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Preliminary Mathematical Model for Jet Fuel Exacerbated Noise-Induced Hearing Loss

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14. ABSTRACT
Laboratory studies support the potential for jet fuel to promote noise induced hearing loss. Noise alone induces hearing loss due to loss of hair cells in the cochlea, associated with oxidative stress. Jet fuel toxicity in association with noise may be at least partially explained by increased free radical production and oxidative stress at the cellular level, resulting in hair cell dysfunction and loss. This project combines a physiologically-based pharmacokinetic (PBPK) model to describe jet fuel component concentrations in the cochlea with pharmacodynamic (PD) models of free radical formation in the cochlea by both noise and jet fuel components, and mathematical models to predict the combined impact on hair cell functionality and loss. Further development of this preliminary combined PBPK-PD model of JP-8 induced hearing loss with noise will provide the basis for estimating the potential risk to humans exposed to the same chemical and sound scenarios in occupational settings.

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PREFACE

Funding for this project was provided through Defense Health Program (DHP) from the Surgeon General's (SG) Air Force Medical Support Agency (AFMSA) (SG5I/Medical Research and Innovations/Force Health Protection). This research was conducted under contract FA8650-10-2-6062 with the Henry M. Jackson Foundation for the Advancement of Military Medicine. The program manager for the contract was David R. Mattie, PhD (711 HPW/RHDJ), who was also the technical manager for this project. All animal data used in this project were from published or soon to be published literature sources.

The authors would like to acknowledge Jeffrey W. Fisher, PhD (Food and Drug Administration/National Center for Toxicological Research, Jefferson AK) for providing the pharmacokinetic model code and Lawrence D. Fechter, PhD (Jerry Pettis Memorial VA Medical Center, Loma Linda CA, retired) for providing data utilized in his publication figures (Fechter *et al.*, 2012).

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1.0 SUMMARY

There is a growing body of laboratory studies supporting the potential for jet fuel to promote hearing loss when exposures also include noise. Noise alone induces hearing loss, which is largely due to loss of hair cells in the cochlea, and is associated with oxidative stress. Jet fuel toxicity in association with noise may be at least partially explained by increased free radical production and oxidative stress at the cellular level, thereby exacerbating the reduction in functionality and loss of hair cells. The objective of this project is to develop preliminary mathematical models for predicting jet fuel induced potentiation of noise induced hearing loss.

Preliminary mathematical models were developed to describe jet fuel component pharmacokinetics, concentrations of jet fuel components at the target site (cochlea), free radical formation in the cochlea by both noise and jet fuel components, and the combined impact on hair cell functionality and loss. Jet fuel itself is a complex mixture of aromatic and aliphatic hydrocarbons, and free radical generation by jet fuel may be due to a number of chemical components within that mixture. An existing physiologically-based pharmacokinetic (PBPK) model for jet fuel that strategically lumps similar constituent chemicals into several representative groups has been adapted to include a cochlear compartment.

The effective jet fuel constitutive tissue concentrations are then used to drive a pharmacodynamic (PD) model predicting the concentration of reactive oxygen species (ROS) due to both noise exposure and the presence of jet fuel components in the cochlea. This, in turn, is used to drive a model for noise/fuel induced cellular damage and hearing loss. Development of the combined PBPK-PD model of JP-8 induced hearing loss with noise will provide the basis for estimating the potential risk to humans exposed to the same chemical and sound scenarios in occupational settings.

The model is preliminary, and is subject to further refinement and validation. Additional mechanisms by which chemical contaminants can disrupt hearing may also play a role in jet fuel exacerbated noise induced hearing loss; these mechanisms potentially include disruption of intracellular calcium homeostasis, receptor distribution, membrane fluidity, or efferent pathways synapsing at the cochlea.

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2.0 INTRODUCTION

Laboratory animal experiments, along with industrial epidemiological studies, have identified several chemical contaminants that can lead directly to hearing loss from the chemical exposure alone, or can increase noise induced hearing loss (NIHL) with concurrent exposure (Fechter *et al.*, 2007a). Common solvents such as toluene, ethylbenzene and p-xylene are known to cause hearing loss at higher concentrations and enhance noise-induced hearing loss at lower concentrations (Fechter *et al.*, 2007b).

The jet fuel JP-8 contains a maximum of 25 percent aromatics by specification (Fechter *et al.*, 2012). A percent of these aromatics include solvents such as those listed above that are known to impact hearing loss. The U.S. Department of Defense (DoD) has shown interest in whether exposure to jet fuel in noisy situations is damaging to the hearing of its personnel.

A study by Kaufman *et al.* (2005) indicated that U.S. Air Force (USAF) personnel with JP-4 and/or JP-8 exposure may indeed have increased hearing loss. Most jet fuel exposures in the epidemiological survey were estimated to be less than a third of the occupational exposure limit for JP-8 at that time (350 mg/m^3). Subjects with three or more years of exposure to jet fuel had a 70 percent increased chance of hearing loss, after age adjustment. However, this epidemiological study surveyed only a small number of personnel and encountered significant difficulty in finding USAF personnel that are unexposed to JP-8.

There is a growing body of laboratory studies supporting the potential for jet fuel to promote hearing loss when exposures include noise. Fechter *et al.* (2007a) were the first to show that exposure to JP-8 followed by exposure to damaging noise resulted in decreased auditory function as well as cochlear outer hair cell (OHC) loss in Long-Evans rats. JP-8 nose-only inhalation exposures were 1000 mg/m^3 for 4 hours on 1 or 5 days, immediately followed by 97, 102 or 105 dB noise for 4 hours. Later that year, Fechter *et al.* (2007b) found that a mixture of toluene and ethylbenzene may be at least partially responsible for the NIHL enhancement by JP-8. Another study by Fechter *et al.* in 2010 compared JP-8 and a synthetic jet fuel made by the Fischer-Tropsch process, which does not produce aromatic hydrocarbons. The synthetic fuel did not increase NIHL, while male Long-Evans rats showed increased NIHL when exposed to 1000 mg/m^3 JP-8 for 4 hours per day for 5 days, followed daily by an hour of 100 dB (damaging) noise. Fechter *et al.* (2012) have shown that subchronic (6 hours/day, 5 days/week, 4 weeks) nose-only JP-8 jet fuel exposure increased susceptibility of male and female Fischer 344 (F344) rats to non-damaging noise (85 dB, which does not cause hearing loss alone). Effects included impaired auditory function as well as loss of OHC. Intermittent noise (102 dB for 15 minutes of every hour) along with 1000 mg/m^3 JP-8 resulted in significant hearing decrements in male rats but not female rats. JP-8 exposure alone (up to 1500 mg/m^3) does not affect hearing in F344 rats.

Chemically enhanced hearing loss is generally accompanied by damage to outer hair cells of the cochlea. Researchers have hypothesized several specific mechanisms of toxicity to hair cells, including disruption of intracellular calcium homeostasis (Liu and Fechter, 1997), disruption of membrane fluidity (Campo *et al.*, 2001; Liu *et al.*, 1997), and disruption of efferent pathways

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synapsing at the cochlea (Lataye *et al.*, 2000). Free radical generation and oxidative stress as a possible pathway was investigated by Fechter (1999) and Rao and Fechter (2000).

Fechter (2005) presented evidence that confirms the role of oxidative stress in the production of hearing loss by both carbon monoxide and by acrylonitrile when noise is present at the time of chemical exposure. Therefore, oxidative stress is a likely candidate for the role of jet fuel in mediating noise-induced hearing loss. This hypothesis is further supported by evidence that oxidative stress is linked to JP-8 exposure itself. Boulares *et al.* (2002) indicated that JP-8 toxicity might be at least partially explained by increasing oxidative stress at the cellular level. In an *in vitro* experiment, RLE-6TN cells from rat lung epithelium exposed for 12 hours to 80 μg/mL JP-8 responded with apoptotic changes. This effect was enhanced by pretreatment with buthionine sulfoximine and hydrogen peroxide; both of these pretreatments would be expected to enhance the extent of oxidative stress. JP-8 reduced cellular glutathione (GSH) levels by approximately 40% after only one-hour of exposure. The damage induced by JP-8 in these epithelial cells was reduced or inhibited by adding exogenous GSH or by treating with L-N-acetylcysteine, an antioxidant drug. Similarly, Fechter *et al.* (2007a) found a significant depletion of GSH in the rat liver following JP-8 nose-only inhalation of 1000 mg/m^3 for 4 hours.

2.1 Objective

For the project herein, the working hypothesis is that free radical generation and oxidative stress combine to form the primary mechanism through which ototoxicants disrupt hearing and through which they potentiate the effects of noise on hearing loss. This hypothetic working relationship is outlined in Figure 1. The objective of this project is to develop preliminary mathematical models for predicting jet fuel induced potentiation of noise induced hearing loss. In order to determine the mechanistic and pharmacodynamic basis for this toxicity, dosimetric predictions of JP-8 at the target site, the cochlea, should be made via physiologically based pharmacokinetic (PBPK) model predictions. The effective JP-8 constitutive tissue concentrations will then be used to drive pharmacodynamic (PD) equations relating the predicted concentration of reactive oxygen species (ROS) and sound induced cellular damage. Development of a PBPK-PD model of JP-8 induced hearing loss with noise will provide the basis for estimating the potential risk to humans exposed to the same chemical and sound scenarios in occupational settings. Modeling oxidative stress at the cochlea, in addition to noise, as a pharmacodynamic dose metric for predicting the dose-response mechanisms of JP-8+noise induced hearing loss will provide the basis for extrapolation of the effects observed in rat studies to potential human exposures. Our quantitative model for oxidative stress is based on that developed in another context by Byczkowski *et al.* (1996, 1997).

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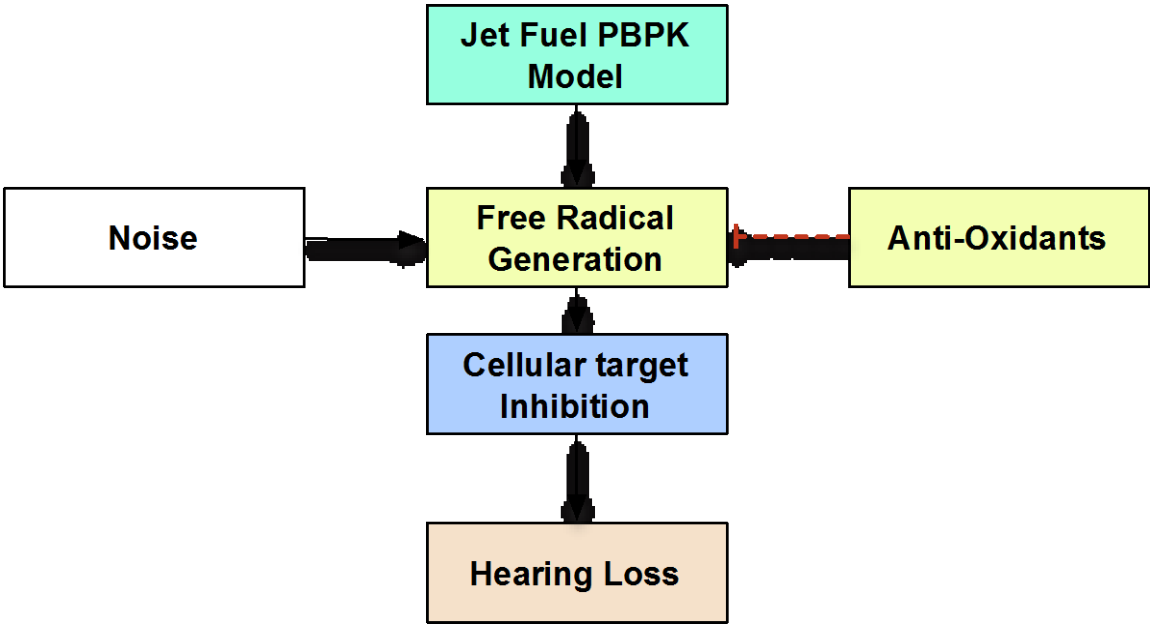

Figure 1. Schematic of overall scope of project

This project essentially has four parts:

1. the fuels PBPK model,
2. the oxidative stress: free radical generation model,
3. the oxidative stress: cellular target inhibition model, and
4. the integrated model, linking fuels exposure to hearing/hair cell loss.

3.0 JET FUEL PBPK MODEL

PBPK models utilize a series of mathematical equations to represent tissues and processes found in the body. The kinetics (absorption, distribution, metabolism and elimination) of a chemical or chemicals in the body are simulated using these equations. PBPK models are useful for predicting the chemical dose to a specific tissue of interest, depending on the purpose of the PBPK model (ATSDR, 2005; U.S. EPA, 2006).

3.1 Mixtures of Chemicals: Surrogates and Lumping

Individual PBPK models for a number of key components, including nonane, decane and toluene have been developed (Robinson and Merrill, 2007; Merrill *et al.*, 2008; Kenyon *et al.*, 2008; respectively). Running single models, however, does not accommodate interaction between components, especially competition for metabolism or cellular effect. To describe mixtures, frameworks in which models for an arbitrary number of specific jet fuel components can be combined via competitive metabolic inhibition in the liver have also been developed, including toluene, ethylbenzene and xylene interaction models (Dennison *et al.*, 2005; Haddad *et al.*, 1999,

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Tardif *et al.*, 1993, 1995, 1997), gasoline models (Dennison *et al.*, 2003, 2004a, 2004b, 2005) and a JP-8 interaction model with ethylbenzene and m-xylene (Campbell and Fisher, 2007).

Jet fuels, however, are complex mixtures of hundreds of compounds (Ritchie *et al.*, 2003) and are impossible to model chemical by chemical in an interaction model. Surrogates of representative classes have been utilized in the petroleum remediation industry to facilitate site cleanup (Edwards *et al.*, 1997). Fuel models use chemical and physicochemical characteristics of surrogate or marker chemicals to describe the kinetics of multiple chemical categories or lumps (Campbell and Fisher, 2007; Dennison *et al.*, 2003, 2004a, 2004b, Martin *et al.*, 2012).

For this project, analysis of jet fuel kinetics is based on the JP-8 PBPK model of Martin *et al.* (2012). The model describes kinetics of multiple fuel concentrations utilizing a chemical lumping strategy to combine similar hydrocarbon components (see Figure 2). Six marker chemical models are combined with three lumped chemical models and linked at the liver to account for competitive metabolic inhibition. Individual PBPK models are developed for each marker and lump; the PBPK model simultaneously describes nine interacting components.

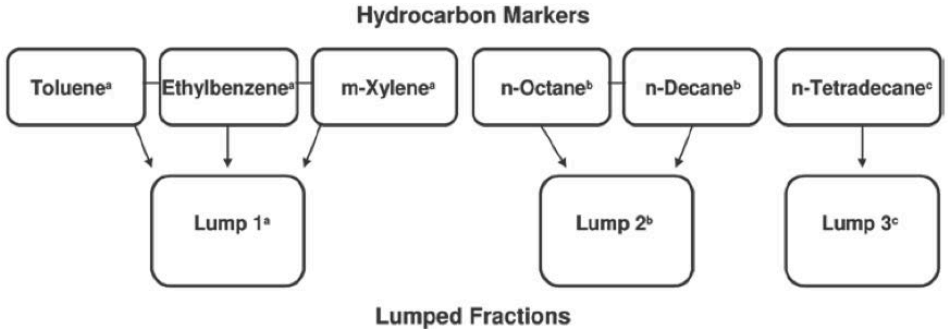

Figure 2. Conceptual representation of the PBPK model for JP-8 (from Martin *et al.*, 2012)

3.2 Modeling Approach

In order to facilitate PBPK predictions of cochlea jet fuel component concentrations, a cochlea had to be added to the existing model by Martin *et al.* (2012) (Figure 3). Since this model is actually a simultaneous solving of nine component models, equations for a cochlea had to be added nine times.

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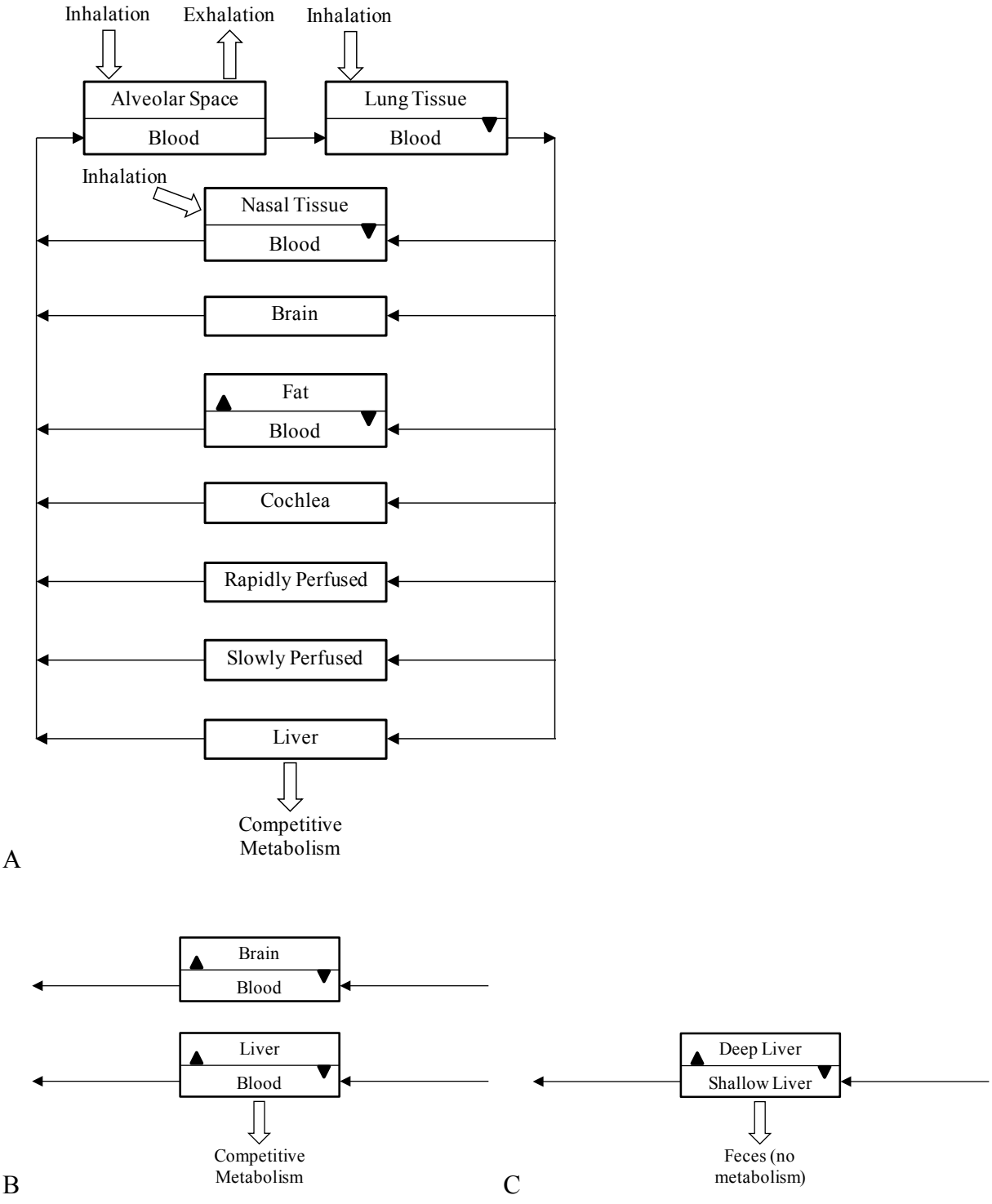

Figure 3. Schematic of PBPK model with cochlea compartment adapted from Martin *et al.* (2012). Schematic A details the basic model construction for aromatic components (toluene, ethylbenzene, xylenes and Lump 1). Blood flows (arrows) enter and exit compartments (boxes) representing tissues. Diffusion into and/or out of tissue compartments is represented by up or down triangles. Schematic B represents the changes to Schematic A in the models for the mid-range aliphatic constituents (octane, decane and Lump 2). Schematic C details the changes in the models for the high molecular weight aliphatics (tetradecane and Lump 3).

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Most of the components in the Martin *et al.* (2012) model are described using flow-limited distribution, indicating that the delivery of the chemical to the tissue and the tissue:blood partition coefficient (PC) are the factors that control how much of the chemical enters the tissue. This basic approach assumes that the blood equilibrates well with the tissue in question (Levitt, 2010). Some of the components in the Martin *et al.* model (octane, decane, tetradecane, Lumps 2 and 3, see Figure 2) are considered diffusion-limited into certain tissues (fat, brain, liver), meaning that the blood leaving the tissue has not equilibrated with the entire tissue. Factors that affect chemical diffusion into a tissue can include size of the molecule, whether the molecule is protein bound, charge of the molecule, etc. (Levitt, 2010). Diffusion limitation is described in a model by factors known as perfusion-area cross products. Because little is known about chemical partitioning in the cochlea, it was assumed to be flow-limited at this time.

For a compartment to be described in a PBPK model, the volume of the tissue (fraction of body weight), the blood flow to the tissue (fraction of cardiac output) and partitioning of the chemical into the tissue (PC, unitless) must be determined. Blood flow and tissue volume are physiological parameters (species and tissue specific). PCs are physicochemical specific parameters, meaning that they are specific to the tissue, species and compound in question.

The volume of the cochlea (expressed as the mass of the cochlea in relation to the bodyweight, since many tissues have a similar density to water (i.e., 1 g = 1 cm^3)) was determined using preliminary data from a kinetics study performed at the Naval Medical Research Unit-Dayton (NAMRU-D) (personal communication, Dr. Lisa Sweeney, NAMRU-D, WPAFB OH). Although the kinetics portion of the study is not yet complete, the bodyweights and combined cochlea weights of the male and female Fischer 344 rats utilized in the study were available to assist in the addition of a cochlea compartment to the model. Table 1 shows the weights and fractions determined from the kinetic study.

Table 1. Bodyweight and combined cochlea weight and fractions from F344 rat kinetic study

	Male		Female		Male and Female	
	Mean	SD	Mean	SD	Mean	SD
Number of animals	97	NA	97	NA	194	NA
Bodyweight (g)	219	11	143	5	180	39
Cochlea weight (g)	0.08	0.04	0.13	0.05	0.10	0.05
Cochlea volume (fraction of BW)	0.00034	0.00016	0.00089	0.00033	0.00061	0.00038

Notes: BW = bodyweight; NA = not applicable; SD = standard deviation

The Martin *et al.* (2012) model was developed for male F344 rats, so male combined cochlea and bodyweight data were utilized for baseline values. Given the obvious bodyweight differences between male and female F344 rats, male rat physiological parameters were utilized in the current model. PBPK models are designed to accommodate different physiological states such

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as lower bodyweights or higher fat percentages due to sex differences, so female bodyweight data can easily be used in the future.

Surprisingly, female F344 rat cochleas were larger than male cochleas. Although the standard deviation between the two groups overlap, the large numbers of animals in the two groups make the combined cochlea weights statistically different ($p<0.001$). The direction of the size difference between rat male and female cochleas was more surprising. Human cochleas are known to be highly variable in size (Erixon *et al.*, 2008; Escude *et al.*, 2006) with male cochleas being statistically larger than female cochleas (Escude *et al.*, 2006). Literature on rat cochlea size and sexual differentiation were not located. The larger size of the female cochlea is suspect; necropsy technicians may have been excising larger chunks of tissue due to the technical difficulty of working with a significantly smaller head for the female rats. Further research into cochlea size for F344 and other strains of rats would be indicated, especially if sensitivity analyses conducted on future versions of the model indicate that the model endpoint is sensitive to this parameter.

Values for blood flow in the cochlea were located through literature sources and are detailed in Table 2. All values were measured utilizing a microsphere method; two studies utilized radiolabeled microspheres (Hillerdal, 1987; Hillerdal *et al.*, 1987), while Larsen *et al.* (1984) used a non-radioactive microsphere method. Since the values were similar and the units equivalent, an average was used for the calculation of fraction of cardiac output, which included both cochlea (Table 2).

Table 2. Blood flow values for rat cochleas

Blood Flow	Method	Reference
1.64 μL/min/cochlea	radiolabeled microsphere	Hillerdal, 1987
1.44 μL/min/cochlea (right) 1.54 μL/min/cochlea (left)	radiolabeled microsphere	Hillerdal *et al.*, 1987
1.47 mg/cochlea/min (right) 1.82 mg/cochlea/min (left)	non-radioactive microsphere	Larsen *et al.*, 1984
AVERAGE		
1.58 μL/cochlea/min		
0.000095 L/hr/cochlea		
0.00004 (fraction of rat cardiac output for 2 cochleas)		

Notes: hr = hour; min = minute

Finally, in order to add a compartment to a PBPK model, partition coefficients must be determined. PCs may be measured *in vivo*, measured *in vitro* or predicted *in silico*. Literature searches have not returned measured PCs for any chemical into cochlea tissue. Further, tissue composition information for cochlea is not available. Proportions of protein, lipid, phospholipids and water in a tissue may be utilized to predict PCs for a chemical (Schmitt, 2008). Given the lack of PC information for the model and the relatively slow blood flow measured (Table 2), the

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cochlea was assumed to be a slowly perfused tissue and that the PCs utilized in the model for such tissues would suffice for a preliminary model. Further research into cochlea tissue content would be recommended for future modeling efforts.

4.0 FREE RADICAL GENERATION SUB-MODEL

4.1 Chemically-Induced Free Radical Production

Production of pro-oxidant chemical-derived free radicals was estimated by a linear algorithm as follows (Equation 1).

$$\text{Pro-oxidant chemical } C \xrightarrow{k_i} FR \xrightarrow{k_t} \text{Non-radical products} \qquad \textit{Equation 1}$$

FR is a steady state concentration of pro-oxidant chemical-derived free radicals, C is local (cochlear) pro-oxidant chemical concentration, k_i is a rate constant of free radical formation from the pro-oxidant chemical (1/hour) and k_t is the lumped rate constant of free radical recombination and quenching by the biological system (1/hour). The rate constant for quenching of free radicals by biological systems was estimated at 200.0 1/((μmol/L)*hour) (Byczkowski *et al.*, 1997). Rate constants of free radical formation are chemical-specific (Byczkowski *et al.*, 1997).

A square-root algorithm (in which two free radical molecules are produced from one pro-oxidant molecule) has been presented previously (Byczkowski *et al.*, 1997) and compared directly with the above linear model for the specific case of trichloroethylene (TCE) (Byczkowski *et al.*, 1996, 1999) (see Figure 4). Although the square-root algorithm is better able to predict thiobarbituric reactive substance (TBARS) production associated with free radical generation (Byczkowski *et al.*, 1997), the advantage in fitting these (indirect) data appears not to carry over to direct FR measurements of Figure 4, and to be outweighed by the additional complexity involved in such a square-root algorithm. Hence, the linear algorithms expressed in Equations 2 and 3 are used in the present report. For these equations, steady state concentrations of free radicals are assumed.

$$\frac{dFR}{dt} = kC - k_t FR = 0 \qquad \textit{Equation 2}$$

$$FR = kC/k_t \qquad \textit{Equation 3}$$

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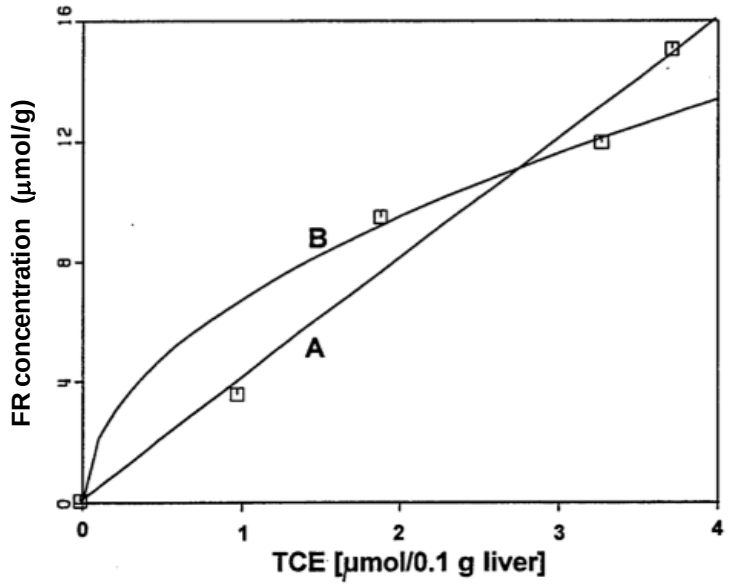
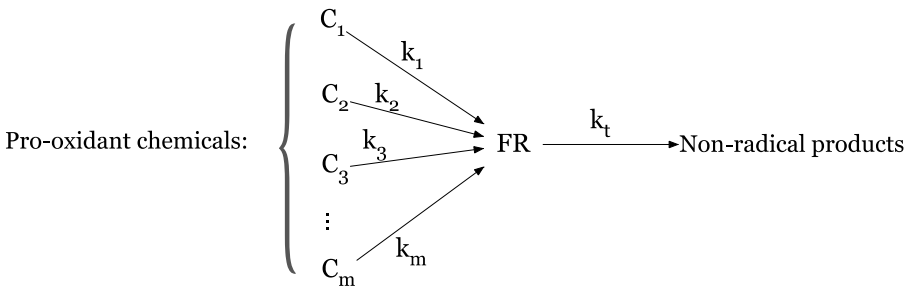


Figure 4. Calibration of the algorithm describing concentration of free radicals under steady-state conditions with experimental data. The figure, taken from Byczkowski *et al.* (1997), depicts free radical generation (concentration of N-tert-butyl-α-nitrone (PBN)-reactive free radicals, µmol/g liver) by different concentrations of TCE (µmol/0.1 g liver) in mouse liver slices using an electron paramagnetic resonance (EPR)/spin-trapping method (Steel-Goodwin *et al.*, 1996). The squares represent actual average experimental data points, after subtraction of physiological background levels of free radicals produced in the absence of TCE. The continuous lines are computer-generated simulations involving either (A) the linear algorithm or (B) the square root algorithm.

Clearly, Equations 1 through 3 are oversimplifications of the biological processes involved in jet-fuel induced free radical formation. Jet fuel is a complex chemical mixture rather than a single pro-oxidant chemical.

4.2 Mixture-Induced Free Radical Production

Given the nature of jet fuel, as discussed in Section 3.1, let us assume that each component of a mixture, either as a single chemical, or as a lumped component as described above, produces free radicals with a rate constant k_i (Equation 4).



Equation 4

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The total free radical accumulation is then given by the sum of the products of the rate constants and the concentration of the components ($\sum_{i=1,m} k_i\, C_i$) (Equations 5 and 6). Again, steady state concentrations of free radicals are assumed.

$$\frac{dFR}{dt} = \sum_{i=1,m} k_i C_i - k_t * FR = 0 \qquad \textit{Equation 5}$$

$$FR = \left(\sum_{i=1,m} k_i C_i\right)/k_t \qquad \textit{Equation 6}$$

4.3 Noise-Induced Free Radical Production

Production of free radicals by noise was estimated by a similar linear algorithm as follows (Equation 7 through 9).

$$\text{Noise level at cochlea } N \xrightarrow{k_N} FR \xrightarrow{k_t} \text{Non-radical products}$$

Equation 7

$$\frac{dFR}{dt} = k_N N - k_t FR = 0 \qquad \textit{Equation 8}$$

$$FR = k_N N/k_t \qquad \textit{Equation 9}$$

FR is a steady state concentration of pro-oxidant chemical-derived free radicals, N is local (cochlear) nose intensity level, k_N is a rate constant of free radical formation from the noise (1/hour) and k_t is the lumped rate constant of free radical recombination and quenching by the biological system (1/hour). Again, steady state concentrations of free radicals are assumed.

Equations 7 through 9 are oversimplifications of the biological processes involved in noise induced free radical formation. First, lumped noise exposure is shown as a single noise intensity level N, whereas in reality it is made up of a complex spectrum of frequencies, each with its own specific propensity to generate free radicals and create localized damage in the cochlea.

Second, noise-induced free radical formation in the cochlea is a complex, multi-factorial process that may involve glutamate excitotoxicity, excessive metabolic demand and reduced metabolic efficiency, lipid peroxidation, blood vessel disruption, ischemia followed by reperfusion injury, and multiple interactions and feedback loops between these processes. These complex interactions are discussed further below in Section 5.1 and Figure 5 (Henderson *et al.*, 2006; Poirrier *et al.*, 2010).

4.4 Combined Chemical/Noise-Induced Free Radical Production

When both noise and a pro-oxidant chemical are present, Equations 2 and 5 can be combined (Equations 10 and 11), assuming that their effects on free radical formation are independent.

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$$\frac{dFR}{dt} = k_i C + k_N N - k_t FR = 0 \qquad \textit{Equation 10}$$

$$FR = (k_i C + k_N N)/k_t \qquad \textit{Equation 11}$$

When noise is combined with a mixture of chemicals, Equations 12 and 13 are written.

$$\frac{dFR}{dt} = \sum_{i=1,m} k_i C_i + k_N N - k_t FR = 0 \qquad \textit{Equation 12}$$

$$FR = \left(\sum_{i=1,m} k_i C_i + k_N N\right)/k_t \qquad \textit{Equation 13}$$

4.5 Synergism between Noise and Chemical-Induced Free Radical Formation

In order to take into account the likely synergism between chemical and noise in local free radical production at the cochlea, an interaction term is introduced as a cross-product (k_xC.N) (Equations 14 and 15).

$$\frac{dFR}{dt} = kC + k_N N + k_x C.N - k_t FR = 0 \qquad \textit{Equation 14}$$

$$FR = (kC + k_N N + k_x C.N)/k_t \qquad \textit{Equation 15}$$

In general, synergism (supra-additivity) occurs when the production of free radicals in the presence of both chemical and noise is greater than the sum of free radical production in the presence of the same amount of chemical or noise separately. In terms of our cross-product, this occurs when the coefficient $k_x > 0$.

For the case of synergism in the presence of a chemical mixture, there will be a cross-product for each chemical component of the mixture, with a coefficient k_{xi} (Equations 16 and 17).

$$\frac{dFR}{dt} = \sum_{i=1,m} k_i C_i + k_N N + \sum_{i=1,m} k_{xi} C_i.N - k_t FR = 0 \qquad \textit{Equation 16}$$

$$FR = \left(\sum_{i+1,m} k_i C_i + k_N N + \sum_{i=1,m} k_{xi} C_i.N\right) k_t \qquad \textit{Equation 17}$$

5.0 CELLULAR TARGET INHIBITION – HEARING LOSS SUB-MODEL

The deterministic module estimated inhibition of uniform cellular targets by pro-oxidant chemical-derived free radicals (concentration FR) as an exponential decay function (Byczkowski *et al.*, 1997) (Equation 18).

$$FR + Cellular\ Target \xrightarrow{k_d} Inactive\ Target \qquad \textit{Equation 18}$$

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It was assumed that homogenous cellular targets have uniform sensitivity to FR. k_d is the rate constant for cellular target inactivation by free radicals. Remaining activity is calculated in Equation 19.

$$I_n = I_0\, exp\left(-k_d.FR.t_p\right) \qquad \textit{Equation 19}$$

I_n is remaining activity, expressed as a fraction of remaining active cellular targets, relative to the amount before inhibition; I_0 is initial concentration of active cellular targets (assumed 100%); and t_p is time of exposure to free radicals at local steady state concentrations. Thus, I_n is linked algebraically with FR. Rearranging Equation 19 gives Equation 20. f_n is the observed fractional hearing loss.

$$FR = -\frac{ln\left(\frac{In}{I_0}\right)}{\left(k_d t_p\right)} = -\frac{ln(f_n)}{\left(k_d t_p\right)} \qquad \textit{Equation 20}$$

It is now possible to relate fractional hearing loss back to the specific pro-oxidant stress that produced the free radicals. For a single pro-oxidant chemical, for example, combining Equations 3 and 20 gives Equations 21 or 22.

$$C = \left(\frac{k_t}{k_i}\right).FR = -k_t\, ln(f_n)/\left(k_i k_d t_p\right) \qquad \textit{Equation 21}$$

$$f_n = exp - \left(\frac{k_i k_d t_p C}{k_t}\right) \qquad \textit{Equation 22}$$

For a mixture of chemicals, using Equation 6 instead of Equation 3 gives Equations 23 or 24.

$$\sum_{i=1,m} k_i C_i = k_t.FR = -k_t\, \frac{ln(f_n)}{\left(k_d t_p\right)} \qquad \textit{Equation 23}$$

$$f_n = exp - \left(\frac{k_d t_p\left(\sum_{i=1,m} k_i C_i\right)}{k_t}\right) \qquad \textit{Equation 24}$$

Similarly, for noise-induced free radical formation, combining Equations 9 and 20 results in Equations 25 or 26.

$$N = \left(\frac{k_t}{k_N}\right).FR = -k_t\, \frac{ln(f_n)}{k_N k_d t_p} \qquad \textit{Equation 25}$$

$$f_n = exp - \left(\frac{k_N k_d t_p N}{k_t}\right) \qquad \textit{Equation 26}$$

Finally, for a combination of noise and chemical exposure, assuming independence, combining Equations 11 and 20 forms Equations 27 or 28.

$$k_i C + k_t N = k_t FR = -\frac{k_t\, ln(f_n)}{\left(k_d t_p\right)} \qquad \textit{Equation 27}$$

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$$f_n = exp - \left(\frac{(k_i C + k_N N) k_d t_p}{k_t}\right) \quad \textit{Equation 28}$$

In the case of synergism between noise and chemical, combining Equations 15 and 20 gives Equations 29 or 30.

$$k_i C + k_n N + k_x C.N = k_t FR = \frac{-k_t \, ln(f_n)}{(k_d t_p)} \quad \textit{Equation 29}$$

$$f_n = exp - \left(\frac{(k_i C + k_N N + k_x C.N) k_d t_p}{k_t}\right) \quad \textit{Equation 30}$$

Finally, for synergism between noise and a chemical mixture, combining Equations 17 and 20 results in Equations 31 or 32.

$$\sum_{i=1,m} k_i C_i + k_n N + \sum_{i=1,m} k_{xi} C_i.N = k_t FR = \frac{-k_t \, ln(f_n)}{k_d t_p} \quad \textit{Equation 31}$$

$$f_n = \exp - \left(\frac{\left(\sum_{i=1,m} k_i C_i + k_N N + \sum_{i=1,m} k_{xi} C_i.N\right) k_d t_p}{k_t}\right) \quad \textit{Equation 32}$$

Equation 32, therefore, describes the fractional cell death or hearing loss expected from the combined exposure to both noise and a chemical mixture, where there is also a synergistic effect between each chemical and noise. For the case of jet fuel, it is assumed that its effects can be adequately described by suitable lumping of component chemicals (Figure 2), so the subscript "i" describes each lumped component. For each (steady-state) noise/jet fuel exposure concentration combination for which hearing loss has been measured, Equation 30 can be written. If sufficient combinations are measured, the unknown parameters $k_1 \ldots k_m$, k_n and $k_{x1} \ldots k_{xm}$ can be fitted to the hearing loss data. Note that the product $k_d * t_p$ and k_t, representing the strength of the free radical interaction with its cellular target, and the first-order decay rate of the free radical are all chemical- (and noise-) independent. In addition, k_t has been estimated as 200.0 (1/((μmol/L)*hour)) (Byczkowski *et al.*, 1997).

5.1 Estimate of Cellular Target Effects: Cell Death

The effect of noise on free radical generation and outer hair cell death in the cochlea is a complex process involving a number of steps as shown in Figure 5 (Henderson *et al.*, 2006). Generally, damage to the cells of the cochlea can lead to both temporary threshold shift (TTS), as a result of reversible changes and repair processes, as well as permanent threshold shift (PTS). In addition, cell damage often continues following exposure, often for as many as 30 days post-exposure. Noise-induced damage may include loss of protein transduction channel permeability in cell membranes of OHC stereocilia; removal of stereocilia tips from insertion points in the tectorial membrane; pillar cell damage; damage to dendritic terminals of auditory nerve afferent fibers via glutamate excitotoxicity as a result of noise-induced overstimulation; swelling due to ion influx; temporary loss of dendrites; and blood vessel damage (stria vascularis) with reductions in cochlear blood flow, which in turn mediates further damage as a result of reductions in oxygen availability and reperfusion injury (Henderson *et al.*, 2006).

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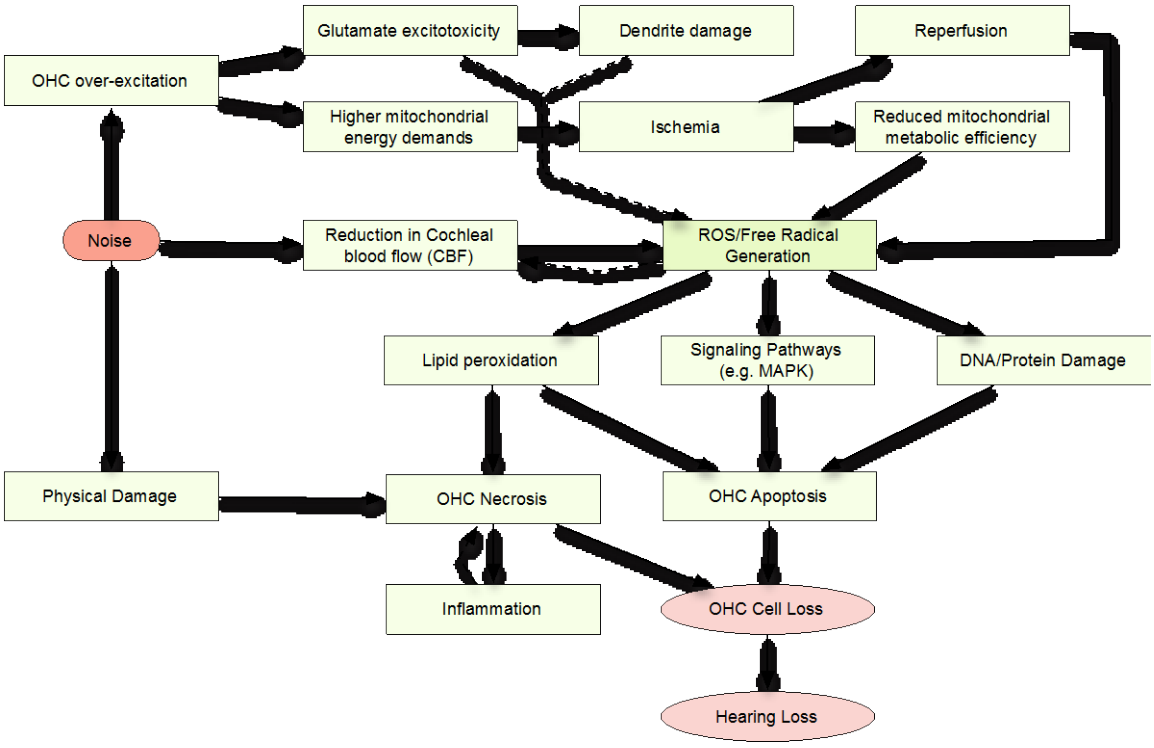

Figure 5. Mechanisms for noise-induced OHC loss

The OHC of the cochlea are particularly sensitive to noise-induced damage, and much of the damage to OHCs is likely due to oxidative stress. Free radicals and lipid peroxidation has been observed *in vivo* following noise exposure, and furthermore ROS have been localized in OHCs. In addition, paraquat-derived superoxide exposure has also been observed to lead to hearing loss. The observed continuation of cell damage after cessation of noise may be due in part to ROS-induced lipid peroxidation. OHCs have high energy demands, and noise exposure exacerbates the already high aerobic metabolic demands on mitochondria in OHCs. Together with reduced oxygen availability as a result of reduced flow (due to reversible blood vessel damage), this leads to reduced metabolic efficiency and higher levels of superoxide production. Returning the blood flow to normal can even lead to free radical generation and reperfusion damage. Thus a network of sometimes self-reinforcing processes forms, leading to ROS-induced hair cell damage as shown in Figure 5 (Henderson *et al.*, 2006).

Cell death occurs either by necrosis or apoptosis. The former is associated with cell swelling and bursting, and initiates an inflammatory response, typically leading to the death or damage of neighboring groups of cells. During apoptotic cell death, on the other hand, the cell membrane remains intact as the cell contents are systematically disassembled. It appears that both apoptosis and necrosis are initially involved in the creation of the OHC lesion, but with the passage of time, apoptosis dominates (Hu *et al.*, 2002).

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OHC death as a result of noise (or noise plus fuel) exposure is the combined consequence of a number of sequential and parallel processes. Hearing loss is a consequence of OHC cell loss, but also a consequence of hair cell damage that may not be manifest as cell death at a particular time point. In addition, damage to other cell types and structures in the cochlea also contribute to hearing loss. Thus, in fitting simple cellular target inhibition models such as those outlined above in Equations 15, 22, 24, or 26 to cell-loss data, many simplifying assumptions are necessary. Further assumptions are necessary in fitting such equations to hearing-loss data; however, such model fits provide initial quantitative descriptions of the process that can be progressively refined.

5.2 Application of Hearing Loss Model: Comparison with Data

Figures 6 and 7 show the effects of a four-week exposure to fuel and noise separately on OHC loss in the cochlea of rats. Figure 8 shows the effect of a combined four-week continuous noise and fuel exposure on the OHC loss. Figure 9 shows the effect of intermittent noise only, fuel only and combined intermittent noise and fuel on OHC loss of female and male rats.

In principle, f_n can be calculated for each of these data sets, using the following equations, and data represented in the following Figures 6 through 9.

- Equation 22 (or 24 for a mixture) for chemicals alone: Figure 6,
- Equation 26 for noise alone – Figure 7, and
- Equation 32 for synergy between noise and chemicals – Figures 8 and 9.

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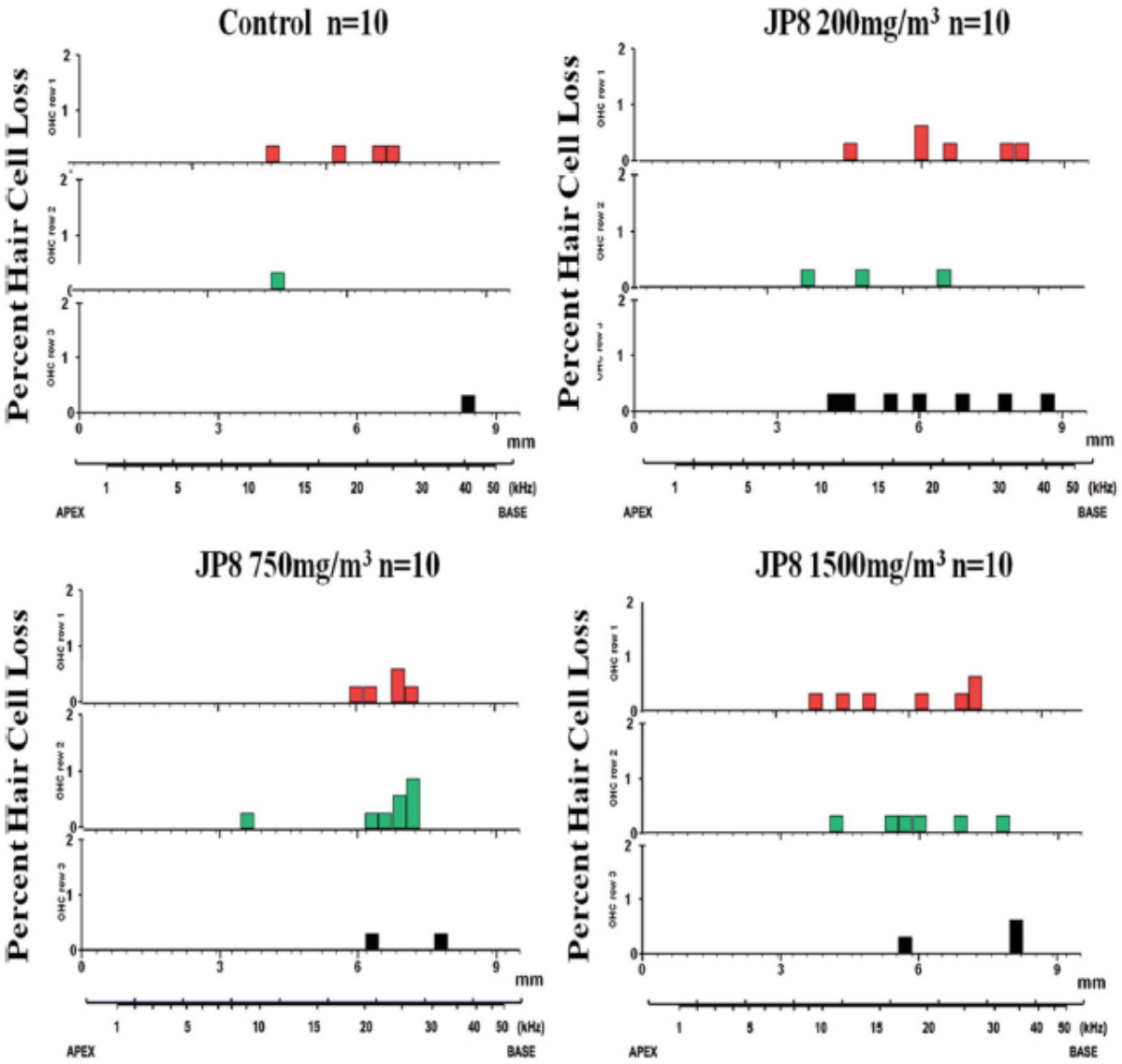

Figure 6. Effect of 4-week exposure to JP-8 on outer hair cell loss in F344 rats. Rats (5 male and 5 female per group) were exposed to an aerosol and vapor mixture of 0, 200, 750, or 1500 mg/m^3 JP-8 fuel for 6 hours daily, 5 days/week for 4 weeks. Red, green and black represent the three rows of OHC in the cochlea. The X axis represents location along the basilar membrane of the cochlea and, therefore, sensitivity to tone frequency (0 to 50 kHz). JP-8 alone did not result in impairment of hearing or OHC loss (Fechter *et al.*, 2012).

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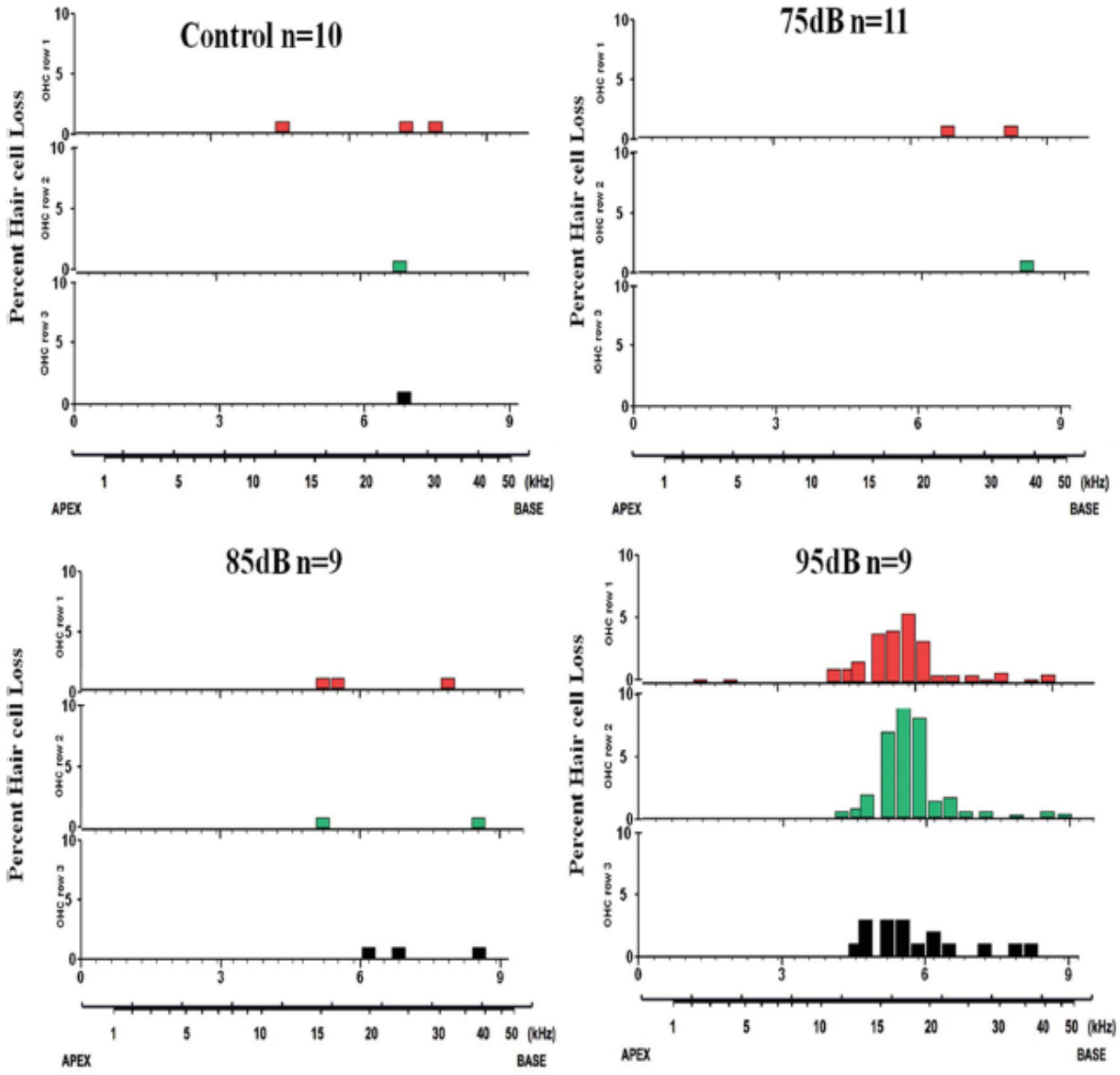

Figure 7. Effect of 4 week exposure to noise on hair cell loss in F344rats. Rats (approximately 5 male and 5 female per group) were exposed to continuous noise at control (no added noise), 75, 85 or 95 dB levels for 6 hours daily, 5 days/week for 4 weeks. Red, green and black represent the three rows of OHC in the cochlea. The X axis represents location along the basilar membrane of the cochlea and, therefore, sensitivity to tone frequency (0 to 50 kHz). Noise at 85 dB was non-damaging, while noise at 95 dB resulted in significant OHC loss, especially in the middle range (Fechter *et al.*, 2012).

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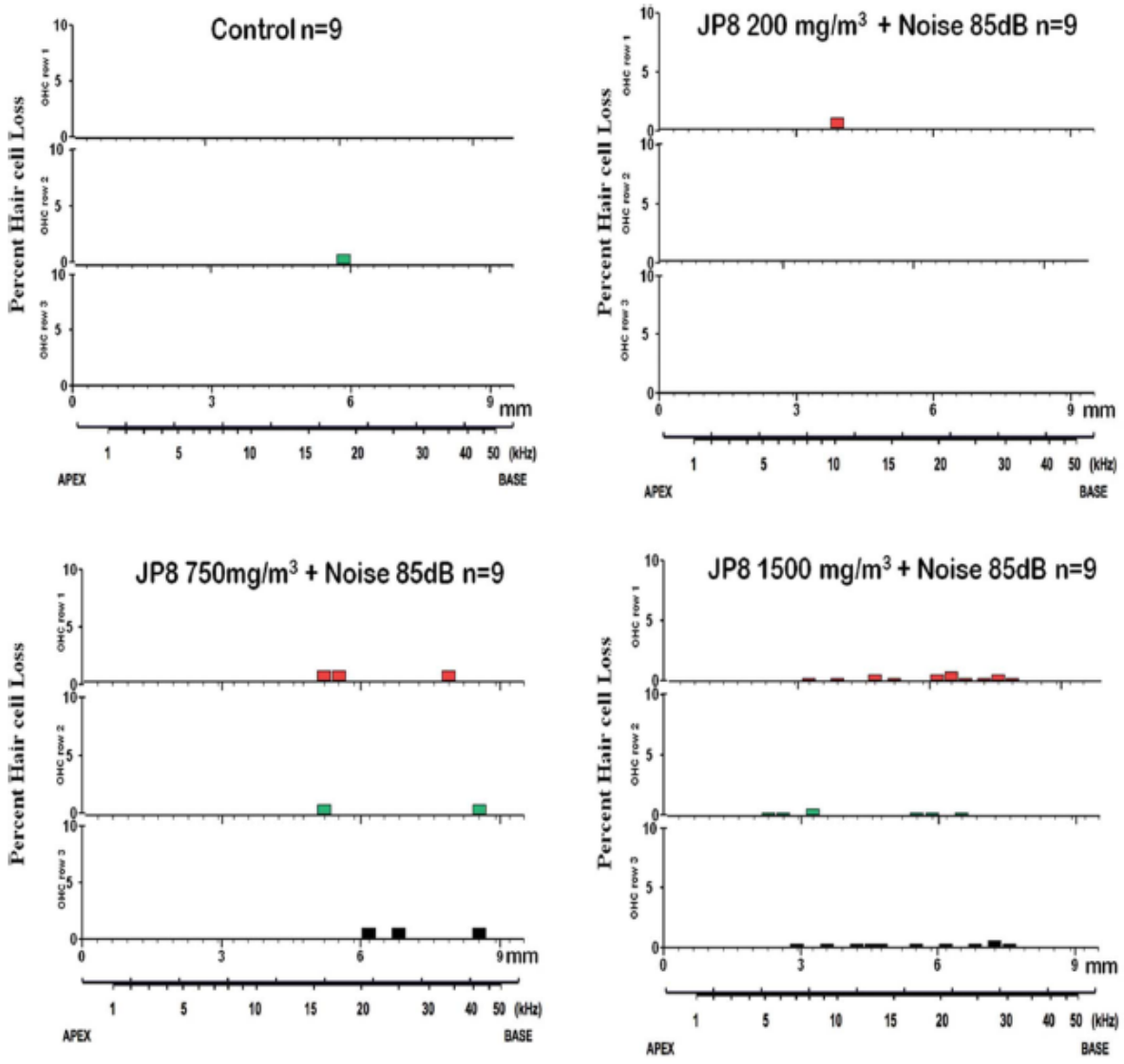

Figure 8. Effect of 4-week exposure to JP-8 and continuous noise (85 dB) on outer hair cell loss in F344 rats. Rats (5 male and 5 female per group) were exposed to an aerosol and vapor mixture of 0, 200, 750, or 1500 mg/m^3 JP-8 fuel for 6 hours daily, 5 days/week for 4 weeks. Simultaneously, rats were exposed to continuous noise at 85 dB (non-damaging noise). Red, green and black represent the three rows of OHC in the cochlea. The X axis represents location along the basilar membrane of the cochlea and, therefore, sensitivity to tone frequency (0 to 50 kHz). Combined exposure of 1500 mg/m^3 JP-8 and 85 dB noise resulted in significantly impaired auditory function and increased OHC loss (Fechter *et al.*, 2012).

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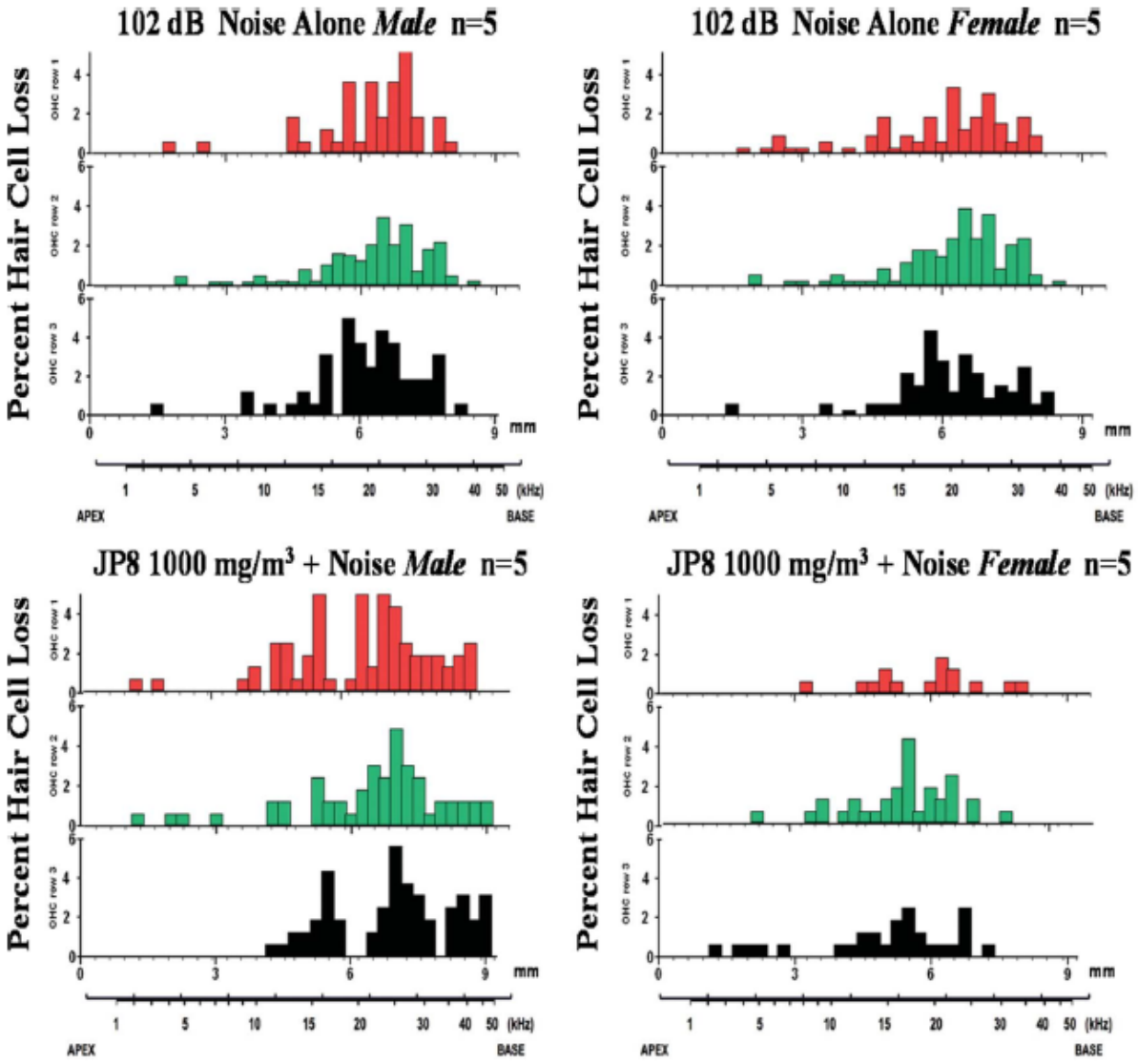

Figure 9. Effect of 4-week exposure to JP-8 and intermittent noise (102 dB) on outer hair cell loss in F344 rats. Rats (5 male and 5 female per group) were exposed to an aerosol and vapor mixture of 1000 mg/m^3 JP-8 fuel for 6 hours daily, 5 days/week for 4 weeks. Rats were also exposed to intermittent noise at 102 dB for 15 minutes of each hour (90 minutes total/day). Red, green and black represent the three rows of OHC in the cochlea. The X axis represents location along the basilar membrane of the cochlea and, therefore, sensitivity to tone frequency (0 to 50 kHz). Combined exposure of 1000 mg/m^3 JP-8 and 102 dB intermittent noise resulted in increased OHC loss in male rats, especially in the higher frequency regions (Fechter *et al.*, 2012).

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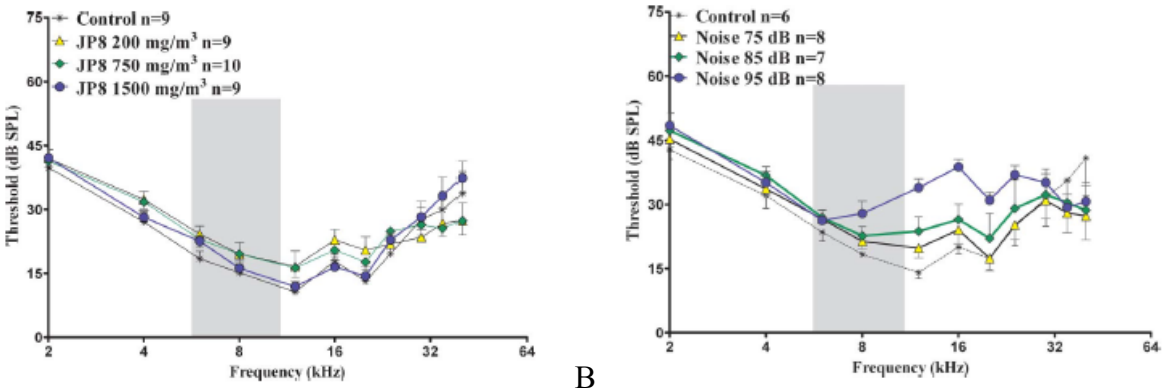
5.3 Correlation with Hearing Loss f_n

Figure 10 shows the effects of a 4-week exposure to fuel and noise separately on the auditory thresholds of rats. Figure 11 shows the effect of a combined 4-week continuous noise and fuel exposure on the auditory thresholds of rats. Figure 12 shows the effect of intermittent noise, fuel and combined intermittent noise and fuel on the auditory thresholds of male and female rats.



A B

Figure 10. Effect of a 4-week exposure to JP-8 (left) or noise (right) on auditory thresholds in F344 rats. (A) Rats (approximately 5 male and 5 female per group) were exposed to an aerosol and vapor mixture of 0, 200, 750, or 1500 mg/m^3 JP-8 fuel for 6 hours daily, 5 days/week for 4 weeks. (B) Rats were exposed to continuous noise at control (no added noise), 75, 85 or 95 dB levels for 6 hours daily, 5 days/week for 4 weeks. Auditory threshold measurements are assessed 4 weeks following the final exposure. The shaded area denotes the frequencies used in the noise exposure. JP-8 alone did not result in an increase of threshold while noise at 95 dB resulted in an increased threshold of hearing (Fechter *et al.*, 2012).

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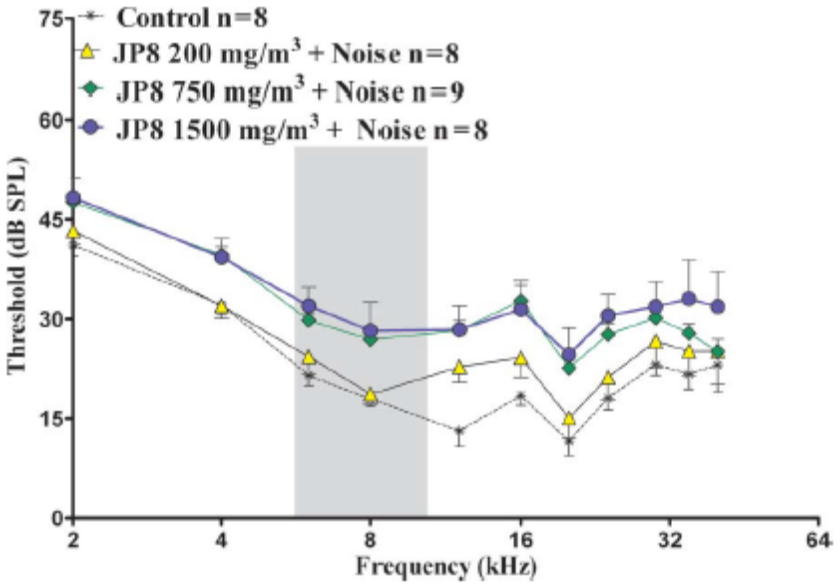

Figure 11. Effect of a combined 4-week exposure to JP-8 and continuous noise on auditory thresholds in F344 rats. Rats (approximately 5 male and 5 female per group) were exposed to an aerosol and vapor mixture of 0, 200, 750, or 1500 mg/m^3 JP-8 fuel for 6 hours daily, 5 days/week for 4 weeks. Simultaneously, rats were exposed to continuous noise at 85 dB. Auditory threshold measurements are assessed 4 weeks following the final exposure. The shaded area denotes the frequencies used in the noise exposure. JP-8 (150 and 1500 mg/m^3) and noise together increased hearing thresholds (Fechter *et al.*, 2012).

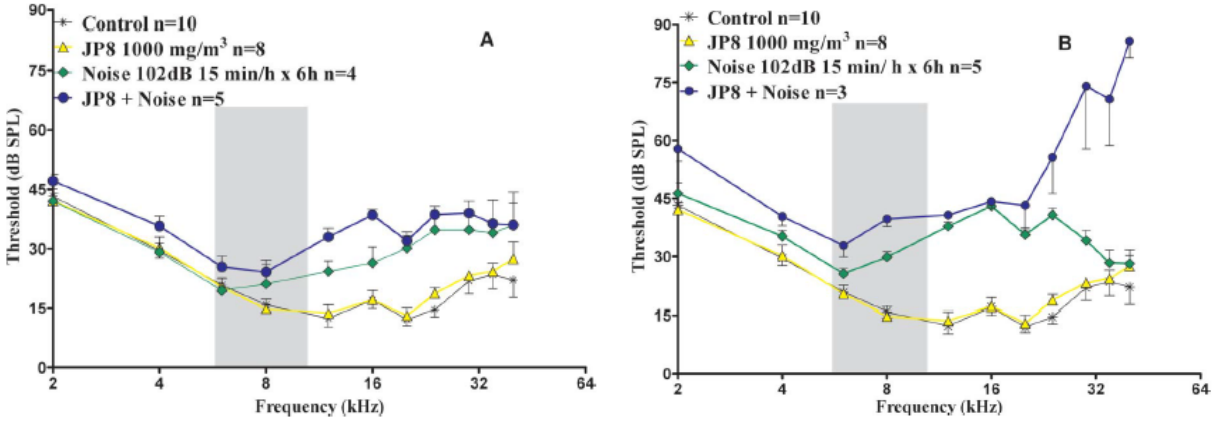

Figure 12. Effect of a combined 4-week exposure to JP-8 and intermittent noise on auditory thresholds in (A) female and (b) male F344 rats. Rats (approximately 5 male and 5 female per group) were exposed to an aerosol and vapor mixture of 1000 mg/m^3 JP-8 fuel for 6 hours daily, 5 days/week for 4 weeks. Simultaneously, rats were exposed to intermittent noise (15 minutes of every hour, 90 minutes total) at 102 dB. Auditory threshold measurements are assessed 4 weeks following the final exposure. The shaded area denotes the frequencies used in the noise exposure. JP-8 and intermittent noise together increased hearing thresholds, especially in male rats at higher frequencies (Fechter *et al.*, 2012).

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6.0 CONCLUSIONS

6.1 Implications of Hearing and Hair Cell Loss Data

Figures 6 through 12 indicate that concurrent JP-8 and noise exposure has a greater effect on hearing thresholds than on OHC losses in F344 rats. This may be due to a number of factors.

- OHCs may be damaged sufficiently to cause hearing loss, but may not (yet) be dead. OHCs are considered present if the stereocilia, the cuticular plate or the cell nucleus are visible microscopically (Fechter *et al.*, 2012). Therefore, damaged cells were counted as present, even if they may not have been functional.

- Hearing loss and cell death may have different time courses. The study design attempts to capture those differences by measuring hearing in live rats (data not reproduced here, Fechter *et al.*, 2012), measuring hearing in a terminal process four weeks following exposures (Figures 10 through 12) and counting OHC loss also at four weeks (Figures 6 through 9). However, it is not certain that the complete time course of cell death was encompassed. (Fechter *et al.*, 2012)

- Additional non-linearities may exist between cell damage and hearing loss. It may only take a small number of OHC to be killed before leading to a relatively large increase in hearing loss.

- Cells other than OHCs may have been impacted, leading to hearing loss. Toluene has been shown to depress the auditory nervous system in Long-Evans rats, interfering with muscle contractions which help to dampen noise (middle ear acoustic reflex), which could explain increases in NIHL with concurrent toluene exposure (Venet *et al.*, 2011).

- Cells outside of the ear may have been impacted. Brain stem effects (central auditory dysfunction) have been implicated in rats exposed to jet fuel or jet fuel plus noise exposure without apparent reductions in hearing acuity (personal communication, O'neil Guthrie, Loma Linda Veterans Association for Research and Education, Redlands CA).

6.2 Further Model Development

The present technical report describes the initial iteration of a model that links the kinetics of jet fuel with its potential effect on the outer hair cells of the cochlea when combined with noise exposure. It is assumed that interaction and loss of function is the result of free radical production and oxidative stress. This initial combined PBPK/PD model necessarily makes a number of simplifying assumptions. This is characteristic of the development of biologically based models, which typically follows an iterative process. Once the model is developed and parameterized, it is tested against available data and, if necessary, modified to take into account discrepancies and deficiencies indicated by the additional experimental data. The current model

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is very preliminary and consists of a number of parts or modules, each of which could be subject to further model development, as outlined below.

Research allowing determination of chemical partitioning into cochlea tissue is needed to further any PBPK model that includes a cochlea. Similarly, cochlea mass data in F344, other rat strains and other species need to be quantified in order to verify the fraction of bodyweight value necessary for this type of modeling. Most importantly, kinetic data from more than one study are needed to characterize the kinetics of any chemical in the cochlea and to test predictions made by the PBPK model.

A major complicating factor in the development of a PBPK model for jet fuel is the fact that it is a complex mixture of hundreds of interacting components. As Fechter *et al.* (2007b) point out: "A significant difficulty in assessing the risk of hearing loss from solvent exposure […] is that aromatic hydrocarbons as a class do not uniformly disrupt hearing, but, rather, demonstrate agent-specific effects". It will therefore be necessary at some point to identify potential "bad actors" in this process, and focus our PBPK model on these (and the potential effects of other mixture components (e.g., synergism) on these bad actors).

The model for oxidative stress outlined above may also need to be extended to take into account other processes involved. As mentioned in Sections 4.3 and 5.1, such additional processes may include glutamate excitotoxicity, excessive metabolic demand and reduced metabolic efficiency, lipid peroxidation, blood vessel disruption, ischemia followed by reperfusion injury, and multiple interactions and feedback loops between these processes (see also Figure 5).

In addition to refinement of the present PD model of oxidative stress, consideration needs to be given to the incorporation of additional modes of interaction between noise and jet fuel exposure (see Section 6.3).

6.3 Alternative and Additional Mechanisms

As noted in Section 2.0, there are additional possible mechanisms involved in the biological impact of both noise and chemical exposure on hearing loss, as well as in their possible interaction. Alternate hypotheses advanced for mechanisms by which chemical contaminants can disrupt hearing include disruption of intracellular calcium homeostasis (Liu and Fechter, 1997), disruption of membrane fluidity (Campo *et al.*, 2001; Liu *et al.*, 1997) and disruption of efferent pathways synapsing at the cochlea (Lataye *et al.*, 2000).

6.4 Additional Data

Additional data are being collected to further assess the likely health impact of joint noise/jet fuel exposures on hearing loss. For example, studies are being conducted with Long Evans rats (as opposed to the F344 rats used in the studies discussed above). Preliminary results indicate that Long-Evans rats may be less sensitive than F344 rats to the noise and fuel combination (personal communication, O'neil Guthrie, Loma Linda Veterans Association for Research and Education,

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Redlands CA). This suggests the need to address potential species/strain differences in our modeling effort and how these will be taken into account in extrapolating our models to humans.

There is also an effort, currently in its planning stages, to collect epidemiological data from Air Force personnel exposed to noise and/or jet fuel; hearing loss is assessed yearly in personnel routinely exposed to noise. Such data would be extremely useful for assessing the need for continued development of our model by providing direct evidence of a health issue in the target population. In addition, the data would be invaluable in the validation stages of our future model development.

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LIST OF ACRONYMS

μmol	micromole
AFMSA	Air Force Medical Support Agency
dB	decibels
DHP	Defense Health Program
DoD	Department of Defense
EPR	electron paramagnetic resonance
F344	Fischer 344 rat strain
g	gram
GSH	glutathione
kHz	kilohertz
L	liter
m^3	cubic meter
mg	milligram
NAMRU-D	Naval Medical Research Unit-Dayton
NIHL	noise induced hearing loss
OHC	outer hair cell
PBN	N-tert-butyl-α-nitrone
PBPK	physiologically-based pharmacokinetic
PC	partition coefficient
PD	pharmacodynamic
PTS	permanent threshold shift
ROS	reactive oxygen species
SG	Surgeon General
TBARS	thiobarbituric reactive substance
TCE	trichloroethylene
TTS	temporary threshold shift
USAF	United States Air Force

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