG) published a series of monographs outlining approaches for assessing the health impact of complex mixtures of hydrocarbons including JP-8 in the environment (Weisman (ed.), 1998; Potter and Simmons, 1998; Gustafson *et al.*, 1997; Edwards *et al.*, 1997; Vorhees *et al.*, 1999). Central to the approach of this group is the notion of a relatively small number of fractions whose components are expected to behave similarly in the environment. These fractions consist of compounds that have similar relevant physico-chemical properties, such as equivalent carbon number, molecular weight, boiling point vapor pressure, water solubility, octanol-water partition coefficient and Henry's Law constant.

Not all of these classification criteria are relevant for a chemical's behavior in the body (and additional parameters may be important), but this approach can form the basis for the classification of petroleum mixtures into a relatively small number of classes of similarly behaving chemicals. The TPHCWG classified fractions into six aliphatic and seven aromatic classes. Fractions relevant for JP-8 include the effective carbon (EC) classes: aliphatics EC >6-8, EC >8-10, EC >10-12, EC >12-16, EC >16-21; and aromatics EC >7-8, EC >8-10, EC >10-12, EC >12-16. In this classification, nonane can be considered representative of EC >8-10 aliphatics.

The specific details of such classification schemes are quite arbitrary, and other alternative classification schemes have been proposed. For example, the Massachusetts Department of

Environmental Protection (MA DEP) issued an interim final petroleum policy document that groups carbon number (C) C9 – C17 alkanes/cycloalkanes together (for the purpose of determining a group RfD), and specifically identifies nonane as a reference compound for this group (MA DEP, 1994).

The US EPA issued draft guidelines for the conduct of health risk assessments of chemical mixtures (EPA, 1999). These guidelines stress the importance of physiologically-based pharmacokinetic (PBPK) modeling in interpreting experimental data, stating that evaluation of risk posed by exposure to multiple chemicals can only come about with a solid understanding of the toxicity of chemical agents and the factors that control their absorption, metabolism, distribution and elimination. Further, the use of PBPK models, together with data from chemical interaction studies, could "form the very basis of mechanistic risk assessment methods for complex chemical mixtures" (EPA, 1999).

The purpose of this paper is to begin such a PBPK modeling process for JP-8 by describing a PBPK model for nonane. Unpublished studies on nose-only uptake of nonane in rats from our laboratory will be described, and these data together with literature derived information will be used to develop and validate the nonane model.

The model will also provide a first "module" for a more comprehensive mixtures model for JP-8 in which the behavior of individual components are modeled in the presence of potential interactions with other components of the fuel, such as competitive metabolic inhibition of one component by the others. In order to form the basis for such a comprehensive mixture model,

the PBPK model developed here for nonane needs to be readily extendible to other components of the mixture. It thus needs to have an underlying physiological structure that is sufficiently general and yet complex enough that it can form the basis for PBPK models for other JP-8 components, as well as their interactions. Individual chemical models developed on such a basis are considered to "harmonize" with one another.

How complex does such a harmonized model structure need to be? We explore this question below with reference to the development of a PBPK model for nonane. Parallel efforts with respect to decane (Merrill *et al.*, 2008) and naphthalene are also underway in our laboratory, and for tetradecane and substituted naphthalenes at the University of Georgia (Dr. Jeffrey W. Fisher, personal communication).

Nonane in breath is a good biomarker for JP-8 aliphatic exposure. We now have some preliminary knowledge of ambient air levels of nonane under specific occupational settings, as well as body burden of nonane as reflected in pre- and post-exposure breath measurements of aircraft maintenance personnel (Pleil *et al.*, 2000). The PBPK model will be used as the basis for extrapolating to humans with the determination and substitution of appropriate species-specific parameters. This human-based model takes an input (exposure to ambient nonane) and predicts an output (body burden of nonane), which can be compared with data.

Acute neurobehavioral and lung immune suppression effects have been observed as a result of exposure to JP-8 (Baldwin *et al.*, 1998a, b; Bhattacharya and Smith, 1998; Kaufman *et al.*, 1998; Zeiger and Smith, 1998). In addition, nonane has been observed to distribute preferentially into

rat brain tissue (Zahlsen *et al.*, 1990). We included a brain compartment in the present model, so that we may have a future tool for predicting brain levels of nonane.

It is not clear at this point, however, which component of JP-8 (or combination of components) may be leading to the observed health effects, so a dose-response analysis becomes problematical. Once a "bad actor" is identified, it can be modeled (in the presence of other components), then its target site dose can be predicted and correlated with the observed effects. However, we need to begin modeling individual candidates in order to start to identify potential bad actors. For example, if a particular component does not get to a target site in sufficient quantities, it cannot be a strong candidate for "bad actor" status. This leads to a "chicken and egg" scenario in terms of identifying which components of the jet fuel are responsible for the observed adverse health effects. A PBPK model of individual components, together with their interactions, is a necessary first step in resolving this dilemma.

When modeling individual components in this way, with a view to ultimately combining them in a single interacting model for the mixture, we need to ensure that the models will fit together to comprise a coherent model for the fuel as a whole. Thus, the individual models need to have a similar underlying structure, based on a common physiology. All the models generally need to be developed to the same level of detail, so that when the underlying conditions are changed (such as blood flow to specific tissues, or breathing rate), the individual models respond in a similar manner, and in such a way that their interactions can be maintained. Thus individual models must generally have the same compartments (at least for those tissues in which interactions between chemicals occur), and have the potential at least to model the behavior of

the chemicals in each of those compartments in a similar way, albeit with quantitative chemical-specific parameters (e.g., partition and diffusion coefficients). Care must be taken to ensure that "lumped" compartments such as rapidly or slowly perfused tissues, take into account differences in volume and flow if tissues are taken out and modeled separately for one chemical, but not another. Such a coherent suite of individual chemical PBPK models are called "harmonized" in the present context.

HARMONIZED MODEL FRAMEWORK

Harmonization may be considered to be the imposition of a common model structure for a number of similar but distinct chemicals. In this context, two models are harmonized if their model structure is described mathematically in the same way (for example with tissues described as having flow limited and/or diffusion limited processes), but with different values for the chemical specific parameters (for partition and/or diffusion coefficients, for example). Physiological parameters, such as tissue volumes and blood flows, should be the same for all chemicals in a set of harmonized models. In cases in which the behavior of a chemical is sufficiently different from the others that novel processes need to be introduced, a "forced" harmonization may still be possible in which an inclusive model structure is developed, but in which some components "drop out," for example by having certain (chemical specific) parameters set to zero. Such a contingency plan may still allow a harmonized set of models to be developed that would include tetradecane and/or substituted naphthalenes if their individual model descriptions turn out to be too different from the existing model structures.

The first step in the development of a composite mixture model is to computationally combine two or more "harmonized" single-chemical PBPK models together into a single composite model in which the chemicals interact in a specific way. For example, Krishnan and co-workers (e.g., Haddad *et al.*,1999) have combined PBPK models for benzene, toluene, ethylbenzene and xylenes (BTEX) compounds together via their metabolic interaction in the liver (competitive metabolic inhibition of cytochrome P450-2E1). Such an interaction can, in principle, be extended to any number of compounds (for which individual single chemical PBPK models are available) that interact via the same pathway, forming a composite simple mixture model ("n-chem" model). It is of course desirable in such a composite model that the underlying single chemical models have the same physiological structure, so that physiological changes, such as changes in cardiac output or tissue blood flows, will have an overall effect that is readily predictable from parallel effects on the single chemical models.

In the current paper, we propose a generic PBPK model structure where tissues are represented as compartments in which chemicals distribute via the combined effects of diffusion limited uptake from the blood and possible diffusion into deep tissue (global tissue model). Both of these processes will not necessarily be needed for all tissues, but they should be available in the generic "harmonized" model structure. Such a model structure also serves as a basis for model refinement and improvement. Additional aspects of developing a harmonized model structure for the kinetic behavior of JP-8 components include integration with exposure models of the lung and skin currently being developed in other laboratories, and considerations of binding to circulating plasma proteins. The necessity for, and impact of, deep tissue compartments together with multiple interconnected fat compartments, will also be discussed below. Finally, additional

interactions, such as up/down-regulation of metabolic enzymes responsible for the metabolism of other components, and altered partitioning due to chemical interactions in the blood and tissue may ultimately be needed in a comprehensive composite model structure, although they have not been addressed at this point.

Simple Tissue Model (Flow Limited)

It is envisioned that a "global" model for lipophilic volatile organics will involve tissue compartments that potentially incorporate both diffusion limited uptake into the tissue together with distribution into a "deep" tissue compartment as follows. The standard equation describing uptake (partitioning) from blood into tissue (in this case fat) used in the standard Ramsey-Andersen (1984) PBPK model is:

$$V_f \frac{dC_f}{dt} = Q_f \left(C_a - \frac{C_f}{P_{fb}} \right) \qquad (1)$$

where V_f is the fat volume, C_f is the concentration in fat (assumed to be equilibrated with the venous (outflow) blood concentration C_v via the fat:blood partition coefficient P_{fb}), C_a is the arterial blood concentration, and Q_f is the blood flow to the fat tissue.

Diffusion Limitation into Tissue (Fat)

In some cases, simple partitioning (flow limitation) is not sufficient to characterize uptake into tissues (e.g., when uptake is restricted (slowed) either by a physical barrier (membrane) or due to the limitation of uptake due to the tissue's properties). Such uptake restriction, whatever the cause, is often termed "diffusion-limited uptake." Figure 1 shows axial and radial transport

governing mass balance in the fat compartment under these conditions. Mass balance requires that the net gain of nonane in fat $(V_f\, dC_f/dt)$, equals the net loss $Q_f(C_a - C_v)$ from blood, and that this equals the net flux $K(C_v - C_f/P_{fb})$ between blood and fat (Figure 1), where K is the "diffusional" transfer coefficient (L/hour) (Andersen *et al.*, 2001):

$$V_f \frac{dC_f}{dt} = Q_f \left(C_a - C_v\right) = K\left(C_v - \frac{C_f}{P_{fb}}\right) \qquad (2)$$

Substituting for $C_v = (Q_f C_a + KC_f/P_{fb})/(K + Q_f)$ from the right equation (2) into the left equation gives:

$$V_f \frac{dC_f}{dt} = \left(\frac{Q_f K}{Q_f + K}\right)\left(C_a - \frac{C_f}{P_{fb}}\right) \qquad (2a)$$

Note that for very rapid diffusion ($K >> Q_f$), the right hand side of equation (2a) reduces to the familiar flow-limited equation (1).

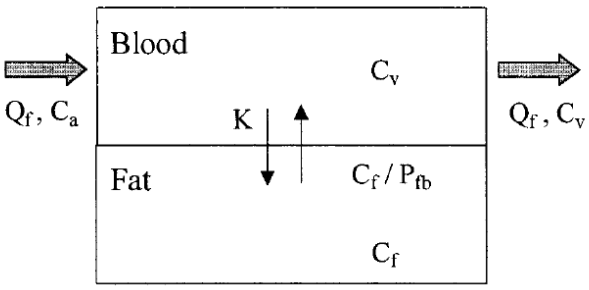

Figure 1: Axial and radial transport governing mass balance in the fat compartment (Andersen *et al.*, 2001)

Deep Tissue Compartment

In some cases, distribution into a single homogeneous compartment representing the tissue is not sufficient to describe the time-course of uptake. In particular, uptake may be better fit with a process that involves uptake into a relatively accessible "shallow" compartment from the blood, combined with movement from the shallow compartment into a slower "deep" compartment in the tissue. This most likely occurs in the fat, where yellow or brown fat may be more readily reached from the blood than the very poorly perfused white fat. In the proposed nonane model, as well as in the decane model (Merrill *et al.*, 2008), we did not find the deep fat compartment necessary to describe the fat kinetics. Hence, the diffusion rate into the deep fat was set to zero, yet this model structure was maintained for future analyses of other JP-8 components that are highly lipophilic. When data describing the simultaneous uptake of multiple components of JP-8 become available, we expect to see selective uptake or "competitive partitioning" into fat of the various components. This will likely necessitate a "deep" fat compartment to describe kinetic changes from single- to multiple-chemical diffusion-limited uptake. Figure 2 shows distribution to the deep tissue compartment of an organ. The governing equation in this case is:

$$V_t \frac{dC_t}{dt} = Q_t \left(C_a - \frac{C_t}{P_{tb}} \right) - D_t \left(\frac{C_t}{P_{tb}} - \frac{C_{dt}}{P_{dt:b}} \right) \qquad (3)$$

where D_t is the diffusional clearance from tissue *t* to deep tissue *dt*, and where, as before, the *P* parameters represent partitioning into these tissues (Andersen *et al.*, 2001).

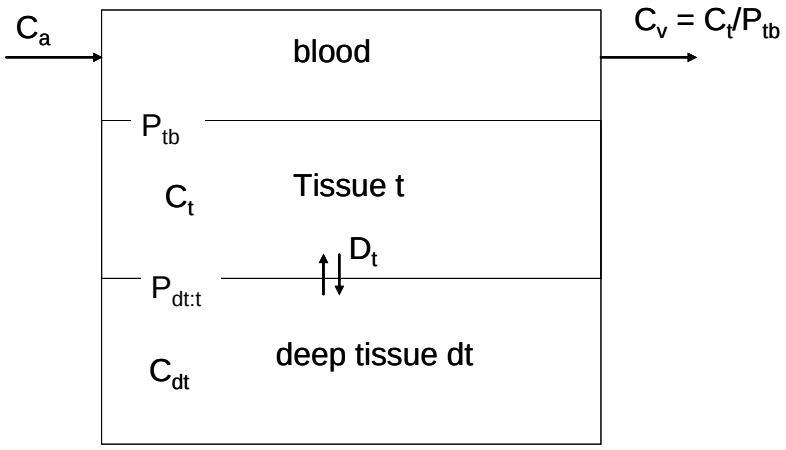

Figure 2. Schematic representation of deep tissue compartment

Combined Diffusion Limitation and Deep Tissue (Global Tissue Model)

Combining both diffusion limitation and movement into a deep tissue compartment gives the following equation:

$$V_t \frac{dC_t}{dt} = \left(\frac{Q_t K_t}{Q_t + K_t} \right) \left(C_a - \frac{C_t}{P_{tb}} \right) - D_t \left(\frac{C_t}{P_{tb}} - \frac{C_{dt}}{P_{dt:b}} \right) \qquad (4)$$

where K_t is the "diffusional" transfer coefficient (L/hour) and D_t is the diffusional clearance from tissue to deep tissue. This is the general form for a generic tissue in a global model, and is the basis for a harmonized framework for highly lipophilic volatile organic compounds such as JP-8 components.

Multiple Fat Compartments

Despite the added complexity, it is becoming apparent that multiple fat compartments are needed for single-chemical models in order to adequately describe the data. In PBPK models, fat is generally described as a single (or sometimes two) homogeneous, poorly perfused compartment, which is assumed, like other tissues, to be in equilibrium with venous blood at a concentration determined by the fat:blood partition coefficient. For highly lipophilic compounds, such as those under consideration here, this description may not be accurate. Unlike other tissues such as liver or brain, fat is distributed throughout the body. Some fat is indeed concentrated in specific locations (such as perirenal fat), but other fat deposits are distributed throughout other tissues. It is feasible to consider that lipophilic compounds that distribute preferentially into fat may do so directly from the associated tissues without first going into the blood. Further, the fat in the body (even those deposits associated with specific tissues) may be considered at least partially "connected" in that material may move from one tissue region to another wholly within the fat, rather than first partitioning out into the blood (which such highly lipophilic compounds are indisposed to do).

Thus, based on these considerations, we may devise an alternative model structure for such compounds that involves an expanded description of the fat using an intertissue diffusion rate. Such a structure is shown in Figure 3. Transfer by intertissue diffusion means that a small part of both rapidly and slowly perfused tissues are slower to reach equilibrium because the chemical is continuously lost to adjacent fat. As a result there is also a decrease in the amount returning from these tissues to blood and the lungs. This phenomenon may explain certain behaviors observed for decane, a very similar compound that differs by only one carbon number. The

slight increase in decane concentrations of the liver and other rapidly perfused tissues and the large increase in fat decane concentrations seen from Day 1 to Day 3 in the study by Zahlsen *et al.* (1990, see Merrill *et al.*, 2008). Without this intertissue diffusion, both would reach steady state much sooner.

Figure 3. Schematic representation of multiple fat compartments, including those associated with specific tissues such as the liver

Integration with Exposure Models

In addition to inhalation, dermal absorption is a significant route of exposure, and jet fuel components may be both absorbed systemically and have local skin effects such as irritation. One of the driving factors for skin irritation is the concentration of certain components at particular skin cell types in certain skin layers following dermal exposure to JP-8. In order to quantify these local skin concentrations, a model that incorporates spatial heterogeneity as well

as time-dependence needs to be developed and implemented. Such a model should elucidate potential links among exposure-time, concentration, skin depth and the onset of skin irritation, and will complement the dermal absorption and skin irritation models currently being developed in the laboratories of Dr. James N. McDougal and Dr. James E. Riviere (McDougal and Rogers, 2004; Riviere and Brooks, 2005, 2007; Muhammad *et al.*, 2005).

Protein Binding

Binding to circulating plasma proteins generally restricts the entry of materials into tissues that do not allow passage of large protein molecules. The default assumption is that the circulating free fraction of the compound is the principal determinant for entry into tissues. This, however, is not always the case; when the dissociation rate constant for the protein-chemical complex is sufficiently rapid compared with the transit time through the tissue of interest (and uptake into tissues is fast enough to significantly deplete the free fraction), significant amounts of the originally bound material may be released to replace the free material taken up. In the case of extremely rapid equilibration between bound and free forms of the material, it may make little difference if a particular molecule is originally bound or not, since it may exchange with the protein binding site many times during a single passage through the tissue. In such cases, the total concentration of the chemical, rather than just the free fraction, drives uptake into the tissues.

Most jet fuel components are sufficiently lipophilic that the free compounds partition readily into tissue. Uptake is therefore determined primarily by the equilibrium free fraction and/or the dissociation rate constant.

NONANE PBPK MODEL

The harmonized nonane model was initially developed on the basis of rat nose-only inhalation data collected as described below.

Methods

Test Materials

Chemical:	n-Nonane
Manufacturer:	Aldrich Chemical Co. (Milwaukee, WI)
CAS #:	111-84-2
Mol. Weight:	128.26
Empirical Formula:	$CH_3(CH_2)_7CH_3$
Boiling Point:	151°C

Partition Coefficient Determination

Blood, liver, fat, muscle and brain samples were obtained from female Fischer-344 rats after euthanasia by carbon dioxide (CO_2). Liver, fat, muscle and brain samples were minced. Equilibration vessels were made by placing Teflon-coated rubber septa in crimp-sealed lids of 10 mL (nominal volume) vials. One gram of muscle, 0.75 mL of blood, 0.5 grams of liver, 0.05 gram of fat, or 0.1 gram of brain tissue were placed in the vials and incubated at 37°C for 15 minutes. Nonane partition coefficients were determined at one concentration, 300 ppm. Multiple vials were prepared using blood, liver, fat, muscle and brain. Three empty reference

vials for blood and tissue were also prepared. One mL of chemical from a freshly prepared gas sampling bag was injected into each vial after removing an equivalent volume of air from the vial with a gas tight syringe and side port needle. The entire set of vials were agitated and incubated at 37°C. The experiment was run at 3, 5 and 7 hour incubation periods to determine the optimum equilibration time. The concentration of the chemical in the headspace of the vials was determined by gas chromatography at the end of the incubation period.

Sample analysis was accomplished with a Hewlett Packard 5890 II Gas Chromatograph and a Hewlett Packard Auto Headspace Sampler. A Flame Ionization Detector (FID) was used with helium as the carrier flow (9.0 mL/minute). Auxiliary flow (helium) was 33.5 mL/minute, hydrogen flow was 26 mL/minute and air flow was 310 mL/minute. Injection temperature was 155°C, detector temperature was 255°C and oven temperature was 165°C. The column was a Capillary DB-1, 30 m x 0.539 mm.

The partition coefficients for blood and other tissues were determined using Equation 5:

$$P_{t:a} = \frac{C_{ref} V_{vial} - C_{test} (V_{vial} - V_{test})}{C_{test} V_{test}} \qquad (5)$$

where $P_{t:a}$ is the partition coefficient (*t:a* is tissue:air), C_{ref} is the chemical concentration in the headspace of the reference vial, C_{test} is the headspace chemical concentration of the test vial, V_{vial} is the volume of the empty vial, and V_{test} is the volume of the test liquid (Gargas *et al.*, 1989).

Nose-only Inhalation Study

Time course blood and tissue samples were collected during and following four-hour nose-only inhalation exposures to nonane in female F344 rats. Surgery to cannulate the jugular veins of the rats to permit the serial withdrawal of blood was performed 24 hours prior to exposure using 1.0 mL/kg body weight of 70 mg/mL Ketamine/6 mg/mL Rompum administered intraperitoneally. Twenty animals per concentration were exposed in cone-shaped tubes fitted to a "Christmas Tree" arrangement to allow for homogeneous exposure concentrations for each animal for four hours (see Figure 5). The exposure concentrations were 100, 500 and 1000 ppm measured in the air. Blood samples of 100 μL from each animal were collected prior to exposure (to serve as a control) and during the exposure via the jugular cannula at 30 minutes, 1, 2, 3 and 4 hours after the start of the exposures. At the end of 4 hours, 10 of the 20 rats from each exposure group were euthanized via CO_2 inhalation; fat, muscle and liver tissue samples were collected immediately. From the remaining ten animals, blood samples were collected at 5, 10, 15, 30 minutes, 1, 2 and 3 hours after the exposure ceased. After the final blood sample was collected, the animals were euthanized via CO_2 inhalation and fat, muscle and liver tissues were collected.

Three replicates were collected from each tissue per animal. Blood and tissue sample weights were approximately 0.1 g blood or 0.2 g tissue. Tissues were digested before analysis by injecting 3 mL of 0.4 g NaOH/mL water into the headspace vial. They were mixed on a Haake Buckler Vortex mixer at 75°C until digested, then run on the Tekmar static headspace sampler. Blood samples were run on the headspace sampler untreated. The headspace sampler loop injected samples onto the Varian 3700 Gas Chromatograph, 0.53 mm x 30 m SPB1 column, temperature program 50°C - 10 °C/minute - 90°C, helium carrier 5 mL/minute, flame ionization

detector temperature 300°C, and 25 mL/minute helium make-up gas flow. The Varian 3700 electronic output went to a computer where it was automatically integrated and stored by the Nelson 2600 software program. Standards were prepared in Tedlar bags and diluted into headspace vials. Headspace extraction recovery from blood, fat, muscle, brain and liver were 98 percent, 27 percent, 79 percent, 58.8 percent and 85.7 percent, respectively.

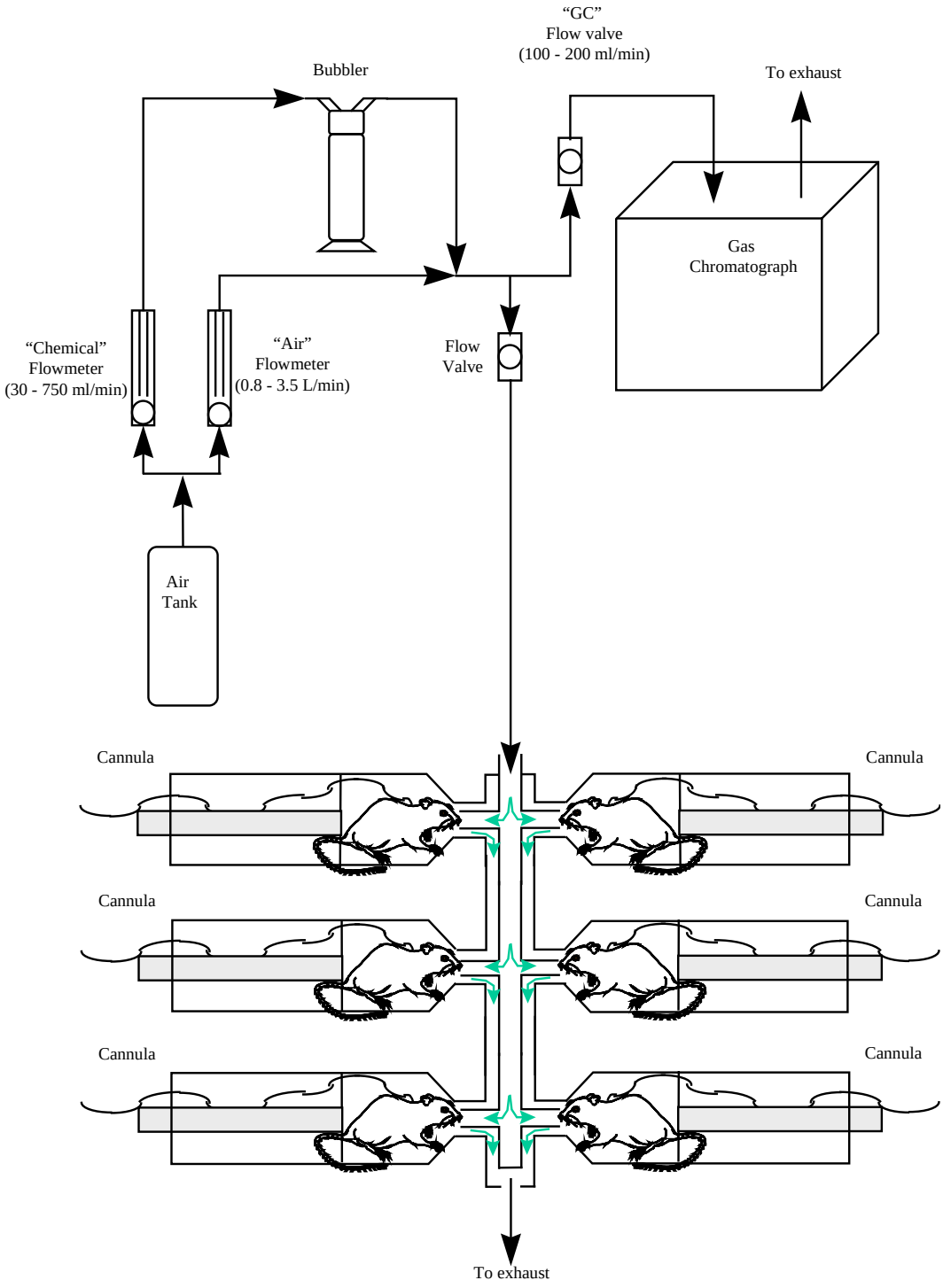

Figure 4: Diagram of nose-only exposure apparatus utilized for this study.
All animals are given the same exposure dose simultaneously. All exhaled air and exhaust is delivered directly to exhaust system and not returned to the animals (not drawn to scale).

Results

Partition Coefficients

Table 1 lists the partition coefficients as determined by the method described. Average values and standard deviations (SD) are reported in this figure. Although most of these results show more variation than is generally preferred, there were technical difficulties as described below.

Nonane proved to be a difficult chemical to work with in the laboratory. Due to its lipophilicity, it has a tendency to "stick" to glass. Inherent within the vial equilibration method, however, is a mechanism to account any loss from the system, such as "sticking" to the glass. Reference vials are treated identically as the sample vials throughout the procedure except that they do not contain tissue or blood. Therefore, any loss to the sample vials should also occur to the reference vials and when the partition coefficient is calculated, system loss has been accounted; however variation tends to increase with compounds that "stick."

Table 1: Summary of blood:air and tissue:air partition coefficients for nonane.

	Blood:Air	**Liver:Air**	**Fat:Air**	**Muscle:Air**	**Brain:Air**
Mean	5.22	7.53	1467.42	6.00	27.04
SD	1.26	2.10	291.53	2.20	7.60

Nose-only Inhalation Study

All data collected during the four-hour nose-only inhalation study are listed in the following set of tables (Tables 2-13). Table 2 depicts the nonane concentration in blood (μg/g) during and after a 100 ppm nonane inhalation exposure. Data are missing data for rat #20; due to a cannula that would not draw, the rat was removed from the study. Similar data are shown for the 500 ppm inhalation exposure in Table 6, and for the 1000 ppm inhalation exposure in Table 10. These data are shown plotted over time in Figures 4-9. Tables 3, 4 and 5 depict the measured tissue concentration in liver, fat and muscle, respectively, following the 100 ppm exposure. Data are missing for Rat #30; again, a non-functioning cannula caused the rat to be removed from the study. Tables 7, 8, and 9 show similar data for the 500 ppm inhalation exposure and Tables 11, 12, and 13 represent the data from the 1000 ppm inhalation exposure.

Table 2: Nonane concentration (μg/g) in blood during and after 100 ppm exposure.
Rats #1-10 were exposed to 100 ppm nonane for 4 hours and then were euthanized. Rats #11-20 were exposed to 100 ppm nonane for 4 hours and euthanized 4 hours post-exposure.

Nonane blood concentrations (μg/g) during 4-hour 100 ppm exposure

Sample Time (hours)	Rat #1	Rat #2	Rat #3	Rat #4	Rat #5	Rat #6	Rat #7	Rat #8	Rat #9	Rat #10
0.5	0.622	0.283	0.336	0.757	0.643	0.242	0.445	0.454	0.583	0.285
1.0	0.694	0.434	0.616	0.756	0.347	0.280	0.503	0.506	0.587	0.550
2.0	0.406	0.699	0.665	0.768	1.021	0.881	0.335	0.351	0.588	0.330
3.0	0.991	0.297	0.774	0.385	0.993	0.839	0.776	0.402	0.677	0.764
4.0	0.355	0.731	0.364	0.922	1.006	0.458	0.754	0.469	0.531	0.237

Nonane blood concentrations (μg/g) during and following 4-hour 100 ppm exposure)

Sample Time (hours)	Rat #11	Rat #12	Rat #13	Rat #14	Rat #15	Rat #16*	Rat #17	Rat #18	Rat #19	Rat #20*
0.5	0.361	0.425	0.400	0.481	0.472		0.467	0.338	0.470	
1.0	0.508	0.520	0.492	0.715	0.641	0.192	0.573	0.180	0.544	
2.0	0.253	0.746	0.599	0.451	0.842	0.438	0.371	0.674	0.266	
3.0	0.798	0.612	0.712	0.860	0.819	0.457	0.792	0.712	0.326	
4.0	0.975	0.764	0.840	0.873	0.961		0.994	0.814	0.904	
4.08	0.465	0.448	0.382	0.487	0.557	0.553	0.185	0.555	0.234	
4.17	0.205	0.325	0.112	0.328	0.456	0.418	0.401	0.481	0.534	
4.25	0.345	0.257	0.275	0.305	0.391	0.303	0.188	0.142	0.526	
4.50	0.074	0.043	0.219	0.114	0.272	0.222	0.270	0.260	0.375	
5.00	0.106	0.089	0.152	0.076	0.178	0.082	0.055	0.052	0.232	
6.00	0.000	0.000	0.039	0.084	0.059	0.000	0.078	0.040	0.032	
7.00	0.000	0.000	0.026	0.000	0.044	0.022	0.041	0.071	0.065	
8.00	0.000	0.000	0.039	0.038	0.000	0.000	0.000	0.042	0.055	

*Blank spaces indicate when blood draws were not possible due to poor cannulas; Rat #20 was completely removed from the study due to a malfunctioning cannula.

Table 3: Nonane concentration (μg/g) in liver following 4-hour 100 ppm exposure.
Rats #1-10 were exposed to 100 ppm nonane for 4 hours, immediately euthanized and tissues were collected. Rats #11-20 were exposed to 100 ppm nonane for 4 hours and were euthanized 4 hours post-exposure.

Nonane liver concentration (μg/g) immediately following 4-hour 100 ppm exposure										
Sample Number	**Rat #1**	**Rat #2**	**Rat #3**	**Rat #4**	**Rat #5**	**Rat #6**	**Rat #7**	**Rat #8**	**Rat #9**	**Rat #10**
1	2.7	2.1	13.1	5.5	5.3	3.4	2.9	0.5	2.1	1.2
2	4.5	3.1	4.9	5.5	4.4	6.1	3.9	2.5	2.0	1.1
3	5.4	3.7	3.7	5.9	6.1	4.0	3.4	3.0	1.8	1.0
Average =	4.2	2.9	7.3	5.7	5.3	4.5	3.4	2.0	2.0	1.1
SD =	1.4	0.8	5.1	0.3	0.9	1.4	0.5	1.3	0.2	0.1
Nonane liver concentration (μg/g) 4 hours after cessation of 4-hour 100 ppm exposure										
Sample Number	**Rat #11**	**Rat #12**	**Rat #13**	**Rat #14**	**Rat #15**	**Rat #16**	**Rat #17**	**Rat #18**	**Rat #19**	**Rat #20***
1	0.9	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	
2	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	
3	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0	
Average =	0.3	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	
SD =	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	

*Rat #20 was completely removed from the study due to a malfunctioning cannula.

Table 4: Nonane concentration (μg/g) in fat following 4-hour 100 ppm exposure.
Rats #1-10 were exposed to 100 ppm nonane for 4 hours, immediately euthanized and tissues were collected. Rats #11-20 were exposed to 100 ppm nonane for 4 hours and were euthanized 4 hours post-exposure.

Nonane fat concentration (μg/g) immediately following 4-hour 100 ppm exposure										
Sample Number	**Rat #1**	**Rat #2**	**Rat #3**	**Rat #4**	**Rat #5**	**Rat #6**	**Rat #7**	**Rat #8**	**Rat #9**	**Rat #10**
1	42.5	3.9	40.7	31.8	21.7	17.6	17.8	12.8	28.8	21.0
2	40.4	46.2	57.9	26.4	10.3	18.3	15.4	10.6	25.5	48.1
3	20.5	37.6	41.0	34.2	32.5	16.0	23.3	14.7	18.4	41.0
Average =	34.5	29.2	46.5	30.8	21.5	17.3	18.8	12.7	24.2	36.7
SD =	12.1	22.4	9.8	4.0	11.1	1.2	4.1	2.1	5.3	14.1
Nonane fat concentration (μg/g) 4 hours after cessation of 100 ppm exposure										
Sample Number	**Rat #11**	**Rat #12**	**Rat #13**	**Rat #14**	**Rat #15**	**Rat #16**	**Rat #17**	**Rat #18**	**Rat #19**	**Rat #20***
1	19.6	17.3	13.3	23.9	28.9	17.4	15.3	8.4	5.7	
2	16.9	6.8	23.8	37.2	9.7	14.1	9.9	11.8	15.7	
3	14.1	8.8	48.2	40.7	28.0	17.0	17.4	13.9	19.7	
Average =	16.9	11.0	28.4	33.9	22.2	16.2	14.2	11.4	13.7	
SD =	2.8	5.6	17.9	8.9	10.8	1.8	3.9	2.8	7.2	

*Rat #20 was completely removed from the study due to a malfunctioning cannula.

Table 5: Nonane concentration (µg/g) in muscle following 4-hour 100 ppm exposure. Rats #1-10 were exposed to 100 ppm nonane for 4 hours, immediately euthanized and tissues were collected. Rats #11-20 were exposed to 100 ppm nonane for 4 hours, and were euthanized 4 hours post-exposure.

Nonane muscle concentration (µg/g) immediately following 4-hour100 ppm exposure										
Sample Number	**Rat #1**	**Rat #2**	**Rat #3**	**Rat #4**	**Rat #5**	**Rat #6**	**Rat #7**	**Rat #8**	**Rat #9**	**Rat #10**
1	4.9	2.2	9.3	2.2	5.7	2.6	1.3	1.4	1.5	1.1
2	3.6	3.0	2.3	2.9	4.2	1.5	1.5	1.4	1.6	1.2
3	4.2	2.3	2.7	3.1	4.7	1.3	1.7	1.2	2.4	3.7
Average =	4.2	2.5	4.7	2.7	4.8	1.8	1.5	1.4	1.8	2.0
SD =	0.7	0.4	3.9	0.4	0.8	0.7	0.2	0.1	0.5	1.5
Nonane muscle concentration (µg/g) 4 hours after cessation of 4-hour 100 ppm exposure										
Sample Number	**Rat #11**	**Rat #12**	**Rat #13**	**Rat #14**	**Rat #15**	**Rat #16**	**Rat #17**	**Rat #18**	**Rat #19**	**Rat #20***
1	1.1	1.0	0.2	1.4	4.6	0.1	0.5	0.2	0.3	
2	0.3	0.5	0.2	0.2	0.3	0.1	0.3	0.8	0.7	
3	0.1	1.4	1.4	2.2	0.1	0.3	0.1	0.3	0.1	
Average =	0.5	1.0	0.6	1.2	1.6	0.2	0.3	0.4	0.4	
SD =	0.5	0.4	0.7	1.0	2.5	0.1	0.2	0.3	0.3	

*Rat #20 was completely removed from the study due to a malfunctioning cannula.

Table 6: Nonane concentration (μg/g) in blood during and after 500 ppm exposure.
Rats #21 - 30 were exposed to 500 ppm nonane for 4 hours and then were euthanized. Rats # 31 - 40 were exposed to 500 ppm nonane for 4 hours and were euthanized 4 hours post-exposure.

Nonane blood concentration (μg/g) during 500 ppm exposure										
Sample Time (hours)	**Rat #21**	**Rat #22**	**Rat #23**	**Rat #24**	**Rat #25**	**Rat #26**	**Rat #27**	**Rat #28**	**Rat #29**	**Rat #30***
0.5	2.661	2.243	2.022	3.162	1.998	1.584	1.698	2.014	2.071	
1.0	1.861	2.506	2.446	1.925	2.795	1.505	1.609	1.725	2.262	
2.0	3.636	4.196	5.971	6.661	3.266	2.465	3.623	3.661	2.630	
3.0	3.179	4.301	3.398	7.520	3.868	2.101	4.124	2.227	3.609	
4.0	6.086	2.007	5.582	6.312	4.233	5.096	3.830	2.243	4.416	
Nonane blood concentration (μg/g) during and following 500 ppm exposure										
Sample Time (hours)	**Rat #31**	**Rat #32**	**Rat #33**	**Rat #34**	**Rat #35**	**Rat #36**	**Rat #37**	**Rat #38**	**Rat #39**	**Rat #40**
0.5	1.406	1.745	1.547	2.061	1.713	3.360	3.235	1.664	1.281	2.493
1.0	2.142	1.403	1.443	1.942	1.122	3.272	2.697	1.877	3.329	3.398
2.0	3.165	3.148	3.535	2.638	3.209	4.178	3.643	5.103	4.967	3.777
3.0	3.270	2.186	2.074	3.255	3.616	3.858	6.010	4.774	5.482	1.818
4.0	4.727	4.082	4.626	3.953	3.304	3.223	6.402	4.109	5.010	4.868
4.08	3.253	3.547	2.855	2.580	3.564	3.943	5.022	3.902	4.489	4.342
4.17	2.870	2.615	2.355	1.847	2.762	1.418	4.038	2.488	3.171	4.282
4.25	1.488	2.043	0.669	1.719	1.707	2.902	1.961	1.957	2.540	2.544
4.50	1.424	1.539	1.591	1.411	1.411	2.017	2.344	1.338	2.145	2.455
5.00	1.031	1.133	1.265	0.663	0.628	1.469	1.099	1.185	1.512	1.851
6.00	0.559	0.693	0.524	0.468	0.130	0.594	0.305	0.255	0.418	0.152
7.00	0.444	0.506	0.398	0.225	0.104	0.508	0.604	0.129	0.292	0.444
8.00	0.322	0.151	0.264	0.318	0.148	0.396	0.281	0.096	0.343	0.190

*Rat #30 was completely removed from the study due to a malfunctioning cannula.

Table 7: Nonane concentration (μg/g) in liver following 4-hour 500 ppm exposure.
Rats #21 - 30 were exposed to 500 ppm nonane for 4 hours, immediately euthanized and tissues were collected. Rats # 31 - 40 were exposed to 500 ppm nonane for 4 hours and were euthanized 4 hours post-exposure.

Nonane liver concentration (μg/g) immediately following 4-hour 500 ppm exposure										
Sample Number	**Rat #21**	**Rat #22**	**Rat #23**	**Rat #24**	**Rat #25**	**Rat #26**	**Rat #27**	**Rat #28**	**Rat #29**	**Rat #30***
1	38.801	38.552	39.813	76.932	48.188	30.8	26.0	24.7	32.0	
2	63.805	52.875	25.684	64.848	39.172	38.4	25.3	26.2	45.2	
3	55.310	36.957	17.446	47.650	55.124	36.2	23.3	27.2	33.5	
Average =	52.6	42.8	27.6	63.1	47.5	35.1	24.9	26.0	36.9	
SD =	12.7	8.8	11.3	14.7	8.0	3.9	1.4	1.3	7.2	
Nonane liver concentration (μg/g) 4 hours after cessation of 500 ppm exposure										
Sample Number	**Rat #31**	**Rat #32**	**Rat #33**	**Rat #34**	**Rat #35**	**Rat #36**	**Rat #37**	**Rat #38**	**Rat #39**	**Rat #40**
1	0.671	lost	lost	0.465	0.289	0.363	0.410	0.380	0.640	0.558
2	0.780	0.465	0.425	0.546	0.363	0.392	0.480	0.274	0.701	0.469
3	0.814	0.432	0.516	0.531	0.329	0.411	0.379	0.441	0.433	0.526
Average =	0.755	0.449	0.471	0.514	0.327	0.389	0.423	0.365	0.591	0.518
SD =	0.075	0.023	0.064	0.043	0.037	0.024	0.052	0.085	0.140	0.045

*Rat #30 was completely removed from the study due to a malfunctioning cannula.

Table 8: Nonane concentration (μg/g) in fat following 4-hour 500 ppm exposure.
Rats #21 - 30 were exposed to 500 ppm nonane for 4 hours, immediately euthanized and tissues were collected. Rats # 31 - 40 were exposed to 500 ppm nonane for 4 hours and were euthanized 4 hours post-exposure.

Nonane fat concentration (μg/g) immediately following 4-hour 500 ppm exposure										
Sample Number	**Rat #21**	**Rat #22**	**Rat #23**	**Rat #24**	**Rat #25**	**Rat #26**	**Rat #27**	**Rat #28**	**Rat #29**	**Rat #30***
1	129.8	146.3	113.5	48.7	118.3	111.3	147.2	220.7	79.8	
2	110.8	67.5	263.6	26.3	287.6	134.5	88.4	173.8	117.3	
3	162.6	57.2	192.3	54.2	231.7	135.6	72.2	113.4	135.4	
Average =	134.4	90.3	189.8	43.1	212.5	127.1	102.6	169.3	110.8	
SD =	26.2	48.7	75.1	14.8	86.3	13.7	39.5	53.8	28.4	
Nonane fat concentration (μg/g) 4 hours after cessation of 4-hour 500 ppm exposure										
Sample Number	**Rat #31**	**Rat #32**	**Rat #33**	**Rat #34**	**Rat #35**	**Rat #36**	**Rat #37**	**Rat #38**	**Rat #39**	**Rat #40**
1	69.9	64.4	84.1	101.8	79.8	75.0	85.0	99.1	41.3	58.3
2	150.1	56.5	84.4	158.4	66.1	70.6	112.4	55.9	42.2	73.0
3	125.7	64.9	87.5	91.2	81.6	66.5	123.8	100.2	37.9	58.4
Average =	115.2	61.9	85.3	117.1	75.8	70.7	107.1	85.1	40.5	63.2
SD =	41.1	4.7	1.9	36.1	8.5	4.3	19.9	25.3	2.3	8.5

*Rat #30 was completely removed from the study due to a malfunctioning cannula.

Table 9: Nonane concentration (µg/g) in muscle following 4-hour 500 ppm exposure.
Rats #21 - 30 were exposed to 500 ppm nonane for 4 hours, immediately euthanized and tissues were collected. Rats # 31 - 40 were exposed to 500 ppm nonane for 4 hours and were euthanized 4 hours post-exposure.

Nonane muscle concentration (µg/g) immediately following 4-hour 500 ppm exposure										
Sample Number	**Rat #21**	**Rat #22**	**Rat #23**	**Rat #24**	**Rat #25**	**Rat #26**	**Rat #27**	**Rat #28**	**Rat #29**	**Rat #30***
1	11.35	17.51	14.29	17.37	14.23	25.92	8.32	14.72	14.92	
2	18.83	14.52	11.57	15.47	29.33	22.58	18.47	14.22	26.46	
3	13.33	18.78	12.46	12.59	13.36	31.49	19.44	17.33	18.93	
Average =	14.50	16.94	12.77	15.14	18.97	26.66	15.41	15.42	20.10	
SD =	3.88	2.19	1.39	2.41	8.98	4.50	6.16	1.67	5.86	
Nonane muscle concentration (µg/g) 4 hours after cessation of 4-hour 500 ppm exposure										
Sample Number	**Rat #31**	**Rat #32**	**Rat #33**	**Rat #34**	**Rat #35**	**Rat #36**	**Rat #37**	**Rat #38**	**Rat #39**	**Rat #40**
1	1.41	0.92	20.67	2.43	6.59	1.87	0.69	4.94	7.54	lost
2	2.17	5.14	1.48	3.35	8.52	1.20	16.61	2.20	4.00	4.32
3	17.67	1.80	3.11	1.32	1.86	6.87	3.30	3.27	1.90	6.68
Average =	7.08	2.62	8.42	2.37	5.66	3.31	6.87	3.47	4.48	5.50
SD =	9.18	2.23	10.64	1.02	3.43	3.10	8.54	1.38	2.85	1.67

*Rat #30 was completely removed from the study due to a malfunctioning cannula.

Table 10: Nonane concentration (μg/g) in blood during and after 1000 ppm exposure.
Rats #41 - 50 were exposed to 1000 ppm nonane for 4 hours and then were euthanized. Rats # 51 - 60 were exposed to 1000 ppm nonane for 4 hours and were euthanized 4 hours post-exposure.

Nonane blood concentration (μg/g) during 4-hour 1000 ppm exposure										
Sample Time (hours)	**Rat #41**	**Rat #42**	**Rat #43**	**Rat #44**	**Rat #45**	**Rat #46**	**Rat #47**	**Rat #48**	**Rat #49**	**Rat #50**
0.5	5.120	7.068	5.460	10.053	6.790	8.768	7.352	5.839	8.337	7.908
1.0	9.544	11.015	10.134	12.581	9.345	10.198	7.032	8.549	9.200	11.689
2.0	6.333	17.248	15.742	12.948	12.235	15.213	8.598	9.720	15.198	18.250
3.0	15.447	17.446	18.646	19.473	14.747	17.392	19.402	12.643	15.959	16.732
4.0	20.068	27.580	21.563	20.499	21.818	21.505	11.286	5.227	9.230	7.302
Nonane blood concentration (μg/g) during and following 4-hour 1000 ppm exposure										
Sample Time (hours)	**Rat #51**	**Rat #52**	**Rat #53**	**Rat #54**	**Rat #55**	**Rat #56**	**Rat #57**	**Rat #58**	**Rat #59**	**Rat #60**
0.5	11.568	17.916	8.289	0.040	6.925	1.827	1.654	5.455	2.450	6.316
1.0	7.421	13.267	4.522	0.000	10.613	3.654	3.650	8.730	2.879	7.818
2.0	25.865	20.851	10.294	0.037	19.497	10.886	7.601	14.207	13.350	14.345
3.0	31.151	28.986	22.551	0.113	17.361	14.236	7.165	13.042	13.571	4.288
4.0	32.923	36.853	13.064	0.075	21.010	4.788	12.504	5.089	6.357	15.615
4.08	23.647	24.722	7.812	0.090	15.679	14.744	7.055	9.865	11.555	7.550
4.17	21.954	21.579	12.894	0.068	17.422	3.767	6.217	2.422	9.735	1.933
4.25	20.888	17.116	2.480	0.180	13.145	5.892	4.884	6.265	6.247	6.608
4.50	4.208	1.989	3.738	0.112	11.882	6.465	3.902	8.869	1.229	3.375
5.00	9.188	9.059	7.028	0.094	6.338	2.544	0.564	1.334	0.709	2.862
6.00	5.680	3.097	3.221	died	2.832	0.412	1.078	3.371	1.445	0.266
7.00	3.386	2.271	1.871		1.746	0.472	0.202	1.228	1.078	1.093
8.00	0.349	1.044	1.478		0.542	0.939	0.812	0.156	0.718	0.143

Table 11: Nonane concentration (µg/g) in liver following 4-hour 1000 ppm exposure.
Rats #41 - 50 were exposed to 1000 ppm nonane for 4 hours, immediately euthanized and tissues were collected. Rats # 51 - 60 were exposed to 1000 ppm nonane for 4 hours and were euthanized 4 hours post-exposure.

Nonane liver concentration (µg/g) immediately following 4-hour 1000 ppm exposure										
Sample Number	**Rat #41**	**Rat #42**	**Rat #43**	**Rat #44**	**Rat #45**	**Rat #46**	**Rat #47**	**Rat #48**	**Rat #49**	**Rat #50**
1	147.4	25.9	132.1	29.6	33.9	47.2	174.9	151.4	156.9	111.2
2	21.9	33.1	124.9	53.4	173.0	148.2	156.4	38.1	132.7	112.7
3	56.8	126.7	140.1	49.5	135.0	130.5	70.5	138.2	28.3	116.8
Average =	75.4	61.9	132.4	44.2	114.0	108.6	133.9	109.2	106.0	113.6
SD =	64.8	56.2	7.6	12.8	71.9	53.9	55.7	62.0	68.3	2.9
Nonane liver concentration (µg/g) 4 hours after cessation of 4-hour 1000 ppm exposure										
Sample Number	**Rat #51**	**Rat #52**	**Rat #53**	**Rat #54***	**Rat #55**	**Rat #56**	**Rat #57**	**Rat #58**	**Rat #59**	**Rat #60**
1	20.74	9.15	1.33		2.65	4.42	2.84	7.77	6.52	2.67
2	16.13	9.01	8.12		4.92	8.77	2.84	6.60	6.18	4.02
3	18.77	12.52	6.71		5.32	2.47	2.63	8.69	7.76	3.60
Average =	18.55	10.23	5.39		4.30	5.22	2.77	7.69	6.82	3.43
SD =	2.31	1.99	3.58		1.44	3.23	0.12	1.05	0.83	0.69

*Rat #54 died during the 4-hour post-exposure period.

Table 12: Nonane concentration (μg/g) in fat following 4-hour 1000 ppm exposure.
Rats #41 - 50 were exposed to 1000 ppm nonane for 4 hours, immediately euthanized and tissues were collected. Rats # 51 - 60 were exposed to 1000 ppm nonane for 4 hours and were euthanized 4 hours post-exposure.

Nonane fat concentration (μg/g) immediately following 4-hour 1000 ppm exposure										
Sample Number	**Rat #41**	**Rat #42**	**Rat #43**	**Rat #44**	**Rat #45**	**Rat #46**	**Rat #47**	**Rat #48**	**Rat #49**	**Rat #50**
1	461.7	333.8	133.6	228.8	163.9	625.2	146.7	353.2	384.3	225.4
2	299.3	287.6	149.2	274.4	117.3	648.0	143.5	289.6	492.9	376.1
3	617.1	292.1	132.1	163.6	283.9	lost	204.7	1648.9	400.6	684.1
Average =	459.4	304.5	138.3	222.3	188.4	636.6	165.0	763.9	425.9	428.5
SD =	158.9	25.5	9.5	55.7	86.0	16.1	34.4	767.1	58.6	233.8
Nonane fat concentration (μg/g) 4 hours after cessation of4-hour 1000 ppm exposure										
Sample Number	**Rat #51**	**Rat #52**	**Rat #53**	**Rat #54***	**Rat #55**	**Rat #56**	**Rat #57**	**Rat #58**	**Rat #59**	**Rat #60**
1	246.9	957.7	355.7		325.3	235.1	296.1	218.7	193.0	207.2
2	178.4	474.9	357.2		323.0	186.6	369.9	239.9	272.1	720.6
3	348.5	563.7	334.4		201.1	273.4	307.2	316.8	138.0	501.1
Average =	257.9	665.4	349.1		283.1	231.7	324.4	258.5	201.0	476.3
SD =	85.6	257.0	12.8		71.1	43.5	39.8	51.6	67.4	257.6

*Rat #54 died during the 4-hour post-exposure period.

Table 13: Nonane concentration (μg/g) in muscle following 4-hour 1000 ppm exposure.
Rats #41 - 50 were exposed to 1000 ppm nonane for 4 hours, immediately euthanized and tissues were collected. Rats # 51 - 60 were exposed to 1000 ppm nonane for 4 hours and were euthanized 4 hours post-exposure.

Nonane muscle concentration (μg/g) immediately following 4-hour 1000 ppm exposure										
Sample Number	**Rat #41**	**Rat #42**	**Rat #43**	**Rat #44**	**Rat #45**	**Rat #46**	**Rat #47**	**Rat #48**	**Rat #49**	**Rat #50**
1	36.28	26.85	34.49	23.99	59.06	70.83	44.35	81.02	44.87	64.07
2	27.01	31.75	37.37	34.43	43.60	60.33	36.25	44.32	41.19	116.81
3	33.08	lost	26.97	47.40	16.18	45.34	38.62	60.06	75.35	31.44
Average =	32.1	29.3	32.9	35.3	39.6	58.8	39.7	61.8	53.8	70.8
SD =	4.7	3.5	5.4	11.7	21.7	12.8	4.2	18.4	18.8	43.1
Nonane muscle concentration (μg/g) 4 hours after cessation of 4-hour 1000 ppm exposure										
Sample Number	**Rat #51**	**Rat #52**	**Rat #53**	**Rat #54***	**Rat #55**	**Rat #56**	**Rat #57**	**Rat #58**	**Rat #59**	**Rat #60**
1	14.10	12.67	25.72		20.81	5.52	15.24	14.52	15.18	17.34
2	21.95	18.94	10.45		30.64	16.77	7.28	12.51	26.19	6.05
3	10.96	52.26	29.49		14.57	16.86	44.41	11.31	40.53	3.28
Average =	15.7	28.0	21.9		22.0	13.1	22.3	12.8	27.3	8.9
SD =	5.7	21.3	10.1		8.1	6.5	19.5	1.6	12.7	7.4

*Rat #54 died during the 4-hour post-exposure period.

Data Analysis

Nonane typically occurs in JP-8 at levels around 0.8 percent and has an octanol:water partition coefficient (K_{ow}) of 5.65. It is metabolized to 2-nonanol by P450 enzymes, and further to 2-nonanone by alcohol dehydrogenase. An earlier analysis of the nonane data is described in a previous Technical Report (Robinson, 2000). The current analysis was improved based on the harmonized decane-nonane model described above in the current report. Figure 6 shows a schematic for this improved harmonized model.

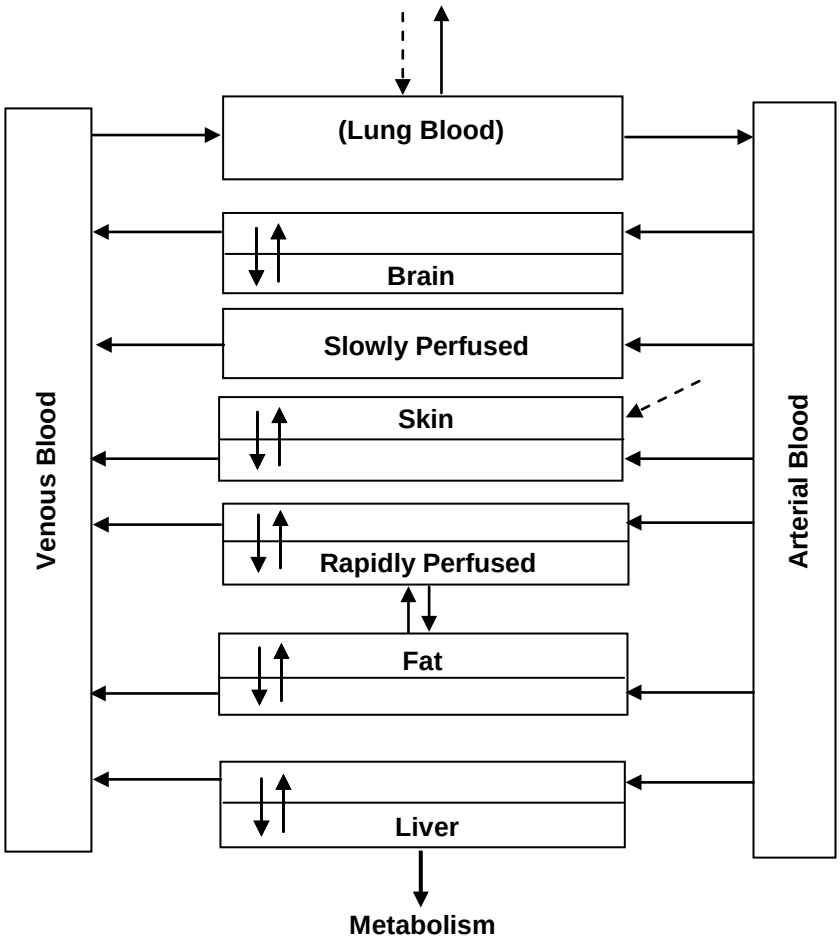

Figure 5. Schematic for the basic harmonized structure of the nonane model. Fat, brain, skin, liver and rapidly perfused tissues are described by diffusion limitation; the slowly perfused tissues are described as flow limited. Exposure routes include inhalation and dermal absorption, as indicated by dashed arrows.

Figure 6 shows model simulations obtained by fitting our harmonized model to the data in Tables 2-13 above, using blood:air and tissue:blood partition coefficients (see Table 1) determined in this study. Physiological parameters used in the fits are shown in Table 14, while Table 15 shows the partition coefficients used and remaining fitted parameters. In these and the

following figures, predicted curves were obtained using the rat physiological and chemical parameters given in Tables 14 and 15.

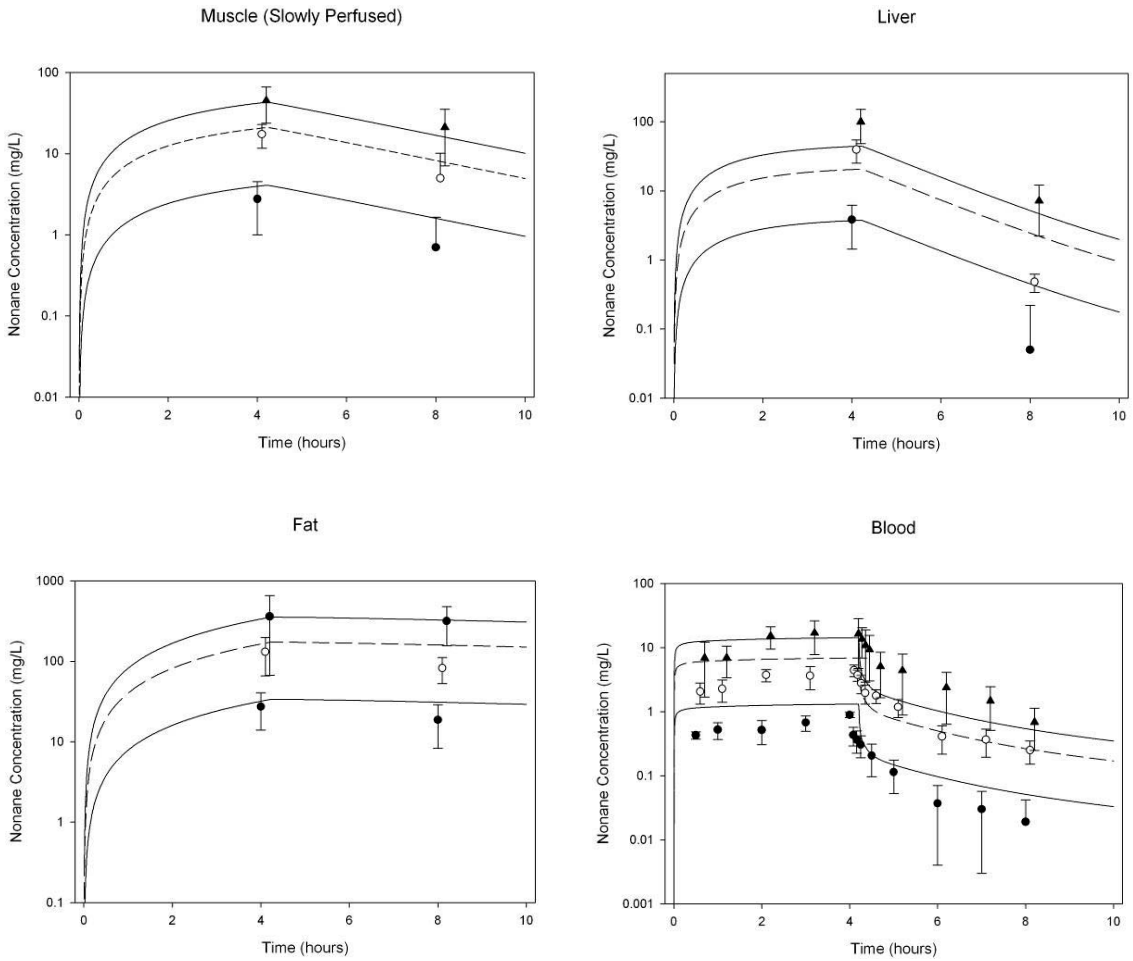

Figure 6. Model simulations (lines) versus measured nonane concentrations in muscle, liver, fat and blood from nose-only exposures to 100, 500 and 1000 ppm nonane for 4 hours. In this and the following figures, data points are given as means and standard deviations.

Table 14. Physiological parameter values

Parameter	Name	Rat
BW	Body weight (kg)	0.3
Q_C	Cardiac output (blood flow) (L/hour-kg)	15
Q_P	Alveolar ventilation (L/hour-kg)	24.8
Blood Flows to Tissues [fraction of Q_C]		
Q_L	Liver	0.25
Q_F	Fat	0.09
Q_{Br}	Brain	0.03
Q_{Sk}	Skin	0.02
Q_R	Rapidly perfused 0.76-*QL*-*QBr*	0.48
Q_S	Slowly perfused 0.24-*QF*-*QSk*	0.13
Tissue volumes [fractions of BW]		
V_L	Liver	0.04
V_F	Fat	0.09
V_{Br}	Brain	0.0057
V_{Sk}	Skin	0.19
V_B	Alveolar blood	0.007
V_R	Rapidly perfused	0.064
V_S	Slowly perfused	0.47
Tissue blood volumes [fractions of tissue volumes]		
V_{LB}	Blood fraction of liver	0.21
V_{FB}	Blood fraction of fat	0.02
V_{SkB}	Blood fraction of skin	0.08
V_{BrB}	Blood fraction of brain	0.03
V_{RB}	Blood fraction of rapidly perfused	0.21[1]
V_{SB}	Blood fraction of slowly perfused	0.04[1]

Source: Brown *et al.* (1997)

[1] based on blood volumes of kidney and muscle for rapidly and slowly perfused, respectively

Table 15. Chemical-specific parameter values

Partition Coefficients	**Value**
P_B Blood/air	5.2[R]
P_L Liver/blood	3.5[Z]
P_F Fat/blood	282[R]
P_{Br} Brain/blood	5.0[S]
P_S Slowly perfused (muscle)/blood	4.0[Z]
P_R Rapidly perfused (kidney)/blood	2.0[Z]
P_{Sk} Skin/blood	4.0[D]
Diffusion Rates	
PA_F permeability area cross product - fat absorption (L/hour) (Zahlsen *et al.*, 1990 data)	0.7[f]
PA_F permeability area cross product - fat absorption (L/hour)	0.8[f]
PA_L permeability area cross product – liver (L/hour)	0.07[f]
PA_{Sk} permeability area cross product – skin (L/hour)	0.4[Df]
PA_{Br} permeability area cross product – brain (L/hour)	0.5 [f]
PA_R permeability area cross product – rapidly perfused (L/hour)	1.0[f]
PA_S permeability area cross product – slowly perfused (L/hour)	0.5[f]
Vmax maximum velocity of saturable metabolism (mg/hour)	0.0[f]
Km Affinity of saturable metabolism (mg/L)	1.5[M]
K_{RF} Rapidly perfused-Fat intertissue transfer rate (L/hour)	0.00001[f]
K_{FR} Fat-Rapidly perfused intertissue transfer rate (L/hour)	0.0005[f]

[R] Robinson (2004)
[M] Mortensen *et al.* (2000)
[D] Based on skin data of Perleberg *et al.* (2004)
[Df] Based on fitted decane concentration in skin (Merrill *et al.*, 2008)
[f] visually optimized to available kinetic data
[S] Based on value for decane Smith *et al*. (2005)
[Z] Zahlsen *et al.* (1992)
Note: The tissue:blood partition coefficient (PC) values used for slowly and rapidly perfused were derived from muscle:blood and kidney:blood PCs, respectively (Smith *et al.*, 2005; Zahlsen *et al.*, 1990; Lof *et al.*, 1999).

Supporting Data and Results

Zahlsen *et al.* (1992)

Rats were exposed in closed chamber to 100 ppm nonane, 12 hours daily for 3 days. Simulations of these data are shown in Figure 7. Predicted curves were obtained using previously fitted values for Vmax (from earlier fitting to in house data) and Km (Mortensen *et al.*, 2000), together with the rat physiological and chemical parameters given in Tables 14 and 15.

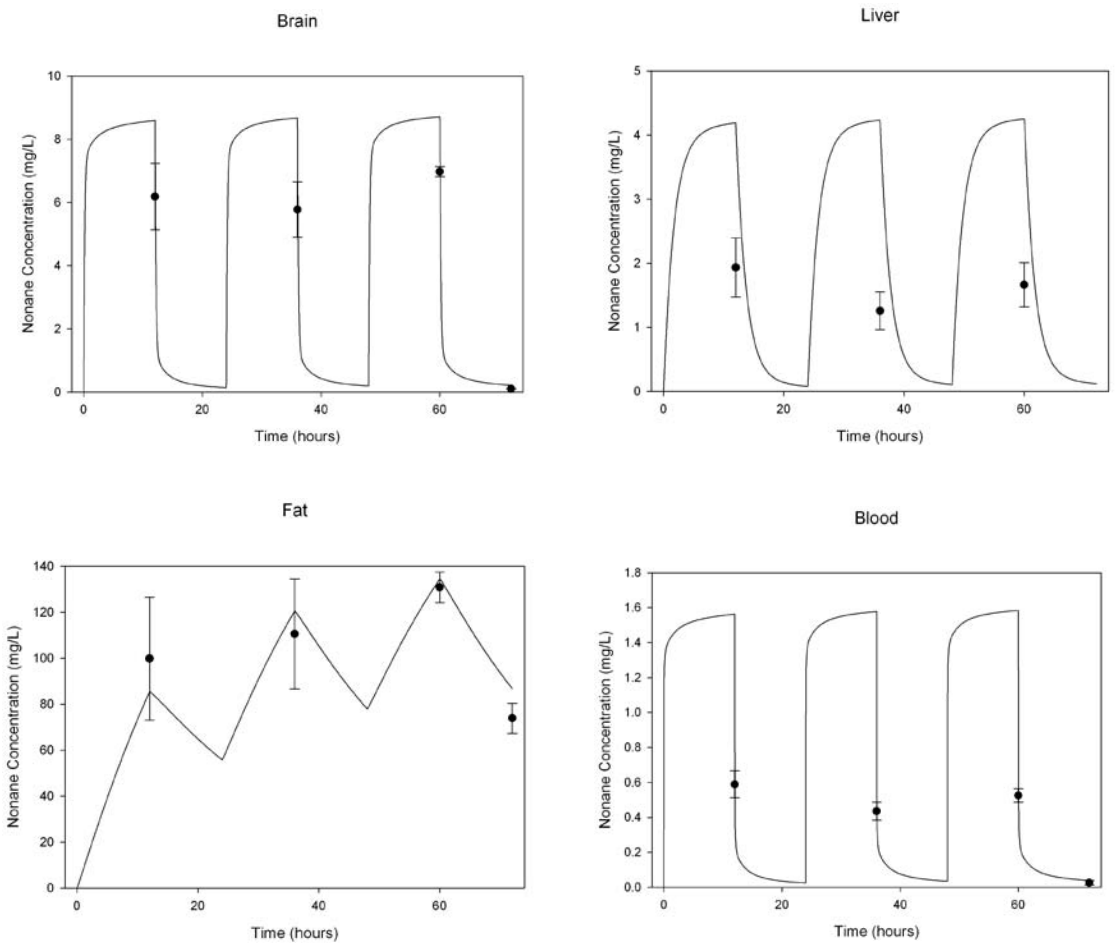

Figure 7. Model simulations (lines) versus measured nonane concentration (circles) in brain, liver, fat and blood in rats exposed in closed chamber to 100 ppm n-nonane, 12 hours/day for 3 days. Data (mean ±SD) are from Zahlsen *et al.* (1992).

Zahlsen *et al.* (1990)

Figures 8 and 9 show rat brain and blood nonane concentrations following exposure at 1000 ppm, 12 hours daily, for up to 14 days.

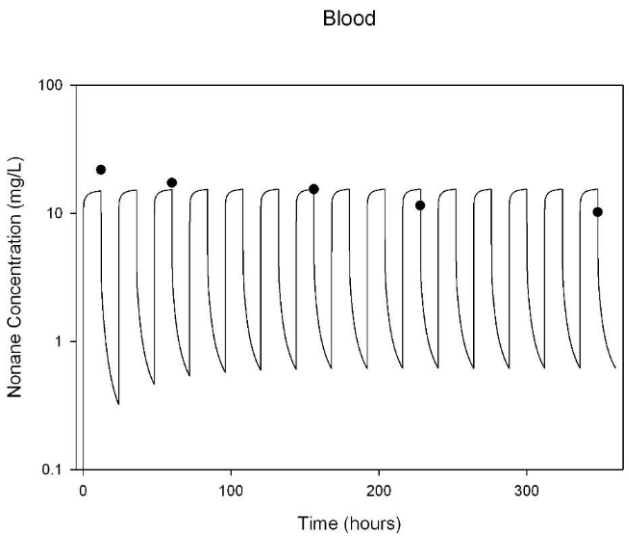

Figure 8. Model prediction of rat blood nonane concentration for the exposure scenario of Zahlsen *et al.* (1990) (1000 ppm, 12 hours daily for 14 days). Data are adapted from Figure 1 of that paper.

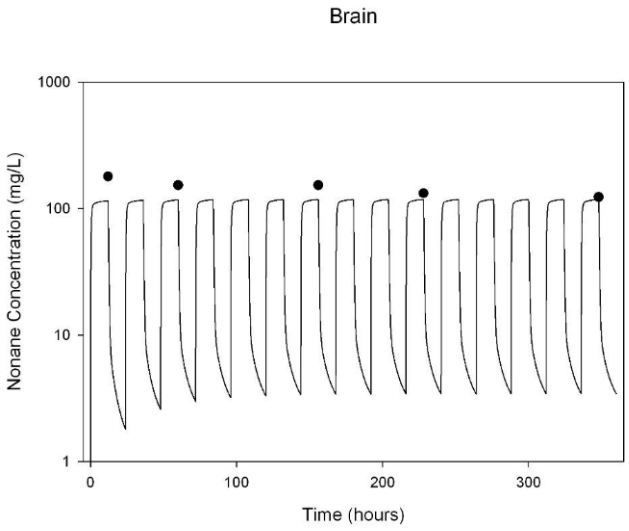

Figure 9. Model prediction of rat brain nonane concentration for the exposure scenario of Zahlsen *et al.* (1990) (1000 ppm, 12 hours daily for up to 14 days) using measured and previously fitted parameter values. Data are adapted from Figure 2 of that paper.

DISCUSSION

Complex mixtures such as JP-8 consist of hundreds, possibly thousands, of individual, though often chemically similar, components. An approach to dealing with such mixtures has been outlined in a previous Technical Report (Robinson, 2004). To facilitate the pharmacokinetic and toxicokinetic analyses of such complex mixtures, a number of simplifying assumptions were made. First, the interconnection of PBPK models of individual substances, via binary mechanisms, is enough to predict the interactions present in the mixture. Secondly, complex mixtures can often be approximated as pseudo-binary systems, consisting of the compound of interest plus a single interacting complex vehicle with well-defined, composite properties. This conceptual approach is applicable to a number of different kinds of potential interaction mechanisms, including substrate metabolism and dermal absorption.

In a complex mixture with components that interact via competitive metabolic inhibition of a specific enzyme, the metabolic rate of each component can be described as if it were part of a binary mixture, with the total mixture concentration replacing that of the single competitor, and the effective inhibition constant given by the (concentration-weighted) harmonic mean of the individual (binary) inhibition constants. For JP-8, with more than 300 components, to describe these processes based on binary interactions alone would require on the order of 10^5 interaction terms. This is clearly too large a number for each to be individually measured.

However, for a sufficiently large number of chemically similar compounds with each making up a small proportion of the total mixture (as is the case with JP-8), an alternative statistical approach can be taken. Some inhibition constants for metabolism of JP-8 components and

similar compounds via P-450 2E1 have been measured in the rat; these values were found to closely approximate a log-normal distribution with a calculated mean of 0.42 mg/L and a harmonic mean of 0.25 mg/L. Based on these characteristics, values for other potential interactions can be inferred statistically (Robinson, 2004). When combined with a detailed PBPK model of a specific component of interest (such as nonane, as outlined above), such an approach describes the behavior of that component in the overall context of JP-8 exposure.

Since partitioning into tissues is a critical determinant of target site dosimetry, it is also important to consider the potential for interaction and interference at this point. There is no direct evidence for the effect of other JP-8 components on the blood:air or tissue:blood partition coefficients for a particular component for most tissues. However, indirect evidence relating to mixture effects on the partitioning of components into the skin as a result of dermal exposure sheds some light on this issue. The following experiments were performed in the laboratory of Dr. Jim Riviere, North Carolina State University (Riviere and Brooks, 2007).

Hydrocarbon partition coefficients were estimated using an inert, silastic membrane-coated fiber (MCF) system. An inert fiber coated with various types of membranes is immersed into the test substance and the amount of absorption is measured using gas chromatograph/mass spectrometry (GC/MS) techniques. For each component, different membranes and different vehicles can be used, each of which can be selected to determine the effect of the vehicle (in this case the other JP-8 components) on their partitioning capacity. Various molecular interactions can be approximated by using different combinations of MCF fibers and solvents. These values are being used by Dr. Subhash Basak (University of Minnesota, personal communication) for

hierarchical QSAR (quantitative structure-activity relationship, HiQSAR) system development to predict skin permeation of chemicals, although they could presumably be used to predict partitioning into other tissues as well. Absorption data from three fibers using three different solvents are being used to develop the *ab initio* calculations using Hartree-Fock (HF) basis sets and density functional theory. Among the other calculations used were EHOMO (energy of the highest occupied molecular orbital), EHOMO–1 (energy of the second highest occupied molecular orbital), ELUMO (energy of the lowest unoccupied molecular orbital), ELUMO+1 (energy of the second lowest unoccupied molecular orbital) and the dipole moment. The EHOMO and HF energy show strong correlations with partitioning, but the other descriptors were only poorly correlated.

Many components of JP-8 are "minor" components, in the sense that they exist individually at very low concentrations, and they are of no particular toxicological concern in themselves. It is not worth developing fully-fledged PBPK models for such compounds, complete with measured blood and tissue partition coefficients, etc. However, it may be useful to have some idea of their kinetic behavior, as a prelude to assessing their potential interactions with components of greater intrinsic concern. For this purpose, a QSAR-based approach for estimating blood and tissue partition coefficients (PCs) may be useful (Basak *et al.*, 2003). In such an approach, PCs are estimated using information on the chemical's structural properties, together with key biological properties of the tissue itself (Poulin and Krishnan, 1995).

Brain Effects

The brain is a target organ for volatile hydrocarbons. White spirits in high concentrations have been shown to elevate the production of reactive oxygen species (ROS) in mammal central nervous systems (CNS) (Lam *et al.*, 1990). Myhre *et al.* (2000) demonstrated an increase in respiratory burst in human granulocytes with increasing doses of n-nonane; however, trimethylcyclohexane (TMCH) was a stronger stimulator of ROS production. In addition, aromatics, such as TMCH, accumulate in the brain to a greater extent than saturated alkanes (Zahlsen *et al.*, 1992). Therefore, in modeling brain dosimetry of JP-8 components, such as nonane and decane, consideration should be given to the potential for such competition between compounds for brain sequestration.

Anesthetic effects, including circulatory alterations, are seen from volatile hydrocarbons. This may result in dose-dependant decreases in the rate of diffusion into non-critical organs, like fat, as blood flow is diverted to critical organs. Significant decreases in flows to kidneys and the gastro-intestinal (GI) tract, with significant increases in flows to brain, heart and liver during ether anesthesia have been reported (Stanek *et al.*, 1988). It is possible that with high or prolonged exposures to nonane, anesthesia induced changes in blood flow may be expected. Lof *et al.* (1999) reported sedation during the first day of exposure at 400 ppm of dearomatised white spirits, which contain significant amounts of both nonane and decane. In simulating the decane data from Perleberg *et al.* (2004), we found that satisfactory predictions of fat concentrations at the high dose could also be achieved either by reducing diffusion into fat tissue or by reducing fat blood flow (from 9 percent to 1 percent of cardiac output; Merrill *et al.*, 2008). It is likely that both effects are occurring, altering both permeability and capillary density in the fat. Capillary de-recruitment causes an increase in inter-capillary distances for diffusion, thus

reducing the effective diffusion rate into fat more at high exposures than at low exposures of the fuel. A harmonized model would take such a process into account as a biological response to fuel exposure.

Human Exposure Predictions

At present we do not have nonane tissue:air partition coefficients for human blood or other tissues. However, we may make extrapolations to human exposure conditions with limited confidence using human physiological values and rat partition coefficients, bearing in mind that this analysis will be modified once human partition data become available. Pleil *et al*. (2000) estimated ambient nonane concentrations for aircraft attendants and fuel tank entry attendants to be 1.8 and 34 ppb, respectively. Measured exhaled breath before and at some undefined period ("shortly") following exposure were used to calculate blood concentrations of nonane in these workers, using the blood:air partition coefficient for rat blood, and were compared with the predictions of an earlier version of the current model (Robinson, 2000). Given the very large uncertainties in the exposures and the calculations, the agreement of the predicted and experimental blood levels (inferred from exhaled air) for the aircraft attendants (1.8 ppb ambient nonane) both before and after exposure is quite reasonable. Blood levels following exposure are, if anything, overestimated by the model. This may be due to the fact that volatile hydrocarbons such as nonane have a greater affinity for rat red blood cells than human red blood cells (Lam *et al.*, 1990), leading to an overestimate of the blood:air partition coefficient, and hence inhalation uptake, for humans based on rat partition data.

Since these workers are likely exposed on a more or less daily basis, blood levels before exposure would be expected to approximate levels predicted some 20 hrs following exposure, as is indeed the case (Robinson, 2000).

On the other hand, blood levels for the tank entry attendants are greatly overestimated by the model (Robinson, 2000). This is likely due to the wearing of respirators by these tank entry workers; aircraft attendants do not wear respirators. If this is indeed the case, respirators seem to reduce blood levels of nonane by almost three orders of magnitude or more, if we allow for the likelihood that dermal exposure likely contributes significantly to blood levels in these individuals.

For purposes of validating human predictions with the proposed model, the Pleil *et al.* (2000) data are not suitable due to these exposure issues. However, we have reasonable confidence that the model can be used for the estimation of human dosimetry based on successful predictions of human blood, fat and exhaled breath achieved with a concurrently developed model for decane (Merrill *et al.*, 2008). The parameterization of the decane model was very similar to the parameterization of this model. As the harmonized model structure is applied to other individual and mixtures of fuel components, we will continue to perform human extrapolations to be compared with the best available data at that time.

CONCLUSION

A harmonized model complex enough to take into account all important physiological processes undergone by any component in an integrated fashion was developed on the basis of observed kinetic behavior of nonane, an aliphatic JP-8 component. Nonane is highly lipophilic and distributes preferentially in brain tissue. Its behavior in the body was described in a PBPK model that includes the blood:air exchange and additional compartments for liver, slowly perfused tissue, rapidly perfused tissue, brain, skin and fat. The model incorporates absorption via inhalation and dermal exposure. It predicts body burdens of nonane under occupational exposure conditions and is consistent with limited occupational body burden data.

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