D-methionine to Reduce Noise-Induced Hearing Loss (NIHL): A Phase 3 Clinical Trial

Sponsored by:
Southern Illinois University School of Medicine

IND Sponsor:
Southern Illinois University School of Medicine

IND #115567
[D-methionine]

Project Director:
Kathleen C. M. Campbell, Ph.D., CCC-A
Professor and Director of Audiology Research
Department of Surgery
Southern Illinois University School of Medicine
Springfield, IL 62794
Phone: 217-545-7310
Fax: 217-545-7771
E-mail: kcampbell@siumed.edu

Principal Investigator:
CPT William P. Grimes, MD
Department of Preventive Medicine
Moncrief Army Community Hospital
Fort Jackson, South Carolina

Medical Monitor:

COL Mark D. Packer, MD
Executive Director, DoD/VA Hearing Center of Excellence
Clinical Assistant Professor, UTSA School of Medicine
Chief of Neurotology & Cranial Base Surgery

Protocol Version 6.2
23 March 2016

Statement of Compliance

The study will be conducted in accordance with the design and specific provisions of this IRB approved protocol, in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with Good Clinical Practice (GCP) and the applicable regulatory requirement(s). The Principal Investigator will assure that no deviation from, or changes to, the protocol will take place without prior agreement from the sponsor and documented approval from the IRB, except where necessary to eliminate an immediate hazard(s) to the trial participants. The Principal Investigator will promptly report to the IRB and the sponsor any changes in research activity and all unanticipated problems involving risk to human subjects, or others.

_____

Principal Investigator (Printed Name) Principal Investigator (Signature)

_____

Date

Investigational Agent: The test drug is D-methionine formulated as an oral suspension.

IND Number: 115567

Protocol History

Version #: 6.2

Version Date: 23 March 2016

Version Number	Amendment Date	Reason for Amendment
2.1	19April2012	
2.2	10August2012	Ft Jackson requested changes to the protocol
3.0	05October2012	Ft Jackson clarifications to the protocol; change in personnel
3.1	5Nov2012	Changes requested per DDEAMC IRB
3.2	30Nov2012	Changes requested per DDEAMC IRB
3.3	07Mar2013	Changes requested per DDEAMC IRB
3.4	01Apr2013	Changes requested per DDEAMC IRB
3.5	01 May2013	Changes requested per DDEAMC IRB
4.0	01 Aug2013	Changes in personnel
4.1	28 Aug 2013	Principal Investigator Change Ft. Jackson clarifications
4.2	08 Jan 2014	Personnel Change for Medical Monitor Added On-site Research Monitor Changes to inclusion criteria
4.3	25 Feb 2014	Added Section 15, 16 per DDEAMC IRB request
4.4	27 June 2014	Fort Jackson requested changes, personnel changes, inclusion/exclusion criteria changes
5.0	30 March 2015	Site PI and other personnel changes Changes to Screening Questionnaire per DSMC
5.1	15 July 2015	Add additional Associate Investigator, minor edits, change of ombudsmen
6.0	15 Dec 2015	Additional Safety Assessments, DSMC Roster Changes, Personnel changes, minor edits
6.1	17 February 2016	Site PI Change
6.2	23 March 2016	Formatting adjustments Added Personnel

Roles and Responsibilities for Clinical Study

Kathleen C.M. Campbell is the Project Director for this clinical study. Dr. Campbell is Professor of Surgery and Director of Audiology Research at Southern Illinois University School of Medicine. The Principal Investigator for this IND application is CPT William P. Grimes, M.D., Department of Preventive Medicine at Moncrief Army Community Hospital, Fort Jackson, South Carolina (SC). Yale University is responsible for data management and statistical analyses. The performance site for this study is Fort Jackson, SC.

NAME AND TITLE	ROLE IN PROJECT	RESPONSIBILITIES
Kathleen C.M. Campbell, CCC-A, PhD/SIU School of Medicine	Project Director	Responsible for overall study management and oversight. Responsible for selecting qualified investigators, providing them with the information needed to conduct investigation properly. Responsible for ensuring that the investigation is conducted according to the signed investigator statement of compliance, investigational plan, and applicable regulations; ensuring proper monitoring; protecting the rights, safety and welfare of subjects; and for the control of clinical supplies under investigation. Maintains records of disposition of test articles, accurate case histories of subjects, progress reports, and assurance of IRB review. Responsible for Final Clinical Study Report.
CPT William P. Grimes, MD, Preventive Medicine Moncrief Army Community Hospital Fort Jackson, SC	Principal Investigator	All investigator responsibilities noted above, including conduct of investigational plan, following applicable regulations, maintaining accurate case histories of subjects. Physician responsible for prescribing study drug and will provide medical care, if needed.
CPT Eric Bunnell, AUD, Deputy Chief, Fort Jackson Army Hearing Program, Moncrief Army Community Hospital Fort Jackson, SC	Co-Investigator, Audiologist	Responsible for performing audiologic examinations.
CPT Jenny Davis, AUD, Ft Jackson Army Hearing Program Manager, Moncrief Army Community Hospital Fort Jackson, SC	Co-Investigator, Supervising Audiologist	Responsible for overseeing all testing and for maintaining all audiologic data files for all subjects enrolled in the study. Assists with on-site study planning and coordination. Responsible for supervising staff audiologist.
Demarcus F. Bush, AUD	Audiologist	Responsible for performing audiologic examinations as needed.
Joseph Milbrandt, PhD/ SIU School of Medicine	Co-Investigator, Clinical Trials Monitor	Duties delegated by Project Director, for instance, ensuring the investigation is conducted according to the signed investigator statement of compliance,

		investigational plan, and applicable regulations; for protecting the rights, safety and welfare of subjects; and for the control of clinical supplies under investigation. Maintains records of disposition of test articles, accurate case histories of subjects, progress reports, and assurance of IRB review.
Carrie Redlich, MD, MPH/Yale	Epidemiologist	Responsible for design and oversight of data management and statistical analyses. Will prepare data for progress reports, FDA submission, publication and presentation.
Martin Slade, MPH Yale	Statistician/Data Manager	Responsible for data management and quality. Will conduct statistical analyses. Will oversee programming and data entry.
Meredith Stowe, PhD Yale	Data Programmer	Responsible for data programming, entry and verification.
Other Personnel		
COL Mark D. Packer, USAF, MD	Medical Monitor (off-site)	Sponsor liaison for study performance site. Off-site medical monitor. Will provide clinical perspective and oversight. Will coordinate with Project Director and PI to conduct appropriate data and safety monitoring.
Daniel Fox, PhD, MPH	Off-Site Study Coordinator	Off-site study coordination/regulatory support. Duties delegated by Clinical Study Manager.
1) Elizabeth Bullock, RN 2) Shelley Laird, LPN	(2) On-site Study Coordinators	Responsible for participant orientation, obtaining informed consent, clinical assessments, distribution of the study drug, distribution/collection of surveys, monitoring of side effects for medical referrals. Responsibilities also include monitoring drug and placebo shipments, tracking distribution of drug and placebo to study participants, maintaining files and confidentiality of records, and scanning/shipping all study data to Yale for statistical analyses. On-site study coordination and study assessments.
Lin Wright Roosevelt Barnwell	Ombudsmen	Will serve as an independent, neutral and impartial mediator of the Soldiers in order to ensure that no coercion is observed for the recruiting and consenting study activities.
Rick Lampe	Regulatory, Manufacturing, Nonclinical and Clinical Consultant/ GCP Auditing	Responsible for overall communications with FDA and assurance that all components are in place for investigator-sponsor and ensure all pertinent regulations are being met; also responsible for ensuring randomization code is properly handled, study balance and SOPs, as needed. Responsible for audit of conduct of the study. Will assist with Final Clinical Study Report.

CLINICAL PROTOCOL

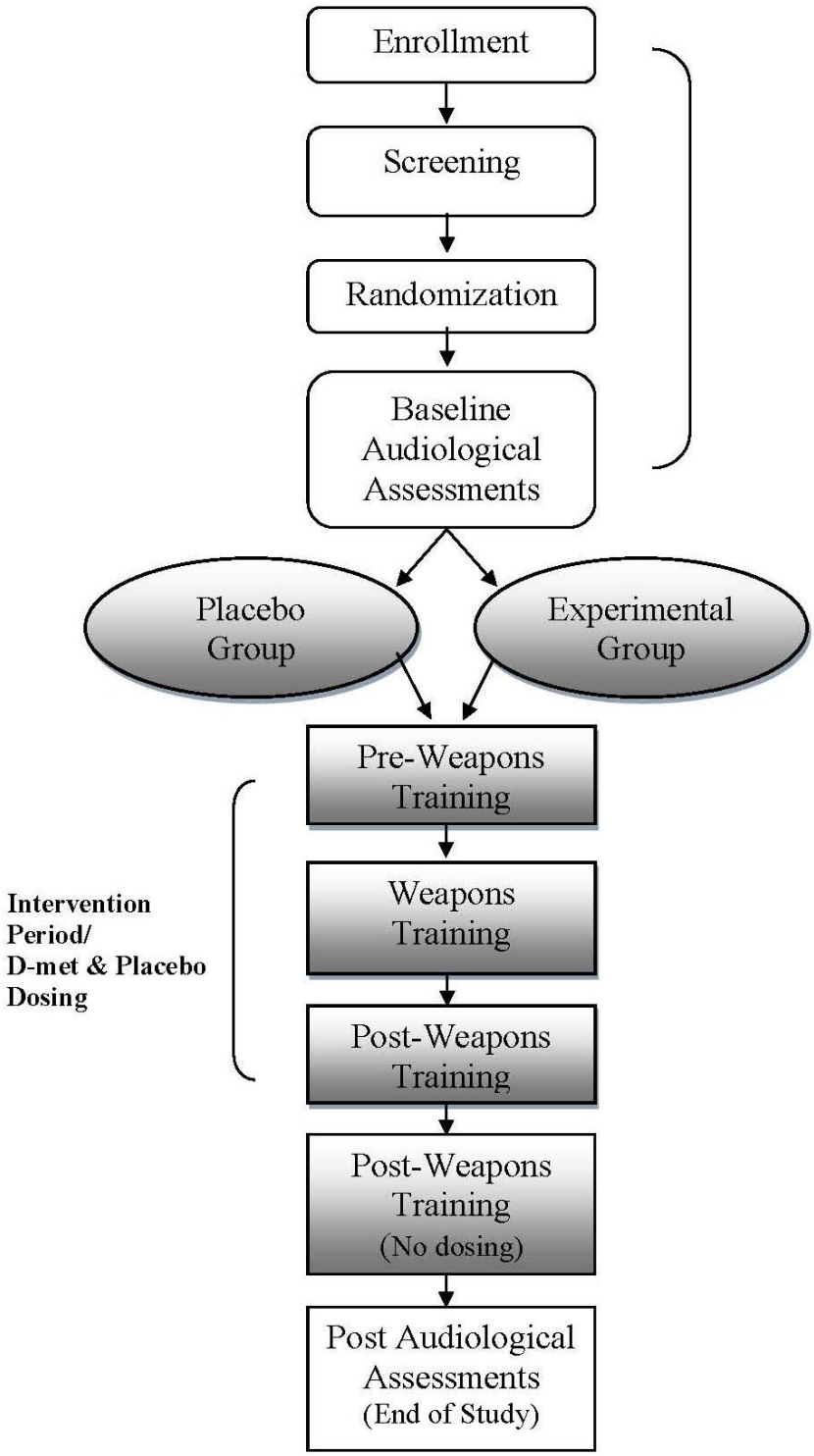
TABLE OF CONTENTS

CLINICAL SYNOPSIS

TITLE OF STUDY	Phase 3 Clinical Trials: D-methionine to Reduce Noise-Induced Hearing Loss (NIHL)
STUDY DESIGN	Randomized, Double-Blind, Placebo-Controlled Clinical Trial of D-methionine to Reduce Noise-Induced Hearing Loss (NIHL)
INVESTIGATOR/ STUDY CENTER	Project Director: Kathleen C. Campbell, Ph.D., CCC-A Professor and Director of Audiology Research Southern Illinois University School of Medicine Principal Investigator: CPT William P. Grimes, M.D., Preventive Medicine, Moncrief Army Community Hospital, Fort Jackson, South Carolina
COMPOUND	D-methionine
ROA	Oral, Liquid Suspension
DURATION OF ADMINISTRATION	18 days
POPULATION	U.S. Army Soldiers (Drill Sergeant School Candidates)
PHASE	Phase 3
OBJECTIVES	**Primary:** (1) To confirm D-methionine safety and tolerability. (2) To determine the effect of D-methionine on preventing or reducing hearing loss due to impulse noise associated with required weapons training. **Secondary:** (1) To determine the effect of D-methionine on preventing or reducing tinnitus due to impulse noise associated with required weapons training.
DESIGN	This is a randomized, double-blind, placebo-controlled study to evaluate the effect of D-methionine on noise-induced permanent hearing loss and/or tinnitus after required weapons training. Subjects will be randomly assigned in a 1:1 ratio to receive up to 100mg/kg/day D-methionine or placebo fractionated into two daily doses.
PLANNED SAMPLE SIZE	600 U.S. Army Drill Sergeant School Candidates
DIAGNOSIS AND KEY SUBJECT SELECTION CRITERIA	Inclusion criteria: 1. Male or female 2. 21 to 45 years of age 3. Negative pregnancy test at enrollment and prior to taking study drug 4. Willing to use an effective method of birth control during the study (Both male and female participants should avoid pregnancy during study) 5. Pure tone air conduction threshold average at 0.5, 1 and 2 kHz of no greater than 40 dB HL bilaterally with no air bone gaps greater than 10 dB and normal otoscopy and tympanometry screens 6. Willing to refrain from using supplements containing or derived from protein while participating in this study 7. Ability to comply with all study requirements Exclusion criteria: 1. History of allergic or idiosyncratic reaction to methionine, amino acid mixtures, nutritional supplements, egg white, or other proteins or food additives 2. Vegetarian (Individual excludes meat and fish from their diet) 3. History of chronic balance disorders 4. Abnormal otoscopic findings, otologic surgery, autoimmune inner ear disease, significant air-bone gaps, abnormal tympanograms or other indication of middle-ear abnormality, history of fluctuant hearing or asymmetric hearing worse than 25 dB at any frequency in either ear, perilymphatic fistula, tumor of the auditory system, or other CNS disorder that is likely to affect hearing

	5. Treatment with intravenous (IV) antibiotics within the past 6 months 6. Any surgical or medical condition which might significantly alter the absorption, distribution, metabolism, or excretion of any drug 7. History of abnormal kidney function or kidney impairment 8. Treatment for alcohol or substance abuse within past 6 months 9. Women of childbearing age who are not using effective contraceptive methods and who may become pregnant during the course of the study 10. Women who are pregnant or breastfeeding. 11. National Guardsmen 12. History of psychotic schizophrenia 13. Exposure within the previous 6 months to systemic ototoxic substances including aminoglycosides or vancomycin 14. Body weight exceeding 225 pounds 15. Renal impairment measured as eGFR < 50 on screening creatinine clearance blood draw
TREATMENTS	Two doses per day of an orange-flavored oral suspension (D-methionine or placebo) starting 3 days prior to weapons training, 11 days during weapons training, and 4 days after completion of weapons training. The total daily dose of D-methionine is up to100 mg/kg fractionated into 2 doses of 50 mg/kg each.
MAIN PARAMETERS OF EFFICACY	Primary endpoint is to **c**onfirm safety and tolerability of up to 100 mg/kg daily given in divided doses approximately 12 hours apart. Primary efficacy endpoint is change from baseline in pure-tone threshold as measured by absolute change and frequency of significant noise-induced threshold shift (STS). Secondary efficacy endpoint is change from baseline scores for the tinnitus scales for both loudness and annoyance.
MAIN PARAMETERS OF SAFETY	Adverse event assessments will be made by study coordinator(s) as study drug is dispensed (twice daily) and at the end of the study. Adverse events will be summarized by treatment group according to the last treatment taken before the Adverse Event (AE) began.
STOPPING RULES	Adverse event resulting in death, emergency surgery, or permanent and irreversible disability, unless determined unrelated to study participation. If an unexpected adverse event occurs in more than 3 subjects, then the risk of the active drug will be compared against risk of the placebo. Significantly higher rates in the active study group, based on severity of the adverse event, could warrant stopping trial.
DATA MONITORING	An on-site research monitor and an off-site medical monitor are assigned to this protocol. The project director, principal investigator, medical monitors, coordinator(s) and data manager of this protocol will schedule regular meetings via video or telephone conference to review study progress and safety data. The regulatory representative will also attend as needed, via teleconference, to determine if there are regulatory concerns or issues to handle based on the meeting content and outcome.
STATISTICAL ANALYSIS	To confirm that the total daily dose of D-methionine 100mg/kg fractionated into 2 doses of 50 mg/kg each is safe and tolerable. To determine if there is significantly reduced change in threshold hearing in each ear, adjusting for handedness, for the D-methionine group as compared to the placebo group at the tested audiometric frequencies. To determine if there is a significantly lower rate of STS (in either ear) for the D-methionine group compared to the placebo group. To determine if there is a significant difference in reported level of tinnitus between the D-methionine and the placebo groups.

Clinical Trial Schema



List of Abbreviations

AAA	American Academy of Audiology
ACE-Mg	ACE-Magnesium
ADR	Adverse Drug Reaction
AE	Adverse Event/Adverse Experience
ANSI	American National Standards Institute
CCR	Center for Clinical Research
CFR	Code of Federal Regulations
COI	Conflict of Interest
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DDEAMC	Dwight D. Eisenhower Army Medical Center
D-met	D-methionine
DFMO	Difluoromethylornithine
DHHS	Department of Health and Human Services
DOEHRSHC	Defense Occupational Environmental Health Readiness System-Hearing Conservation
DSM	Data and Safety Monitoring
FDA	Food and Drug Administration
FWA	Federal Wide Assurance
GCP	Good Clinical Practice
GSH	Glutathione
GSSG	Oxidized Glutathione
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICF	Informed Consent Form
IDE	Investigational Device Exemption
IND	Investigational New Drug
IRB	Institutional Review Board
ISM	Independent Safety Monitor
JC	Joint Commission
MACH	Moncrief Army Community Hospital
MCRD	Marine Corps Recruit Depot
N	Number (typically refers to participants)
NAC	N-acetylcysteine
NB	Narrow Band
NIHL	Noise Induced Hearing Loss
NCI	National Cancer Institute, NIH
NIH	National Institutes of Health
PI	Principal Investigator
PK	Pharmacokinetics
PTA	Pure Tone Average
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SOP	Site Operations Manual
STS	Significant Noise-Induced Threshold Shifts
WHO	World Health Organization

Definitions

Air conduction - AC method of delivering acoustic signals through an earphone; COM: bone conduction

Air-bone gaps – ABG; difference in dB between air-conducted and bone-conducted hearing thresholds for a given frequency in the same ear, used to describe the magnitude of conductive hearing loss

Bel – unit expressing the intensity of a sound to a reference intensity; intensity in bels is the logarithm (to the base 10) of the ratio of power of a sound to that of a reference sound; after Alexander Graham Bell

Bone conduction – BC; method of delivering acoustic signals through vibration of the skull; COM: air conduction

Cochlear hearing loss – hearing sensitivity loss due to hair cell damage or other damage to the cochlea

Decibel (dB) – A unit of measure of Sound Pressure Level (SPL). When used to measure SPL, a dB is equal to 20 times the common logarithm of the ratio of the existing sound pressure to a reference sound pressure of 20 micropascals

Effective Methods of Birth Control for this Study –Subject *"should either abstain from sexual relations or practice a method of birth control while taking part in the study. Except for surgical removal of the uterus, birth control methods such as condoms, a diaphragm or cervical cap, birth control pills, IUD, or sperm killing products are not totally effective in preventing pregnancy"*
"Male study participants should refrain from fathering babies while enrolled in this study."

Hertz (HZ) - A unit of measure of frequency, numerically equivalent to cycles per second

High frequency – HF; audiologically, a nonspecific term referring to frequencies of 2000 Hz or higher.

Impulse Noise – A short burst of acoustic energy consisting of either a single impulse or a series of impulses. The pressure-time history of a single impulse includes a rise of 40 dB or more in 1 second or faster to a peak pressure, followed by a somewhat slower decay of the pressure envelope to ambient pressure, both occurring within 1 second. When the intervals between impulses are less than 500 milliseconds, the noise is considered continuous, except for short bursts of automatic weapons fire which are considered "impulse noise".

Meniere's disease – idiopathic endolymphatic hydrops, characterized by fluctuating or episodic vertigo, hearing loss, tinnitus, and aural fullness

Narrow Band (NB) Noise - Noise in which the acoustic energy is concentrated in a relatively narrow range of frequencies

Otoscope – a speculum-like instrument for visual examination of the external auditory meatus and tympanic membrane

Otoscopy – inspection of the external auditory meatus and tympanic membrane with an otoscope

Perilymph – cochlear fluid, found in the scala vestibule, scala tympani, and spaces within the organ of Corti, which is high in sodium and calcium and has an ionic composition that resembles cerebrospinal fluid
Perilymphatic fistula – abnormal passageway between the perilymphatic space and the middle ear, resulting perilymph leakage at the oval or round window, usually caused by congenital defects or trauma

Potentially Hazardous Noise - Exposure to steady-state noise having an 8-hour TWA noise level of ≥ 85dBA, or exposure to impulse/impact noise levels greater than 140 dB peak SPL, regardless of duration

Pure tone –Sound wave having only one frequency of vibration

Pure-tone air-conduction threshold – lowest level at which a pure-tone stimulus, presented through earphones is audible 50% of the time

Pure-tone average – PTA; average of hearing sensitivity thresholds to pure-tone signals at 500, 1000 and 2000 Hz

Pure-tone bone-conduction threshold – lowest level at which a pure-tone stimulus, presented via a vibrating oscillator, usually placed on the forehead or mastoid, is audible 50% of the time

Significant Threshold Shift (STS) - An average change of plus or minus 10 dB at 2000, 3000, and 4000 Hz, relative to the reference audiogram, in either ear, without age corrections (military definition)

Sound Pressure level – SPL; magnitude or quality of sound energy relative to a reference pressure, 0.0002dyne/cm² or 20μPa

Threshold – level at which a stimulus or change in stimulus is just sufficient to produce a sensation or an effect that is perceived 50% of the time

Tinnitus – sensation of ringing or other sound in the head, without an external cause

Tympanogram –graph of the middle ear immittance as a function of the amount of air pressure delivered to the ear canal. Its purpose is to assess tympanic membrane and middle ear function

List of Figures

1. 0 OBJECTIVES

1.1 Primary Objectives

1.1.1 To monitor for any potential side effects of D-methionine in human subjects. This aim will be accomplished by assessing for side effects each time study drug is dispensed (twice daily by the study coordinator(s) and at the final study visit.

1.1.2 To determine whether administering oral D-methionine can prevent permanent noise-induced hearing loss (NIHL) due to impulse noise associated with required M-16 weapons training. This aim will be addressed by comparing the results of D-methionine versus placebo administration starting 3 days prior to, during the 11 day period of weapons training (Monday-Friday and Monday -Thursday over a 2 week period), and 4 days after weapons training for a total of 18 days of dosing. Pure tone air conduction hearing thresholds will be assessed before and 15-16 days after completion of weapons training (i.e., 11-15 days after the last day of study drug/placebo administration).

1.2 Secondary Objective

1.2.1 To determine whether administering oral D-methionine can prevent tinnitus after required M-16 weapons training. This aim will be addressed by comparing the results of D-methionine versus placebo administration starting 3 days prior to, during the 11 day period of weapons training (over a 2 week period), and 4 days after weapons training for a total of 18 days of dosing. Tinnitus questionnaires will be completed before weapons training and 11-15 days after the last day of study drug/placebo administration.

1.3 Study Design

This is a prospective, randomized, double-blind, placebo-controlled study to evaluate the effect of D-methionine on permanent NIHL after required M-16 weapons training. The study will include 600 U.S. Army personnel enrolled in Drill Sergeant Drill Sergeant School (DSS) scheduled to undergo 9 days of M-16 weapons training over an 11 day period at U.S. Army Basic Combat Training Center of Excellence, Ft Jackson, South Carolina. This study is funded by the Department of Defense and written permission has been obtained from Sonya Cable, LTC, SP, Director, Experimentation and Analysis Element, Office of Deputy Commanding General, Initial Military Training, Fort Jackson, South Carolina and Commandant COL Mark Higdon, MD, of Moncrief Army Medical Center (MACH) and 2nd Commandant CSM Michael McCoy, DSS

2.0 BACKGROUND

2.1 Justification for the Clinical Trial

Although significant progress has been made in developing physical hearing protectors and in controlling work-related noise exposure, permanent noise-induced hearing loss (NIHL) still affects at least 10 million Americans (Lang 1994; Alberti et al., 1998). Further, harmful levels of occupational noise exposure may affect close to 30 million Americans (Rabinowitz, 2000). NIHL is also an international problem. According to the World Health Organization, exposure to excessive noise is the major avoidable cause of permanent hearing loss worldwide (Smith 1998). Recreational activity with firearms, amplified music, motorcycles, and power tools also expose millions of people to sound capable of producing permanent hearing loss (Metternich and Brusis1999; Axelsson et al., 1991; Rabinowitz 2000). Recently, studies have shown that even young children suffer from hearing loss after exposure to sudden noise emitted by toy pistols and firecrackers (Hellstrom 1992; Segal et al., 2003).

Noise is defined as a short burst of acoustic energy comprising either a single impulse or a series of impulses. The pressure-time history of a single impulse includes a rise of 40 dB or more in 1 second or faster to a peak pressure, followed by a somewhat slower decay of the pressure envelope to ambient pressure, both occurring within 1 second. When the intervals between impulses are less than 500 milliseconds, the noise is considered continuous, except for short bursts of automatic weapons fire which are considered "impulse noise" (Stach, 2003).

NIHL generally first affects the high frequency range with a characteristic "notch" at approximately 4 kHz. As the loss progresses, the patient may have difficulty in all listening environments affecting both social and work life; sometimes impacting employability. The financial impact to the government is significant. The U.S. Veteran's Administration alone paid approximately $24 billion dollars in hearing loss compensation from 1970-1990 (Wolgemuth et al., 1995). Noise-induced tinnitus is less well studied but is a frequently reported consequence of noise exposures (Henry et al., 2005). The world-wide social and financial impact of military, industrial, and recreational noise exposure is enormous.

For the military particularly, NIHL has high costs not only in financial but in human terms. Military efficacy frequently depends on hearing. For the dismounted Soldier, Letowski (2003) described hearing as the most important sense for survival. Further, physical hearing protectors such as earmuffs and plugs provide insufficient hearing protection for many military exposures. Taggart 2001 reported that 11% of marines had permanent hearing loss following recruit training even with all hearing conservation protocols in place.

NIHL appears to be a problem for all branches of the military including the U.S. Army (Helfer et al., 2005) U.S. Navy, (Bohnker et al., 2002a,b, 2003, 2004) U.S. Marines (Taggart 2001, Bohnker et al., 2002, Barney and Bohnker 2006) and U.S. Air Force (Ritter and Perkins 2001). Further, NIHL affects not only front line military personnel. In a study of personnel on the flight deck of a Nimitz class aircraft carrier, Rovig et al. (2004) reported high levels of NIHL for engineers (27%) and flight personnel (17%) compared with administrative personnel (4%).

Currently, no FDA approved pharmacologic prevention exists for NIHL. Animal studies have shown that administration of D-methionine can reduce or prevent NIHL (Kopke et al., 2002, Campbell et al., 2007, Campbell et al., 2011). The goal of this IND study is to determine if it has similar efficacy in humans. Although testing for protection from noise-induced tinnitus in animals has not yet occurred, this clinical trial would provide an opportunity to also simultaneously test for protection from noise-induced tinnitus in humans.

The purpose of this specific clinical trial is to determine if impulse-induced permanent NIHL and tinnitus can be prevented in a large cohort of Army personnel, during their required weapons training as a part of Drill Sergeant Instructor Training School at Ft Jackson. This is an ideal subject population comprising highly disciplined and motivated Soldiers who will fire exactly 500 rounds of M-16 weapon fire (156 dB SPL) within a 9 day period of weapons training. Soldiers unavoidably develop permanent NIHL during their course of training, even with the best of physical hearing protection and the most proactive training in its use. Proof of concept data from several studies demonstrates that D-methionine given before and after noise exposure in animals can prevent permanent NIHL. This study is designed to document whether or not it prevents permanent NIHL in Soldiers.

2.2 Noise-Induced Ototoxicity: Cochlear Organ of Corti Effects

In general, acoustic stimulation deflects the stereocilia on top of the hair cells. When the shear forces stretch tip links between adjacent stereocilia, the stereocilias' mechanoelectrical transduction channels can open. This opening causes excitation by ion influx into the cell, thus depolarizing the plasma

membrane, which in turn causes neurotransmitter release. Shear forces in the opposite direction can close the channels (Saunders et al., 1991, Howard et al., 1988, Roberts et al., 1988). Noise exposure can also temporarily decrease cochlear microcirculation (Vertes et al., 1979; Axelsson et al., 1981; Quirk et al., 1992; Henderson and Hamernik, 1995; Miller et al., 1996).

In excessive noise exposure, a variety of cochlear anatomical changes occur. Outer hair cell (OHC) loss and, to a lesser extent, inner hair cell loss, predominantly in the basal portion of the cochlea, are well-established findings (Henderson and Hamernik, 1995). However, some variability across species has been reported (Hamernik et al., 1984). Bohne and Rabbitt (1983) first reported that phalangeal scars will eventually replace degenerated hair cells, but 1 - 2 hours after noise exposure, holes in the reticular lamina may be present where the hair cells were, possibly allowing endolymph infiltration. They hypothesized that subsequent degeneration of supporting cells, nerve fibers and possibly sensory cells may be secondary to the damage caused by potassium rich endolymph contaminating the fluid spaces.

In addition to actual loss of cochlear hair cells, more subtle forms of hair cell damage may include swelling and vacuolization of the hair cells, swelling of the supporting cells, fractures or discontinuities of the stereocilia rootlets, splaying of the stereocilia, deterioration of the stereocilia shafts' actin crystals and loss of tip links (Engstrom et al., 1983; Slepecky 1986; Thorne et al.,1986; Lim 1986; Liberman and Dodds 1987; Liberman 1987; Pickles et al., 1987; Raphael and Altschuler, 1992). Dendritic swelling of the afferent fibers beneath the hair cells may also occur (Robertson 1983; Puel et al.,1996).

Noise induces hearing loss by either mechanically over-stimulating the cochlea or by metabolic processes. Mechanical damage, in addition to metabolic damage, generally occurs when noise intensity levels exceed 125 dB SPL (Henderson and Hamernik, 1995). For noise exposures under 125 dB SPL, damage is for the most part secondary to metabolic processes (Henderson and Hamernik, 1995). For impulse noise and continuous noise above 125 dB SPL, both metabolic and mechanical damage can occur however protective agents have also shown some promise. Partial but not complete protection from PTS and OHC loss have been reported for N-acetyl cysteine (NAC) and acetyl-L-carnitine (ALCAR) (Kopke et al., 2004). However in other studies D-methionine has shown superior protection to NAC for continuous noise and equal or better protection than ALCAR (Kopke et al., 2000, 2002; Campbell et al., 2007, 2011).

2.3 Selection of D-methionine as an Otoprotective Agent Against NIHL

D-methionine was first discovered as an otoprotective agent in 1996 in studies investigating cisplatin-ototoxicity by Dr. Campbell l at Southern Illinois University School of Medicine (Campbell, et al., 1996). Since that time D-methionine has been found to protect hearing from a variety of ototoxins, including noise. D-methionine protects against cisplatin-induced (Campbell et al.,1996, 1999, 2007; Kopke et al. 1997; Reser et al.,1999) carboplatin-induced (Lockwood et al., 2000), aminoglycoside-induced (Sha and Schacht, 2000) and noise-induced hearing loss (Coleman et al., 2002 a,b; Kopke et al., 2002; Campbell et al., 2011).

D-methionine protection from NIHL in animals has been confirmed in other independent labs (Samson et al., 2008; Cheng et al., 2008). However, this would be the first study in humans for D-methionine protection from NIHL and cochlear outer hair cell loss. In studies to date, D-methionine provides virtually complete protection from permanent NIHL and cochlear hair cell loss (Kopke et al., 2002, Campbell et al., 2007; ; Samson et al., 2008; Cheng et al., 2008; Campbell et al., 2011). These studies all consistently report protection from NIHL. No studies report a lack of D-methionine protection or exacerbation of permanent NIHL. Additionally almost complete protection from permanent NIHL in chinchillas has been obtained even when D-methionine is first administered up to 7 hours after noise cessation (Campbell et al., 2010). Protection from temporary thresholds shift has been variable but has not been as carefully studied. No agent has been found to be more effective in animal studies to date. It has been studied for decades in multiple species as a part of nutrition.

Noise exposure increases cochlear reactive oxygen species (ROS) levels (Ohlemiller et al., 1999), thus placing an oxidative challenge on the cochlea. ROS levels, exceeding the cochlea's capability to detoxify or eliminate them, can cause cochlear damage (Huang et al., 2000; Clerici et al.,1995; Clerici and Yang 1996; Seidman et al., 1993). D-methionine may provide prophylactic rescue protection from permanent noise-induced hearing loss primarily through two putative mechanisms: 1) acting as a free radical scavenger and 2) altering glutathione (GSH) levels. Unlike most amino acids, D-methionine is reversibly oxidized (Vogt 1995).

Because noise exposure markedly increases ROS formation (Ohlemiller et al., 1999), D-methionine may protect against NIHL by serving as a free radical scavenger. Because the nearly 4-fold noise-induced cochlear ROS elevation continues for at least 1-2 hours after noise exposure (Ohlemiller, et al., 1999), post-exposure administration of a protective-rescue agent (e.g., D-methionine) can be effective. Campbell et al., (2007, 2011), demonstrated that D-methionine first administered 1-7 hours and then twice a day for an additional 2 days after a 6 hour 4 kHz octave band noise exposure markedly reduced PTS measured 3 weeks following the noise exposure in a chinchilla. Therefore, it is known that D-methionine rescue can occur.

The glutathione pathway is one of the body's major detoxification pathways. Methionine may also act by increasing intracellular reduced glutathione (GSH) (Lu, 1998) and particularly mitochondrial glutathione (Fernandez-Checa et al., 1998). Hyde and Rubel (1995), reported that mitochondrial function appears to regulate the probability of cochlear hair cell survival after noise exposure. Because methionine can increase not only mitochondrial GSH levels (Lu,1998) but also can prevent the efflux of cellular GSH secondary to injury (Ghibelli et al.,1998), methionine administration may increase overall cochlear GSH levels. Further studies are currently being conducted to determine if that is the case. Noise exposure does alter cochlear GSH and oxidized glutathione (GSSG) levels (Bobbin et al., 1995, Yamasoba et al., 1998a; Campbell et al., 2003) and GSH inhibition exacerbates NIHL (Yamasoba et al., 1998b). Consequently, using an agent such as D-methionine that can potentially increase cochlear GSH levels may decrease noise-induced hearing loss via the GSH pathway.

Therefore, D-methionine may ameliorate NIHL by direct free radical scavenging, increasing cochlear glutathione levels or both. Regardless of the mechanism, it is established that D-methionine is an effective agent for NIHL prevention and rescue (Coleman et al., 2002a,b; Kopke et al., 2002; Campbell et al., 2007, 2011; Cheng et al., 2008; Samson et al., 2008). However further studies need to be conducted to move towards clinical trials.

2.4 D-methionine Versus Other Otoprotective Agents

D-methionine can be administered orally as an orange flavored suspension for this clinical study, along with a flavor matched placebo. The volume per dose is approximately a teaspoonful depending on subject weight. This formulation is stable for at least 18 months at up to 40 degrees centigrade. Further, packaging will be in individual doses for easy and contamination free distribution. Animal data for multiple applications including prevention of NIHL, cisplatin-induced hearing loss, aminoglycoside-induced hearing loss and radiation-induced oral mucositis (Campbell et al., 1996, Campbell et al., 1999, Campbell et al., 2003, Campbell et al., 2007; Vuyyuri, et al., 2008; Campbell et al., 2009; Campbell et al., 2011) with this formulation exist and have been reported.

As described above, human safety and efficacy data to prevent cisplatin induced hearing loss and radiation induced oral mucositis have been obtained through ex-US studies. (Campbell et al., 2009; Hamstra et al., 2010). Findings in animals in D-methionine protection against NIHL have been confirmed in independent labs (Campbell, et al., 2007;, Samson et al., 2008; Cheng et al., 2008; Campbell et al., 2010). However, this would be the first study in humans for protection from NIHL. In studies to date, D-

Methionine provides virtually complete protection from permanent NIHL and cochlear hair cell loss in animals (Kopke et al., 2002; Campbell et al., 2007;; Samson et al., 2008; Cheng et al., 2008 Campbell et al., 2011). These results are consistent. All studies show D-methionine protection from NIHL. No studies show a lack of protection or exacerbation of permanent NIHL. Additionally almost complete protection from permanent NIHL has been obtained in chinchillas even when it is first administered up to 7 hours after noise cessation (Campbell et al., 2011). Protection from temporary thresholds shift has been variable but has not been as carefully studied. No agent has been found to be more effective in animal studies to date. It has been studied for decades in multiple species because it is a part of nutrition.

2.4.1 N-acetylcysteine (NAC) - NAC has been the most widely studied agent for protection from NIHL, but results have been variable. Most studies show at least partial protection from permanent NIHL in animals when administered either before or within 24 hours noise exposure (Ohinata 2003; Lorito et al., 2008; Coleman et al., 2007; Bielefeld et al., 2007; Fetoni et al., 2009) but other studies have shown that NAC provides no protection or even exacerbation of NIHL (Duan et al., 2004; Hamernik et al., 2008). NAC appears to work best in combination with other agents. Kopke et al. (2000, 2001), attributed the protection against NIHL to NAC but only found significant protection from NIHL when combined with high dose salicylate. However salicylate itself has otoprotective properties (Yu et al., 1999) and may have contributed to the results observed. Unfortunately, high dose salicylate (aspirin) is probably not advisable for military use because it can increase the risk of bleeding. Some studies do show partial protection from permanent NIHL using NAC in isolation however (Kopke et al., 2005, 2007).

Three human clinical trials with NAC have been conducted but none showed significant protection from NIHL (Toppila et al., 2002; Kramer et al., 2006, and Kopke et al., unpublished data). Toppila et al. (2002, unpublished), used 400 mg NAC per day and Kramer et al. (2006) used 900 mg NAC per day in a double blind placebo clinical trial of 31 normal hearing subjects before and after 2 hours of night club noise. Neither study showed any otoprotection. Another study using 900 mg NAC administered 3 times per day in 566 U.S. Marine recruits at Camp Pendleton exposed to 300 rounds of M-16 weapon fire reported no protection from permanent NIHL. The results have not been published but have been publicly presented. D-methionine and NAC are among the most widely studied protective agents for NIHL. In comparative studies however, thus far D-methionine seems to provide superior otoprotection. Coleman et al., 2002 reported that pre-administration of a combination of low-dose D-methionine and NAC markedly reduced permanent noise-induced threshold shift in chinchillas, but that the protection afforded by the D-Methionine component alone was similar to the combined administration of D-methionine and NAC. However, when the NAC component was delivered alone, no protection was provided. Kopke et al., (2002) reported that both D-methionine and NAC pre- administration (Kopke et al., 2000) could protect against NIHL in the chinchilla. However D-methionine provided superior cochlear outer hair cell protection. Over 90% of outer hair cells were preserved with D-methionine protection as compared to only 50-60% with NAC/salicylate pre-administration. The reason they administered NAC with salicylate is reportedly to improve the stability of NAC, but as reviewed above, the salicylate may have also served as the otoprotective agent.

2.4.2 Ebselen - Ebselen, a selenium containing compound, has also shown some efficacy in animals and is reportedly approaching clinical trials at Camp Pendleton. However, even with 14 days of administration for a single noise exposure the protection from NIHL appears less than in the D-methionine studies (Kil et al., 2007; Kopke et al., 2002, Campbell et al., 2007, 2011) although noise exposure paradigms were not identical. Partial protection from permanent NIHL has been consistently observed across studies in the rat and guinea pig (Pourbakht and Yamasoba 2003; Lynch et al., 2004; Lynch and Kil 2005) with no studies reporting a lack of protection or exacerbation of NIHL. Only one study has been published addressing temporary threshold shift and that study did show significant reduction of temporary threshold shift in the guinea pig (Yamasoba et al., 2005.) Ebselen can be administered orally and they are using a dry blend capsule for clinical studies.

2.4.3 ACE Magnesium (ACE Mg) – Ace-Mg (a combination of beta carotene -the precursor to vitamin A, plus vitamins C and E, and magnesium) is another otoprotective agent for NIHL which can be delivered as a capsule. This agent combination is currently in clinical trials in Florida through a grant from the National Institutes of Health (NIH) grant awarded to Dr. Miller of the University of Michigan. Drs. Miller and Le Prell are the inventors on that patent application but Dr. Campbell is the audiology clinical trials coordinator for those studies. To date, the animal work with ACE Mg seems promising (Le Prell et al., 2007; 2009a,b). While Mg alone or the ACE combination alone did not confer significant hearing protection, the combination of ACE Mg provided partial but significant protection from permanent NIHL in the guinea pig (Le Prell et al., 2007, 2011) and the mouse (LePrell et al., 2009a). It also reduced temporary threshold shift in the guinea pig (Le Prell et al., 2009b). However these findings have not yet been confirmed in research labs other than the inventors' labs.

One limitation of this combination is that it cannot be used in smokers because beta carotene may increase the risk of lung cancer and cannot be used in individuals with gastric disorders because of the Mg content. The Mg content may increase the risk of loose stools which may limit use in some occupations such as the military or certain industries. Thus, even if successful in clinical trials, it cannot be used in all patient populations exposed to excessively high noise levels.

2.5 Distribution of D-methionine

Methionine is an amino acid, and both the D and L isomers been studied for several decades (Block and Bolling 1945) in a wide variety of animal models and in humans as it is part of normal protein intake in the diet with oral intake and also part of parenteral nutrition. The D isomer is more common in fermented proteins such as cheese and yogurt. Thus, an extensive literature exists regarding its distribution, degradation, catabolism, metabolism in many texts (Linder 1985; Kleinman and Lemann1987; Abelow 1998; Stipanuk and Watford 2000; Liberman et al., 2006).

The D-isomer of methionine is primarily distributed in plasma and is excreted in the urine unless it is transaminated to the L-isomer, which can be incorporated into protein formation. Both isomers of methionine are small molecules with wide distribution throughout the body including easily passing through the blood brain barrier.

2.6 D-methionine Safety Factors

Methionine is a micronutrient, and thus it is not alien to the human system. It is present in a wide variety of foods (Friedman, 1999b). Methionine comprises 26 mg/g high quality protein in the diet (National Academy of Sciences 1980). Methionine is used for other purposes and at relatively high doses. The World Health Organization lists methionine as an essential drug for treating acetaminophen overdose (WHO 1997). As an oral antidote, methionine is administered initially at 2.5 g, followed by three more 2.5 g doses at four-hour intervals, for a total dose of 10 g over 12 hours.
Monteagudo et al., (1986) noted that methionine is "remarkably free of side effects" including nausea and vomiting. Di Rocco et al., 1998 administered 3 g L-methionine twice a day for six months to treat vacuolar myelopathy in 12 HIV-infected human adults. Patients tolerated it (6 grams/day) well, other than one complaint of some nausea. DiRocco et al. (1998) further reported that even 20g/day for an adult is safe for chronic administration.

Methionine has been available for decades as an over-the-counter orally administered preparation to reduce urinary odor and dermatitis. For that application in adults, the recommended dosing is 200-400 mg orally three to four times per day (Drug Facts and Comparisons 1991). Most human studies using methionine reported no side effects (Kies et al.,1975; Kaji et al., 1987; Stegink et al., 1986). However,

methionine toxicity can occur with very high dosing of racemic or L-methionine, particularly in the presence of a low protein diet and/or in developing animals as opposed to adults (Benevenga, 1974; Klavins and Johansen, 1965; Daniel and Waisman, 1969; Cohen et al., 1958; Muramatsu et al., 1971; Klavins, et al., 1963).

To date, no side effects have been encountered greater in D-methionine than in placebo groups in the ex-US normal Phase 1 subjects or in Phase 2 clinical trials for either radiation-induced oral mucositis or cisplatin-induced hearing loss (Hamstra et al., 2010). Because the Phase 2 studies were conducted in cancer patients undergoing therapy, some nausea was reported but it was not significantly different between the treated and control groups and it is probably not D-methionine related. No side effects occurred in normal subjects in the Phase 1 study (Hamstra et al., 2010).

2.7 Methionine: Comparison of the D isomer vs. the L isomer

Methionine is a compound known to have relatively low toxicity in humans (Kaji et al. 1987; Kies et al., 1975; Stegink et al., 1986). It is used throughout Europe and India in high doses (total dose of 10 g given over 12 hours) to prevent toxicities associated with acetaminophen overdose (WHO, 2011). It is also used in lower doses (200-400 mg 3 to 4 times a day) to reduce urinary odor and dermatitis. The D-isomer appears to be better tolerated than either the L-isomer or the racemic mixture (Monteagudo et al. 1986; and Stekol et al, 1962).

2.7.1 D versus L Methionine Utilization Varies by Species

Humans and monkeys utilize only 30% of D-methionine but utilize 100% of L-methionine (Baker 2006). This poor utilization by humans and monkeys, specifically of the D isomer of methionine, is in contrast to the excellent utilization of D-methionine in the dog, pig, mouse, rat, rabbit, and chick (Stegink et al., 1980; Cho et al. 1980; Burns and Milner 1981; Baker 2006) [Table 1].

TABLE 1. Relative utilization on sulfur amino acid isomers, analogs, and precursors (Baker 2006)

Amino acid	Chick	Rat	Mouse	Pig	Dog	Human
L-Met	100	100	100	100	100	100
D-Met	90	90	75	100	100[2]	30[3]
DL-Met	95	95	88	100	100	65
DL-OH-Met	80	70	70	80	NA	NA
Keto-Met	90	NA	NA	NA	NA	NA
L-Met sulfone	NA	0	0	NA	NA	NA
L-Met sulfoxide	NA	60	85	NA	NA	NA
N-acetyl-L-Met	100	100	90	NA	100	NA
N-acetyl-D-Met	0	0	25	NA	0	NA
L-Hcy	65	65	NA	NA	NA	NA
D-Hcy	7	NA	NA	NA	NA	NA
S-Methyl-L-Met	+[4]	+[4]	NA	NA	NA	NA
L-Cys	100	100	100	100	100	100
L-cystine	100	NA	NA	100	NA	NA
D-cystine	0	0	0	NA	0	NA
Keto-Cys	NA	0	NA	NA	NA	NA
L-Cysteic acid	NA	0	NA	NA	NA	NA
DL-Lanthionine	35	NA	35	NA	NA	NA
GSH	100	100	NA	NA	NA	NA
N-Acetyl-L-Cys	100	100	100	100	NA	NA
S-Methyl-L-Cys	NA	NA	0	NA	NA	NA
L-Hcy	100	NA	NA	NA	NA	NA
D-Hcy	70	NA	NA	NA	NA	NA
L-Met	100	100	NA	100	NA	100
Taurine	0	0	NA	NA	NA	NA
L-OTC	80	70	NA	NA	NA	NA

[1] Values are expressed as growth efficacy percentages (molar or isosulfurous basis) of the L-isomer, which in all cases is presumed to represent 100% oral utilization (14); NA = data unclear or not available.
[2] Efficacy of D-Met is also near 100% in growing kittens.
[3] Efficacy is about 30% in monkeys also.
[4] Met sparing present in chicks when both dietary Met and choline (or betaine) are deficient; choline sparing definitely occurs in both chicks and rats.

As shown in Table 1, oral utilization of D-methionine in the pig, dog, and growing kittens is near 100%, 90 % for the rat and chick, and 75% for the mouse in contrast to the human and the monkey's utilization rate of only 30%. However for L-methionine, utilization is 100% for all the aforementioned species including the human.

Specifically, the utilization of D-methionine in rats is quite different than in humans. In rats, over 90% of D-methionine is rapidly converted to the L-isomer (Hasegawa et al., 2005). In rats, D-methionine is the most effectively used of all the amino acids and is also toxic but less so than L-methionine when added to the diet at excess levels (Sauberlich 1961; Sugiyama and Muramatsu 1987). Interestingly, transport characteristics in the intestine are similar in rats and humans (Zheng et al., 1994) thus the differences in utilization are not secondary to simple differences in transport across the intestinal epithelium.

D-methionine also has the advantage in humans, in that plasma concentrations of D-methionine peak at a higher level (Stegink et al., 1986) and decline at a slower rate than for L-methionine, possibly because of the lower utilization(see Figure 1 & 2). Thus the D-isomer may be available in the plasma longer to serve as an antioxidant. Yet, nitrogen balances of human subjects fed D-methionine are no different than subjects fed no methionine, although subjects fed L-methionine had significantly higher nitrogen balances (Kies et al., 1973; Zezulka and Calloway 1976).

Figures 1 & 2. Plasma concentrations of D and L methionine

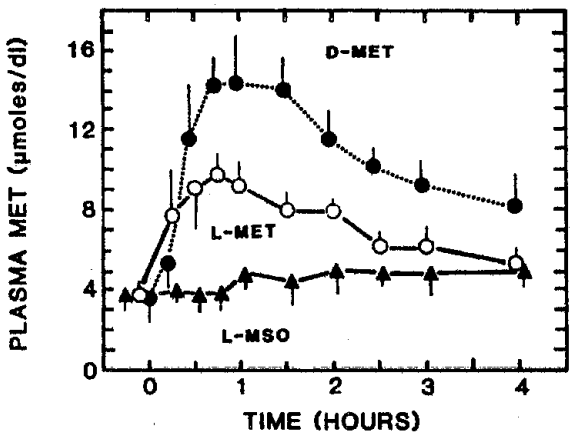


Figure 1. Mean ± SD plasma total free methionine (MET) concentrations (μmol/dl) in 4 normal adults administered 0.0605 mmol/kg body wt of D-methionine (● … ●, D-MET), L-methionine (○ ○, L-MET) and L-methionine-*dl*-sulfoxide (▲ ▲, L-MSO). (Stegink, et.al., 1986)

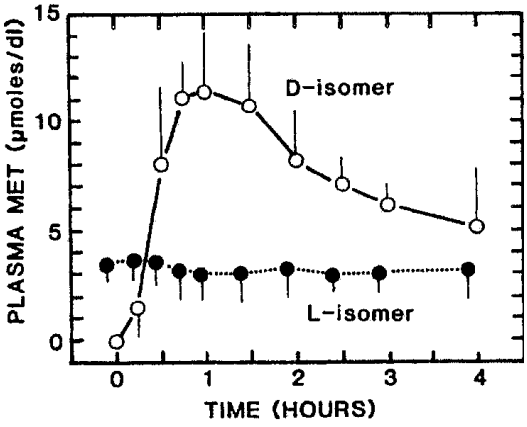


Figure 2. Mean ± SD plasma concentrations of L-isomer (●----●) and D-isomer (○ ○) of methionine in 4 normal adults administered 0.0605 mmol/kg body wt of D-methionine. (Stegink, et.al., 1986)

2.7.2 D versus L Methionine excretion varies by species:

Researchers have found that D-methionine, in humans, whether administered orally or parenterally, is excreted in large quantities in urine (Stegink et al., 1980; Printen et al., 1979; Stegink et al., 1971; Heller et al., 1970; Efron et al., 1969). Some variation exists across human studies, probably secondary to administration method and specific human population, but D-methionine is clearly less well utilized in humans than in rats, chicks, pigs, rabbits and dogs as indicated by preferential urinary excretion of the D-isomer in humans. Term infants excreted 34-45% of the D-isomer from DL methionine containing formula (Stegink et al., 1971). Adult humans excreted 11-37% of oral D-methionine (Zezulka and Calloway 1976 and Boggs 1978). Infused D-methionine in post-surgical patients given complete parenteral nutrition containing DL methionine resulted in a 55-70% excretion rate (Printen et al., 1979). While Brummel et al. reported 33% of infused D-methionine was excreted in normal adults receiving protein sparing regimens. These data are in contrast to the rat in which less than 1% of D-methionine was excreted in rats infused with parenteral solutions of DL methionine (Cho and Stegink 1979). Similarly low excretion levels of D-methionine urinary excretion have been reported in neonatal pigs infused with DL methionine (Cho et al., 1980), and no significant urinary excretion of D-methionine in rabbits or dogs and ingesting oral D-methionine loads (Cho et al., 1980).

The only reported animal species somewhat similar to the human appears to be the monkey, although reported excretion of the D-isomer is still less than in most human studies (Stegink et al., 1971; Zezulka 1978 and Boggs 1978; Printen et al., 1979). Monkeys, unlike rats, chicks, pigs, rabbits, and dogs, process D-methionine more similarly to humans in that D-methionine is preferentially excreted in the urine while L-methionine is not. In 4 normal adult cynomolgus monkeys, Stegink et al., 1980 reported that for an ingested average of 488 micromoles DL methionine per day, mean daily urinary excretion was 67.4 micromoles of which 65.5 micromoles was the D-isomer. Thus, 26.8% of ingested D-methionine was excreted but only .008% of L-methionine was excreted versus only .077% of the L-isomer. Based on

plasma measurements, the authors also concluded that the excretion did not reflect an "overflow amino aciduria". In an additional experiment, the animals were fasted for 8 hours then administered 150 mg (1,006 micromoles) dissolved in 10ml water of D-methionine by feeding tube. They then fasted for an additional 24 hours but with water ad libitum. During this 24 hour period, 173 ± 46 micromoles methionine of which 97% was of the D configuration. Thus 17% of administered D-methionine was excreted.

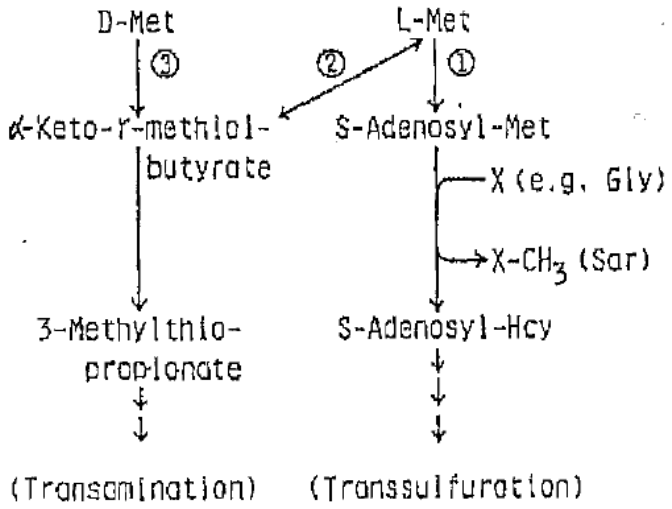
Methionine loading tests (L or DL methionine) have been performed in over 6000 subjects without any serious side effects with one exception (Garlick 2006). Cottington et al., 2002 reported one death that was reportedly secondary to a 10 fold overdose (1 g/kg rather than 100mg/kg for a total dose of up to 80 g) of L-methionine. Methionine loading tests are usually conducted with 100 mg/kg methionine which is about 7 times the daily requirement for total sulfur amino acids (methionine plus cysteine) (Garlick 2006). However, no study reported in the literature has performed D-methionine loading.

2.7.3 D versus L Methionine Safety Comparisons

In the rat, growth depression by D-methionine is significantly less than with L-met (Sugiyama and Muramatsu 1987). However for the chick, Baker and Boebel 1979 reported that L-methionine was superior to D-methionine in promoting growth as did Friedman and Gumbmann 1984 in the rat. However, adverse effects on growth have not been reported in human adults.

The limited capacity of the transulfuration pathway rather than metabolism of methionine in the transamination pathway is primarily responsible for the adverse effects of methionine (Sugiyama and Muramatsu 1987). The limited capacity of the transulfuration pathway is primarily responsible for methionine toxicity which suggests that L-methionine, primarily metabolized through the transulfuration pathway, underlies methionine toxicity. D-methionine is metabolized via the transamination pathway which does not appear to play a major role in toxicity (Sugiyama and Muramatsu 1987). See Figure 3 and 4. D-methionine can contribute to toxicity if converted to L-methionine thus then being metabolized through the transsulfuration pathway (Sugiyama and Muramatsu 1987).

Figures 3 & 4. Effects of experimental diets on the growth (A) and food consumption (B) of rats



Figures 3 & 4 show the metabolic relationship between D and L methionine in mammals (1) Met adenosyltransferase; (2) Met transaminase(s); (3) D-amino acid oxidase (Sugiyama and Muramatsu 1987)

However, because D-methionine is so quickly and effectively converted to L-methionine in the rat, unlike the human and monkey, the toxicities observed in the rat may be the result of the resultant L-methionine rather than specifically the D-isomer (Hasegawa et al., 2005). Similarly, Benevenga 1974 concluded that "the apparent toxicity of D-methionine may be simply due to its conversion to L-methionine".

2.7.4 Potential Risks in Humans

2.7.4.1 Potential Increase in Homocysteine Levels for L-Methionine Administration, Not Investigated for D-Methionine Administration

High dose methionine administration, such as methionine loading, has been reported to increase homocysteine levels (See review by Garlick 2006). However, no studies have reported the relationship of D-methionine to homocysteine levels. Considering that D-methionine is highly excreted in humans, without conversion to the L isomer, and utilization of the D-isomer is only 30% as opposed to 100% for L-methionine it seems unlikely that D-methionine would be metabolized to increase homocysteine levels in the same manner as L-methionine. However, even for L-methionine, B-12 and folate can reduce the correlation of methionine loading to homocysteine formation (Garlick 2006).

2.7.4.2 D L Methionine May Exacerbate Schizophrenia

Baldessarini et al. reported that large doses of DL methionine (4 to 40 g/d of L or DL methionine for 1 week to 2 months) in neuropsychiatric disease, such as, schizophrenia, markedly exacerbated schizophrenics' psychotic symptoms (Garlick 2006). Cohen et al. noted large doses of methionine (5-40 g/d of L or DL methionine) with or without a monoamine oxidase inhibitor, given over periods of 1 week to 2 months have resulted in striking exacerbation of psychotic symptoms in chronic schizophrenic

patients (Garlick 2006). In healthy subjects, doses of 10 g of methionine have been shown to have no effect (Baldessarini et al., 1979).

2.7.4.3 Potential Antidote if Needed

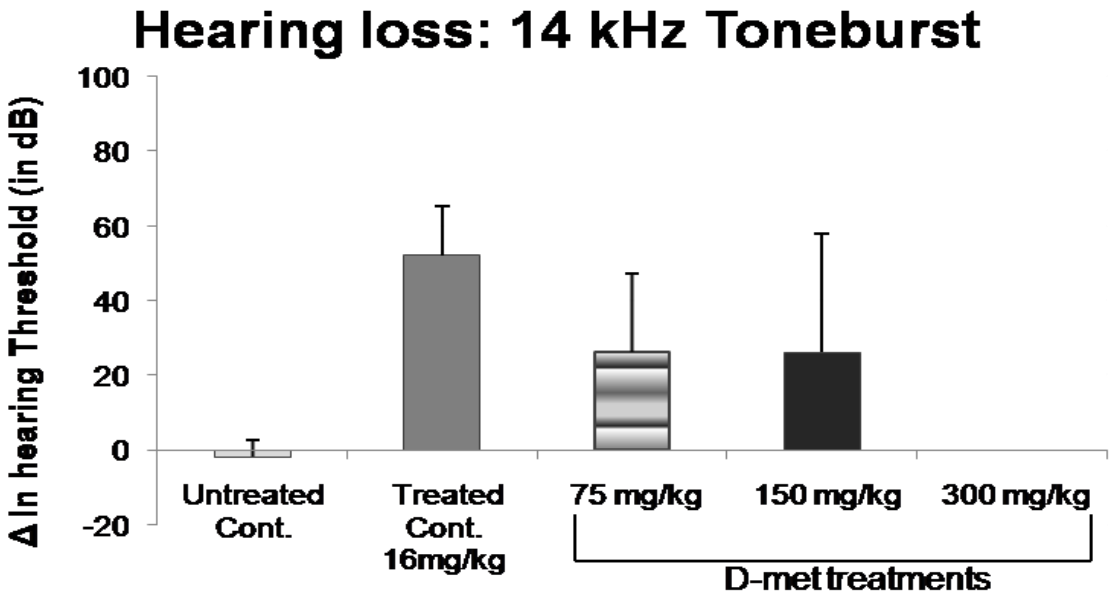
The toxicity of methionine can be alleviated by dietary supplementation with glycine (Benevenga and Harper 1967; Benevenga 1974). However, these studies were conducted with L-methionine and only in animals.

3.0 SUMMARY OF PRE-CLINICAL AND CLINICAL STUDIES

3.1 Previous Pre-Clinical Studies

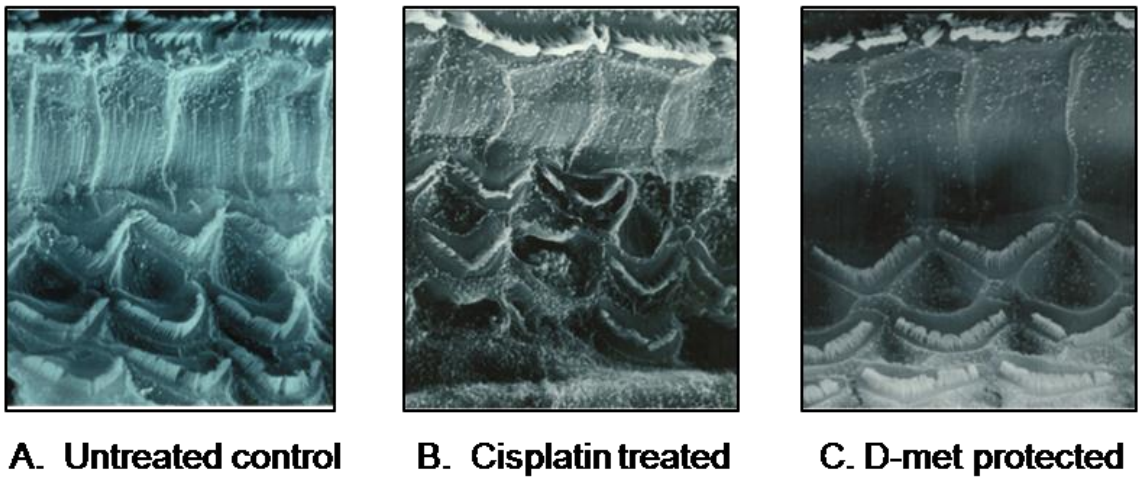
In 1996, it was first reported that D-methionine protected against cisplatin-induced (CDDP) ototoxicity in the rat (Campbell et al., 1996). Figure 5 below shows the change in auditory brainstem response (ABR) thresholds for 14 kHz tone bursts in rats receiving saline injection only (untreated control), 16 mg/kg CDDP (treated control group), and rats receiving either 75, 150, or 300mg/kg D-methionine prior to the 16 mg/kg CDDP.

Figure 5. Protection from cisplatin-induced auditory threshold shift in rats.



Additionally, the literature contains reports that the 300 mg/kg dose of D-methionine prevented CDDP-induced outer hair cell loss. Representative photomicrographs from the basal turn are presented in Figure 6 below.

Figure 6. Protection from cisplatin induced cochlear hair cell loss.



3.2 D-Methionine Protection Against Cisplatin-Induced Hearing Loss in Humans

To date, one study has been conducted in normal human subjects for pharmacokinetics and side effects; two studies evaluating the safety and effectiveness of D-methionine for radiation-induced oral mucositis in humans. A fourth study evaluated the effectiveness of D-methionine protection in cisplatin-induced hearing loss. Findings from the first two studies were recently published (Hamstra, et al., 2010); studies #3 and #4 are in preparation.

- Study #1: Phase 1a: Pharmacokinetic Evaluation of MRX-1024 included 12 normal human adult volunteers.

- Study #2: Phase 1b: Open-label, multiple-dose, phase 1study of MRX-1024 concurrent with radiation therapy with or without cisplatin. Purpose of the study was to evaluate the effect of MRX-1024 on radiation-induced oral mucositis in head and neck cancer. This study included 25 adult subjects with head and neck cancer (15 male/10 female; mean age 47 years).

- Study #3: Phase 2: Multi-center, randomized, double-blind, placebo-controlled, phase 2 study to evaluate the safety and effectiveness of MRX-1024 for mucosal protection in head and neck cancer patients. This study included 58 adult subjects (44 male/14 female; mean age 49 years).

- Study #4: Phase 2: Randomized, double-blind, placebo-controlled, phase 2 study to evaluate MRX-1024 protection in cisplatin-induced hearing loss. This study included 27 adult subjects (6 male/21female; mean age 55 years).

Results from the most recent study, provides some evidence of effectiveness in humans for protecting hearing from cisplatin chemotherapy. In this double blind randomized pilot study, 14 adult patients received 100 mg/kg dose of an oral orange flavored suspension of D-met (MRX-1024) and 13 subjects received flavor matched placebo in equivalent volume prior to each dose of cisplatin. Mean cumulative cisplatin dosing was 263.57 (SD 74.79) in the experimental group and 253.85 (SD 56.94) in the control group. Primary tumor sites ranged from genitourinary tract to head and neck cancers. Six patients in the experimental group and four patients in the placebo group also received radiation to the head and/or neck area for primary tumors in that region. Auditory thresholds were tested bilaterally at 8, 10, 11.2 and 12.5 kHz with a GSI 61 audiometer using a modified Hughson-Westlake technique. Significant threshold

protection was obtained for the frequencies of 10 kHz and 11.2 kHz. No difference in tumor regression was noted between groups.

Although the sample size was small (n = 27), protection from hearing loss at 10 kHz and 11.2 kHz was statistically significant as demonstrated in Figures 7 and 8. For the other two frequencies tested, 8 kHz and 12.5 kHz, the results were completely consistent with the protection observed at 10 kHz and 12.5 kHz, but because of slightly greater variability in the relatively small sample size, the results at those two frequencies did not reach statistical significance (Campbell et al, 2009). While additional audiometric frequency testing would have been desirable; test time was an issue with these patients, so the four most sensitive frequencies were chosen for cisplatin ototoxicity which was the higher frequency range. Nausea was the most common side effect, but was not significantly different between treatment and placebo groups.

Figure 7. Protection from cisplatin induced hearing loss in humans.

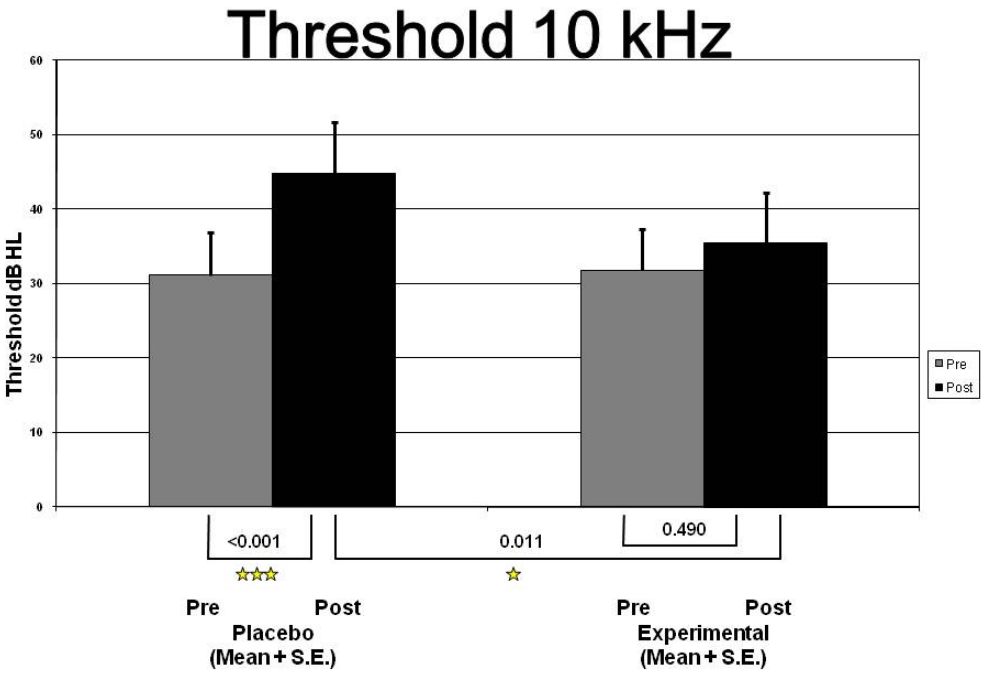
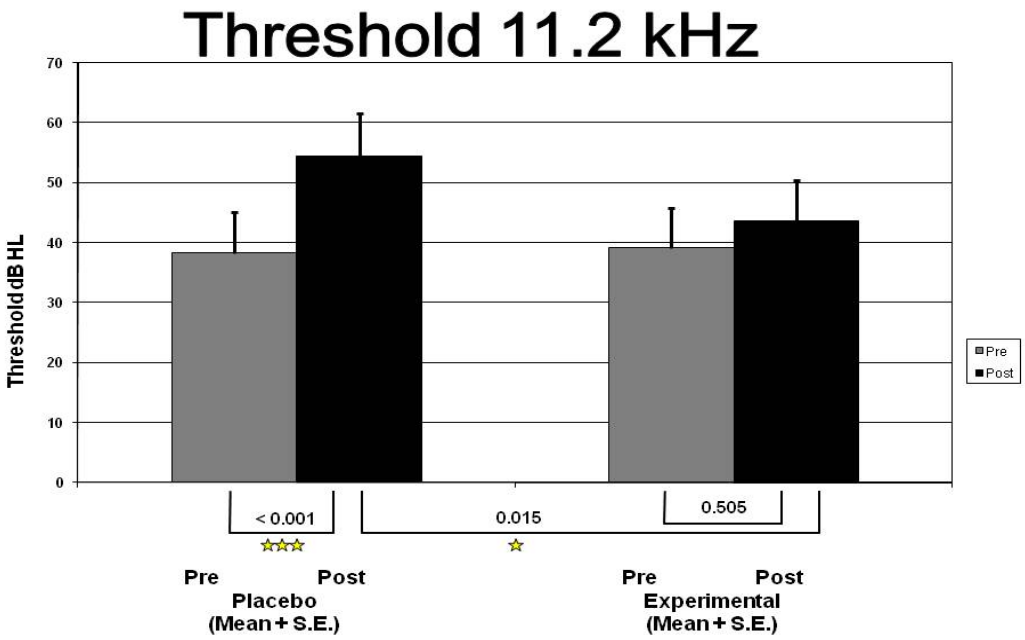


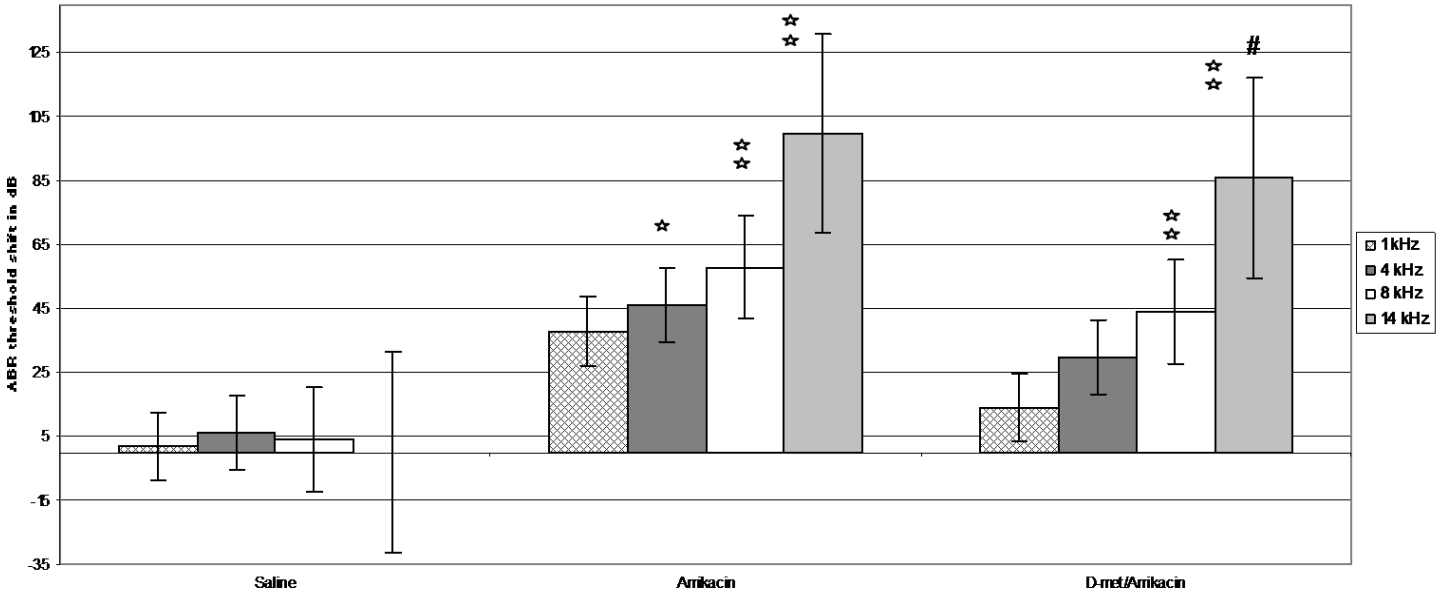
Figure 8. Protection from cisplatin induced hearing loss in humans.



3.3 D-methionine Protection Against Aminoglycoside-Induced Ototoxicity

In another set of experiments (Campbell, 2007), showed that D-methionine partially protected against amikacin-induced hearing loss in guinea pigs. Hartley guinea pigs, 5 per group, received either saline injections (control) 200 mg/kg subcutaneously (s.c.) or amikacin only for 28 days (treated control) or 300 mg/kg ip D-methionine 30 minutes prior to each 200 mg/kg s.c. amikacin injection (experimental group). ABR results for the 1, 4, 8, and 14 kHz stimuli after 28 days are shown in **Figure 9**.

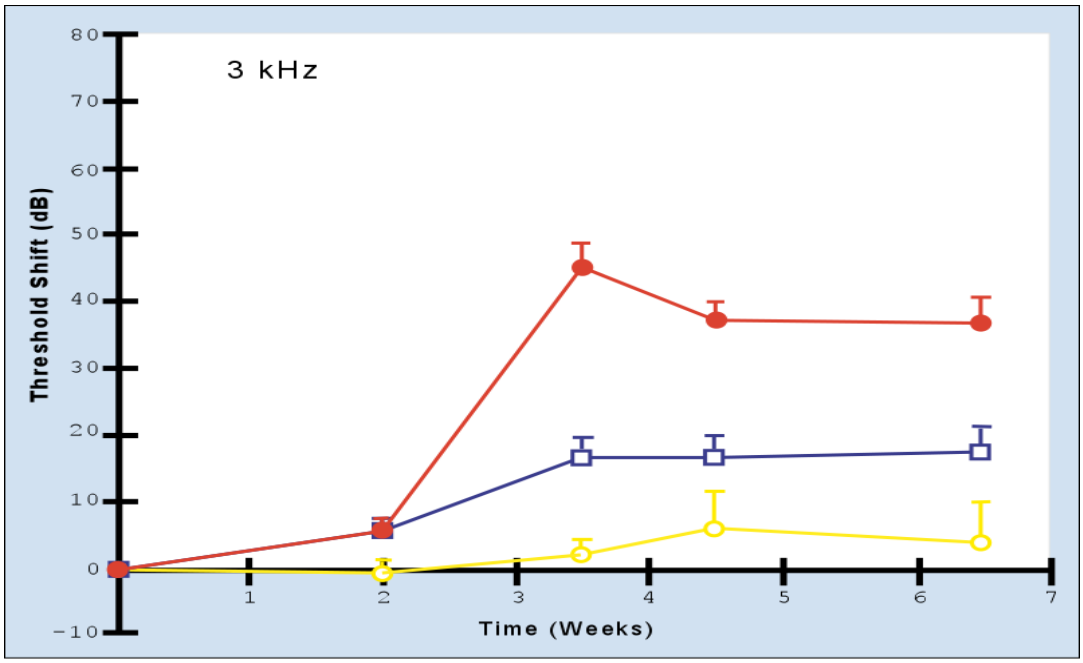
Figure 9. Protection from aminoglycoside-induced auditory threshold shift in guinea pigs.



The left bar in each group is the control, the middle bar amikacin alone and the right bar D-met prior to the amikacin. D-met provided partial but incomplete protection against amikacin-induced ototoxicity. As expected, no threshold shift occurred in the untreated control group, and significant ($p \leq .05$) threshold shift occurred in the treated control group for all stimuli. For 1, and 4 kHz stimuli, ABR threshold elevation was reduced in the D-met treated group with values not significantly different from either the treated or untreated control group, suggesting only partial protection. For the 8 kHz stimulus, the average ABR threshold elevation was somewhat less for the D-met protected group than for the amikacin only group but was significantly greater than for the saline only control group. For the 14 kHz stimulus, the D-met protected group did show significantly less threshold shift than the amikacin only group but protection was still only partial. Although a significant difference between the amikacin only and D-met protected groups was found only at 14 kHz, there was a definite trend toward otoprotection for all tone-burst stimulus conditions with average ABR threshold shift from the D-met protected group was consistently lower than for the amikacin only group but variability (note error bars) may have precluded statistical significance.

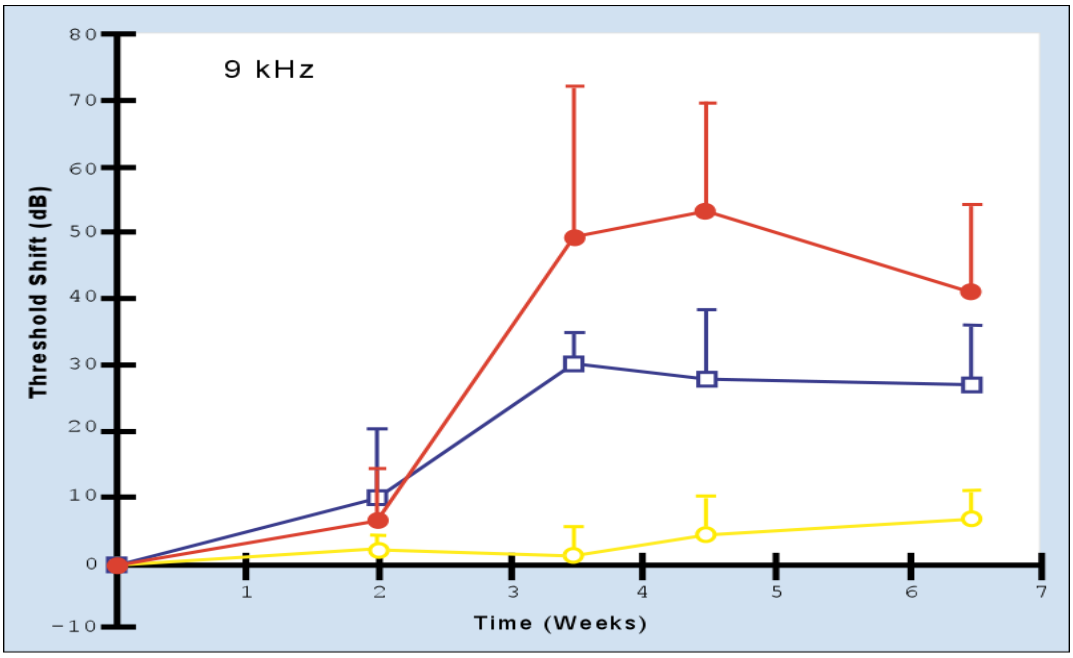
Studies have confirmed that aminoglycoside otoprotection in animals with divided daily doses is as beneficial as increasing the dose. Sha and Schacht (2000) reported that twice daily injections of D-methionine markedly improved D-methionine protection from gentamicin aminoglycoside ototoxicity in a guinea pig model (**Figures 10, 11, & 12** used with permission from Hearing Research and Dr. Schacht) show the protection of twice daily administration of 200 mg/kg D-methionine 7 hours apart, using the same gentamicin model for the ABR stimulus frequencies of 9, and 18 kHz respectively. ABR thresholds (mean plus one SD N=6 per group) were determined from 0 to 6.5 weeks. The top line on each graph (filled circles) represents gentamicin alone. The next line (open squares) represents gentamicin plus twice daily D-methionine. The bottom line (open circles) represents saline controls. Twice daily D-methionine provided significant though incomplete protection at all frequencies. From Sha and Schacht, it is clear that D-methionine protection was greater with two as opposed to one daily injection of 200 mg/kg D-methionine. However, because the D-methionine dose was always 200 mg/kg for each injection, it could not be determined whether the additional D-methionine protection was the consequence of the twice daily dosing strategy or simply increasing the daily dose of D-methionine from 200 mg/kg to 400 mg/kg.

Figure 10. Protection from aminoglycoside auditory threshold shift in guinea pigs: twice daily D-methionine dosing.



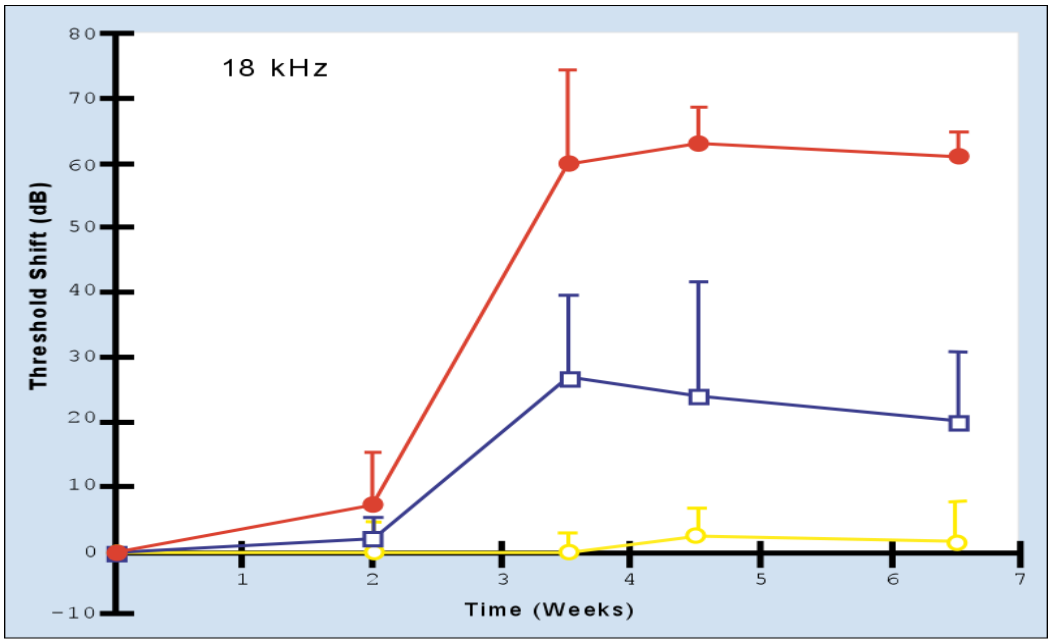
Effect of twice-daily treatment with D-methionine on gentamicin-induced threshold shifts. ABR thresholds were determined from 0 to 6.5 weeks. Values are means S.D. (n=6). Open circles, controls; filled circles, gentamicin alone; open squares. gentamicin plus D-methionine. Gentamicin increased thresholds at 3.5 weeks and later ($P<0.05$). D-methionine attenuated these threshold shifts at 3 kHz at 3.5 and 4.5 weeks ($0.1>P<0.05$). (Sha and Schact, 2000)

Figure 11. Protection from aminoglycoside auditory threshold shift in guinea pigs: twice daily D-methionine dosing.



Effect of twice-daily treatment with D-methionine on gentamicin-induced threshold shifts. ABR thresholds were determined from 0 to 6.5 weeks. Values are means S.D. (n=6). Open circles, controls; filled circles, gentamicin alone; open squares, gentamicin plus D-methionine. Gentamicin increased thresholds at 3.5 weeks and later ($P<0.05$). D-methionine attenuated these threshold shifts at 9 kHz at 3.5 and later ($P<0.05$). (Sha and Schact, 2000)

Figure 12. Protection from aminoglycoside auditory threshold shift in guinea pigs: twice daily D-methionine dosing.



Effect of twice-daily treatment with D-methionine on gentamicin-induced threshold shifts. ABR thresholds were determined from 0 to 6.5 weeks. Values are means S.D. (n=6). Open circles, controls; filled circles, gentamicin alone; open squares, gentamicin plus D-methionine. Gentamicin increased thresholds at 3.5 weeks and later ($P<0.05$). D-methionine attenuated these threshold shifts at 18 kHz at 3.5 and later ($P<0.05$). (Sha and Schact, 2000)

3.4 D-methionine Protection from Noise-Induced Hearing Loss

A number of studies have been conducted investigating D-methionine protection and rescue from NIHL (Kopke, 2002; Campbell et al 2007, Campbell et al, 2011).From studies with D-methionine protection from cisplatin and aminoglycoside protection and in studies of the mechanisms of D-methionine, it has been shown that D-methionine is a potent direct and indirect antioxidant that could protect the auditory system from a variety of insults. Campbell previously collaborated with Dr. Kopke in testing D-methionine protection from noise- induced hearing loss. In 2002, publication of the data and study results, Kopke (2002) showed that D-methionine provided virtually complete protection from noise-induced hearing loss in the chinchilla when it was administered 200 mg/kg twice/day starting 2 days before and continuing after a 6 hour 105 dB SPL 4 kHz narrow band noise for 6 hours. Further, the protection of outer hair cells was excellent.

Figure 13 shows D-methionine protection from permanent noise-induced hearing loss (ABR threshold shifts) in chinchillas 21 days after a 105 dB SPL 4 kHz narrow band (NB) noise exposure for 6 hours.(Kopke et al., 2002). D-methionine was administered with 200 mg/kg ip injections at 12 hour intervals starting 2 days prior to the noise, 1 hour prior to the noise and then at 12 hour intervals for an additional 2 days.

Figure 13. D-methionine protection from noise-induced threshold shift in the chinchilla

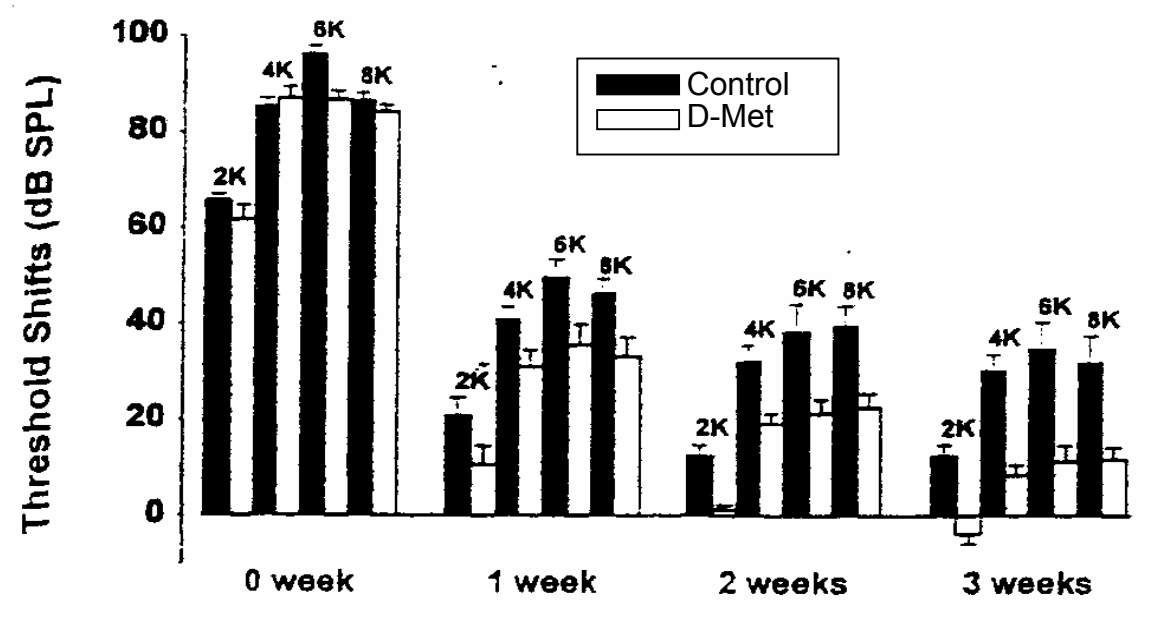


Figure 14. Protection from inner and outer hair cell loss in the same groups of chinchillas as in the **Figure 13** ABR data.

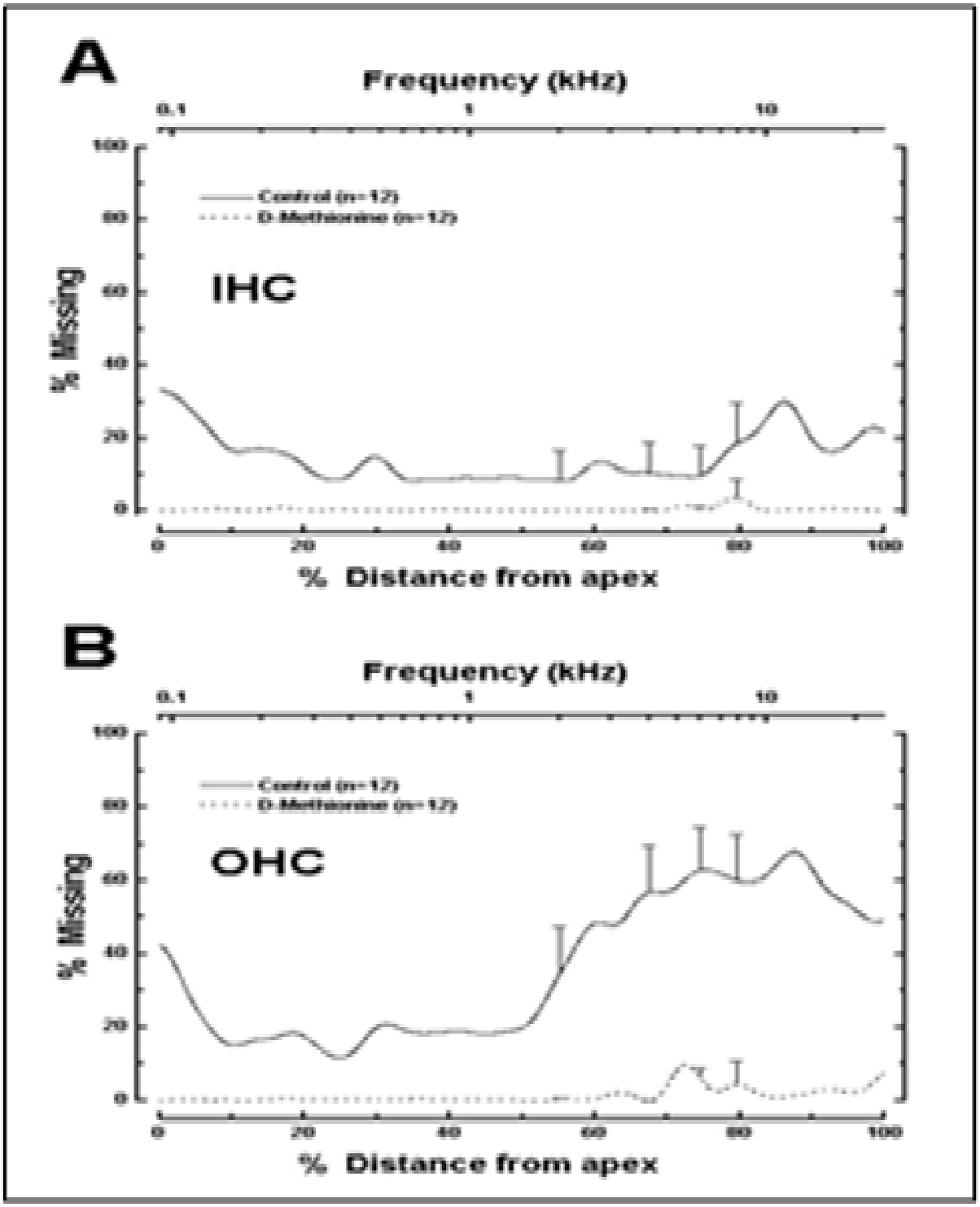
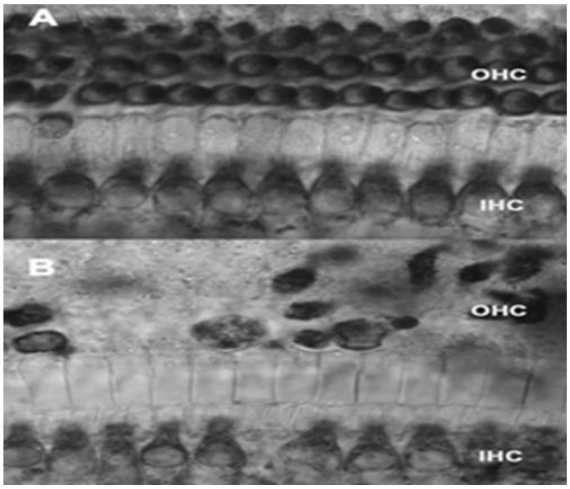


Figure 15. Cochlear outer hair cell protection from noise exposure in chinchillas.



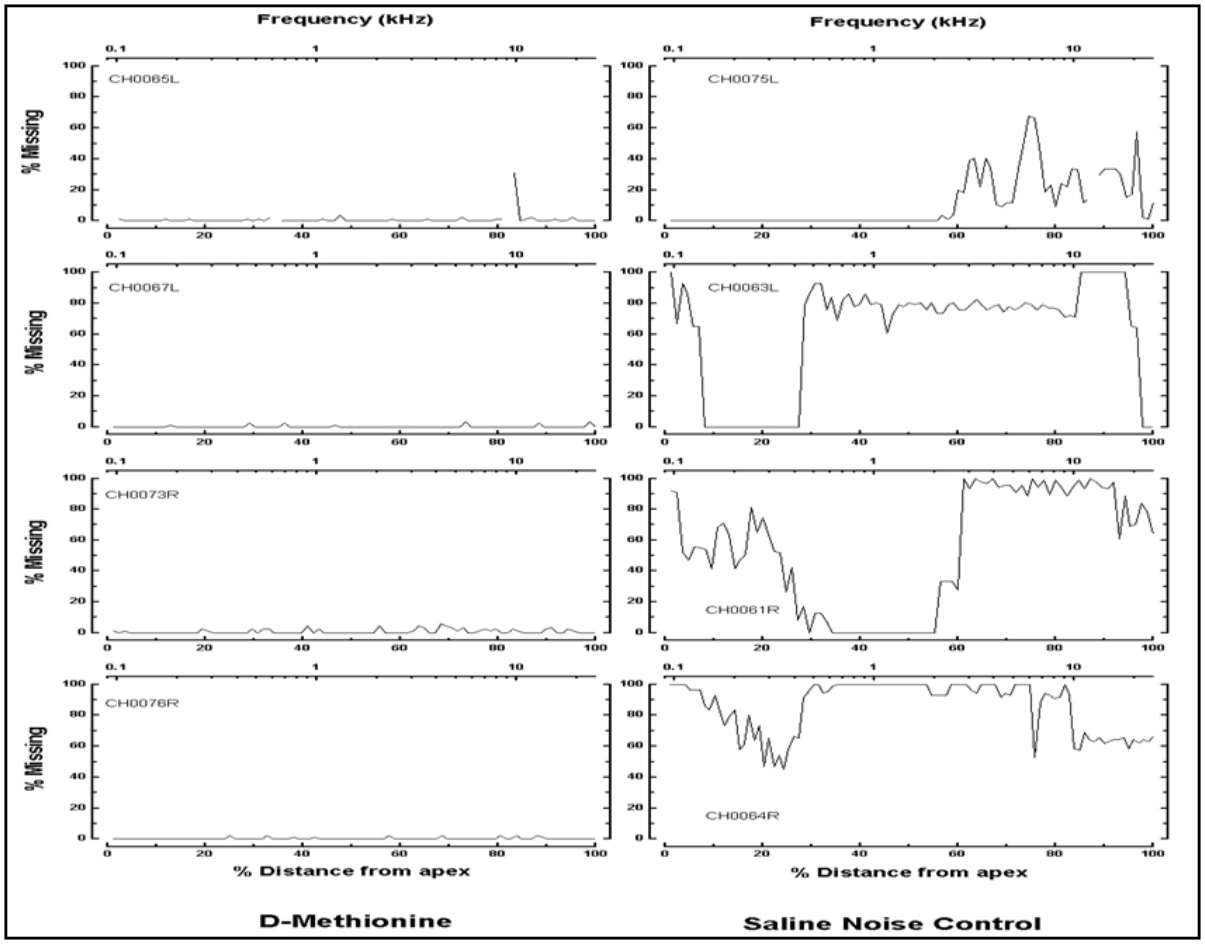
Representative Samples:

A. D-methionine protection

B. No D-methionine protection

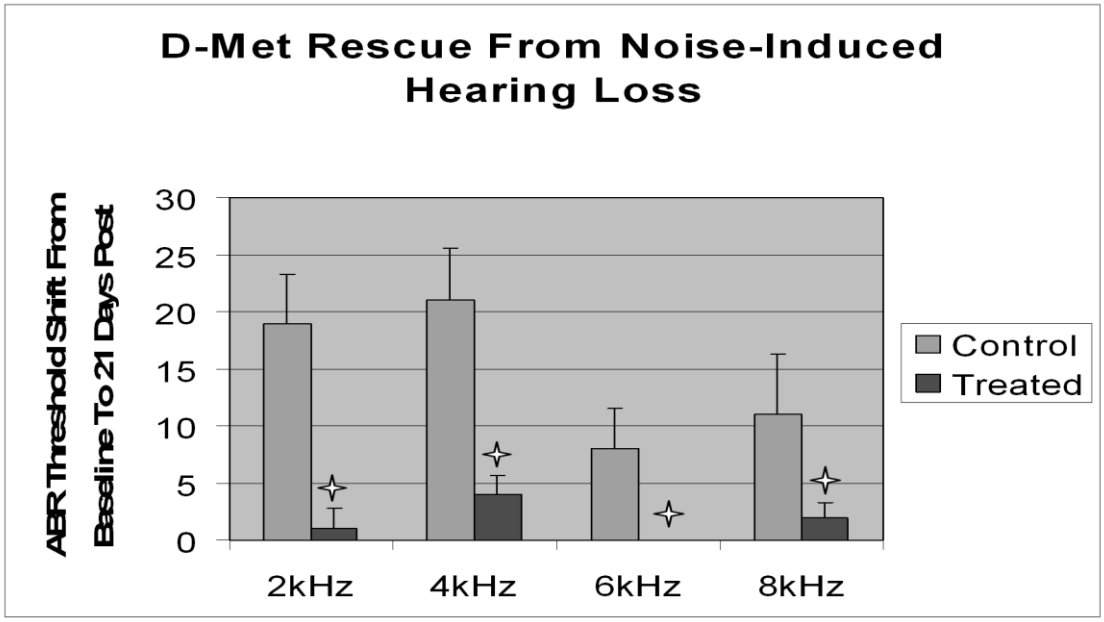
Figure 15 shows a representative example of the photomicrographs of the basal region of the chinchilla inner and outer hair cells, with D-methionine protection (top) and without D-methionine protection (bottom) 21 days after the 6 hour, 105 dB SPL 4 kHz NB noise exposure. Further, protection was consistent (**Figure 16: below**) although the outer hair cell loss varied across unprotected animals (**Figure 17**: **below**).

Figures 16 & 17. Outer hair cell protection in individual chinchillas.



D-methionine can also protect against permanent noise-induced hearing loss in chinchillas exposed to a 6 hour 105 dB SPL continuous noise even when first delivered one hour after noise exposure as demonstrated in **Figure 18** (Campbell et al., 2007). Significance is noted in the next four graphs.

Figure 18. D-methionine rescue from noise-induced hearing loss: D-methionine started 1 hour after noise cessation.



We have now extended these studies to document that D-methionine (200 mg/kg bid) can protect against permanent NIHL even when first administered up to 7 hours after cessation of the 6 hour 105 dB SPL 4 kHz NB noise exposure and then continued for another 48 hours. In **Figure 19 A-D** D-methionine protection from ABR threshold shift is shown for the tone-burst center frequencies of 2, 4, 6 and 8 kHz respectively for the 1,3,5 and 7 hour time delays for D-methionine after the noise cessation (one star indicates $p \leq .05$).

Figure 19 A-D. D-methionine Rescue from Noise-Induced Hearing Loss at Various Time Delays

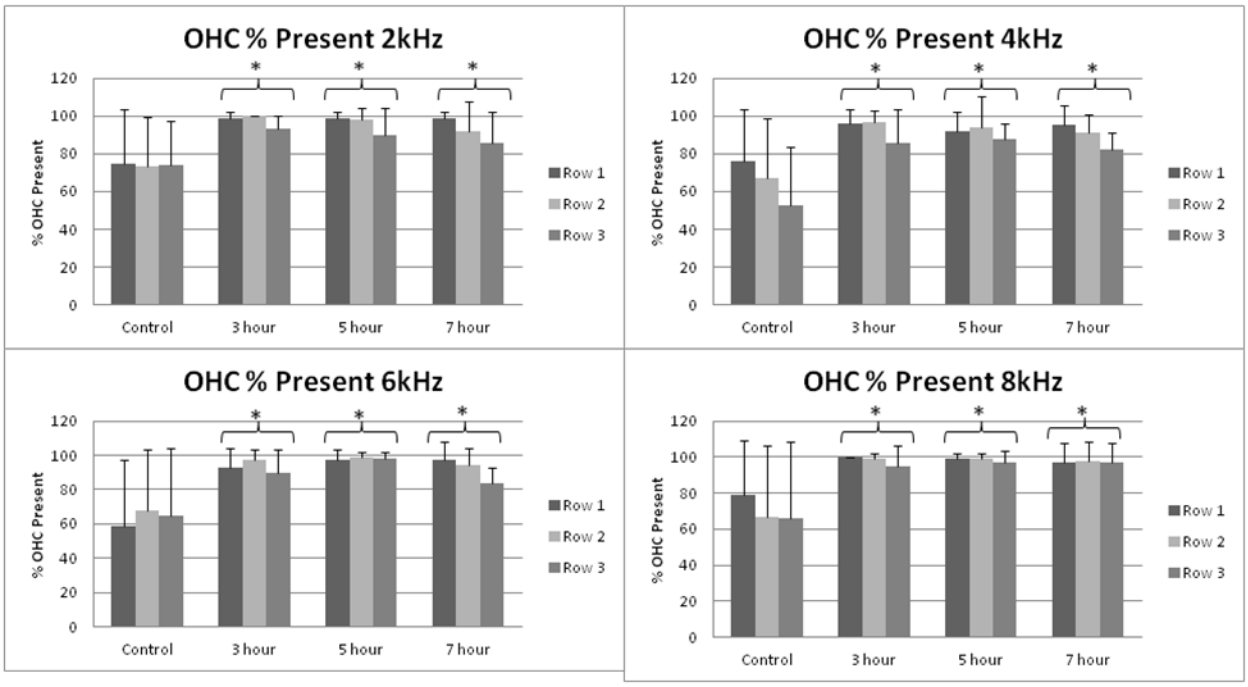


Figure 19. Mean percentage of OHCs present in the 2, 4, 6, and 8 kHz frequency regions. Error bars indicate +1 SD per row of hair cells. Values significantly different than the control group at the 0.05 level, as determined by Tukey's tests, are indicated by *. Histology results are also plotted by individual hair cell row within each region.

The results of outer hair cell analyses for these same animals are consistent with the ABR findings. D-methionine provided statistically significant, almost complete outer hair cell protection, when 200 mg/kg bid D-methionine was first initiated 1, 3 or even 5 hours after the noise cessation (105 dB SPL 4 kHz NB for 6 hours). At 7 hours the protection appeared to be slightly less but was significant at 2, 4, and 8 kHz. In the 4 panels of **Figure 19** the outer hair cell data corresponding to the same frequency regions as in the ABR data of **Figure 20** are presented.

Figure 20. ABR Threshold Shifts 21 days post noise exposure at 2, 4, 6, and 8 kHz.

ABR Threshold Shifts

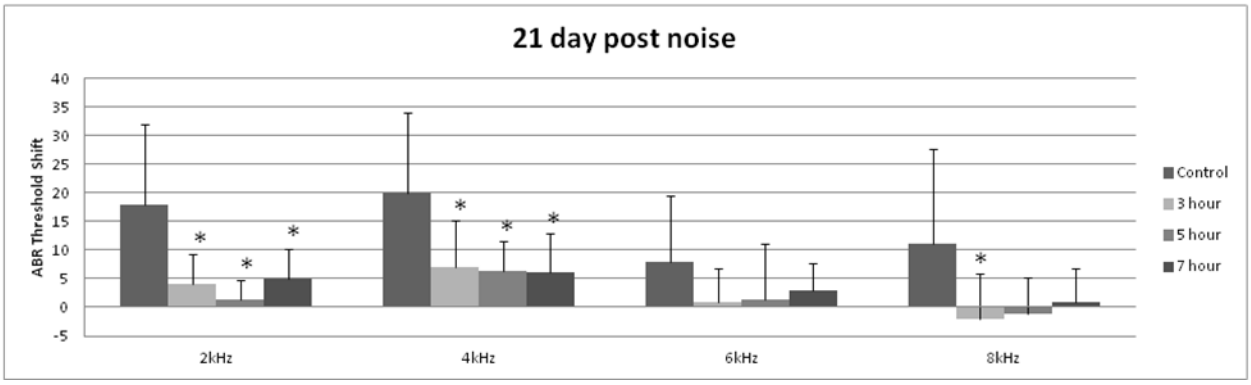
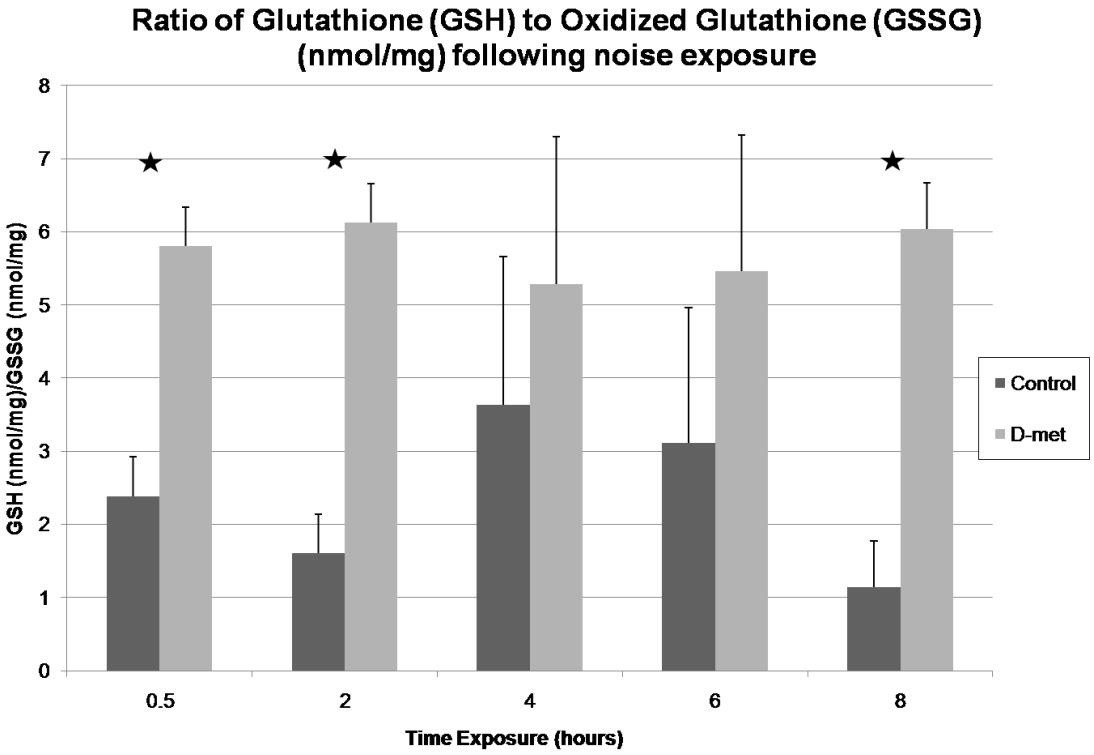


Figure 20. Mean post-noise exposure at Day 21 ABR threshold shifts from baseline at 2, 4, 6, and 8 kHz. Error bars indicate +1 SD. Mean values that are significantly different from the control group at the 0.05 level, as determined by Tukey's test, are indicated by *. No threshold protection was observed 24 hours after noise exposure but D-methionine conferred significant threshold protection for 2 and 4 kHz for all time delay intervals at 21 days. No significant ABR threshold protection was observed at 6 kHz and only for the 3 hour delay at 8 kHz at 21 days possibly because of the minimal threshold shift in the control group for those frequencies.

3.5 D-methionine Affects the Glutathione Pathway in Response to Noise Exposure

We have been investigating the glutathione pathway in D-methionine protection against NIHL. The impact of D-methionine on this pathway may explain, at least in part, why D-methionine protects against so many types of ototoxicity including noise. Ten groups of 3 chinchillas Laniger were exposed to 105 dB SPL NB noise for either 0.5, 2, 4, 6, or 8 hours of noise with either 200 mg/kg ip D-methionine BID or equivalent volume saline delivered for 2 days prior to the noise exposure. Animals were sacrificed immediately after the noise exposure and cochlear tissues were harvested for HPLC analysis of oxidized (GSSG) and reduced (GSH) glutathione levels. Although only 3 animals per cell were used the GSH/GSSG ratio was significantly increased ($p \leq .05$) as demonstrated in (**Figure 21).** The change in the GSH/GSSG ratio resulted from both a significant increase in GSH and a significant decrease in GSSG.

Figure 21. Ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) (nmol/mg) following noise exposure with and without D-methionine.



3.6 Summary

In conclusion, D-methionine can protect against a variety of cochlear insults including noise-induced hearing loss and the concomitant outer hair cell loss.

The purpose of this study is to determine if permanent noise-induced hearing loss (NIHL) and tinnitus can be prevented or reduced in a large cohort of Army personnel during their weapons training as a part of Drill Sergeant Instructor Training School at Ft Jackson. Soldiers at Ft Jackson are required to fire a minimum of 500 rounds of M-16 weapons fire (156 dB SPL) within a 9 day weapons training period., The proof of concept data has been published in several studies demonstrating that D-methionine, given before and after noise exposure in animals, can prevent permanent NIHL. The current goal is to document whether or not it prevents permanent NIHL in a clinical population of Soldiers.

If an oral preparation of D-methionine could prevent noise induced hearing loss and/or tinnitus, potentially millions of Americans and people world-wide could have an improved quality of life by retaining their hearing. Further the financial impact of preventing permanent NIHL and/or tinnitus could provide tremendous cost savings to the military and to industry. This Phase 3 study will enable us to determine the efficacy of oral D-methionine in preventing permanent NIHL and tinnitus in humans. Additionally they will allow us to ascertain any potential side effects. Ultimately these studies are expected to support an FDA approved pharmacologic intervention to prevent permanent NIHL and tinnitus due to impulse noise.

4.0 STUDY PLAN

4.1 Description of Overall Study Design

This is a prospective, randomized, double-blind, placebo-controlled study to evaluate the effect of D-methionine (D-met) on permanent NIHL after required weapons training. The study will include 600 Army personnel enrolled in Drill Sergeant School (DSS) scheduled to undergo 9 days of weapons training over an 11 day period at Ft Jackson, South Carolina. This training requires that they each fire approximately 500 rounds of M-16 weapons fire (156 dB SPL) within a 9-11 day period. Spent cartridges are collected and tallied by DSS leaders to document rounds fired. All subjects will be randomized to two equal arms; one to oral D-methionine (test drug) and the other to flavor-matched placebo. In both arms, there will be no other change in scheduled treatment. This facility trains approximately 2000 drill sergeant instructors annually.

4.2 Study Endpoints

4.2.1 Primary Endpoints

- The first primary endpoint is to confirm safety and tolerability of administering up to 100 mg/kg daily, given in divided doses, twice daily (with morning and evening meal). The other primary efficacy endpoint is change from baseline in pure-tone thresholds as measured by absolute change and frequency of significant noise-induced threshold shift (STS).

4.2.2 Secondary Endpoint

- Secondary efficacy endpoint is change from baseline in scores for the tinnitus scales for both loudness and annoyance.

4.3 Subject Selection

Six hundred military personnel enrolled in the Drill Sergeant School (DSS Candidates) at Ft. Jackson will be recruited for the study. This training requires that each Soldier fire approximately 500 rounds of M-16 weapons fire (156 dB SPL) within a 9-11 day period of weapons training with a possible additional makeup day if necessary to meet training requirements. Ft Jackson makes great efforts to prevent noise-induced hearing loss by means of physical hearing protection for all Soldiers. Every DSS Candidate in training at Ft Jackson is issued physical hearing protection, trained and required to use them at all times on the weapons range. Spent magazines are collected and tallied to document rounds fired.

Study subjects will be randomized to two equal arms: oral D-methionine (test drug) or flavor-matched placebo. All 600 subjects will receive either D-methionine (n = 300) or placebo (n = 300) twice daily for 18 days starting 3 days prior to the weapons training and ending 4 days after cessation of weapons training. Drug will be dispensed in the morning and in the evening at meal times. Drug logs will be kept for every subject, recording every dose taken or missed and whether or not battle plugs were used. Side effects will be assessed each time study drug is dispensed.

Soldiers routinely undergo pre-training audiology assessments that include pure tone threshold, otoscopy and typanometry testing. Study participation will include the following additional audiology assessments: pre-post training pure tone threshold and tympanometry assessments. Pre- post training tinnitus

assessments are not routinely done and are a required assessment for this study which will be conducted by the audiologist and/or study coordinators.

In order to be a Drill Sergeant Instructor, the Soldier must have achieved a rank of E5 (Sergeant) or higher, which generally takes between 4 to 5 years to obtain. Soldiers in DSS training are generally male (87%), between 21 – 45 years of age and racially diverse. In addition, Soldiers are routinely excluded from participation in DSS training if they have any driving under the influence convictions; domestic violence accusations or if they are under any Uniform Code of Military Justice (UCMJ) action or have any felony convictions. Soldiers are routinely screened for drug use by urinalysis and are excluded if test results are positive. They are also excluded if they fail to pass the Physical Training (PT) test, are restricted from any PT activity, or fail to meet weight, height or pregnancy restrictions.

4.4 Inclusion Criteria:

1. Male or female

2. 21 to 45 years of age

3. Negative pregnancy test confirmed by urine sample at enrollment and prior to taking first study drug dose.

4. Willing to use an effective method of birth control during the study (Female participants should avoid pregnancy and male participants should avoid fathering children during study). *Subject should either abstain from sexual relations or practice a method of birth control while taking part in the study. Except for surgical removal of the uterus, birth control methods such as condoms, a diaphragm or cervical cap, birth control pills, IUD, or sperm killing products are not totally effective in preventing pregnancy. Male study participants should refrain from fathering babies while enrolled in this study.*

5. Pure tone air conduction threshold average at 0.5, 1 and 2 kHz of no greater than 40 dB HL bilaterally with no air bone gaps greater than 10 dB and normal otoscopy and tympanometry screens.

6. Willing to limit the use of nutritional supplements containing or derived from protein to 50 grams of protein per day while participating in this study.

7. Ability to comply with all study requirements

4.5 Exclusion Criteria:

Most of these exclusion criteria apply to DSS candidates, but also apply in this study:

1. History of allergic or idiosyncratic reaction to methionine, amino acid mixtures, nutritional supplements, egg white or other proteins or food additives

2. Vegetarian (Individual excludes meat and fish from their diet)

3. History of chronic balance disorders

4. Abnormal otoscopic findings, otologic surgery, autoimmune inner ear disease, significant air bone gaps, abnormal tympanograms, or other indication of middle-ear abnormality, history of fluctuant hearing or asymmetry in hearing worse than 25 dB at any test frequency, or central nervous system disorder that is likely to affect hearing

5. Treatment with intravenous (IV) antibiotics or Carboplatin/Cisplatin within the past 6 months

6. Any surgical or medical condition which might significantly alter the absorption, distribution, metabolism, or excretion of any drug

7. History of abnormal kidney function or kidney impairment

8. Treatment for alcohol or substance abuse within past 6 months

9. Women of childbearing age who are not using effective contraceptive methods and who may become pregnant during the course of the study

10. Women who are pregnant or breastfeeding

11. National Guardsmen

12. History of psychotic schizophrenia

13. Exposure within the previous 6 months to systemic ototoxic substances including aminoglycosides or vancomycin

14. Body weight exceeding 225 pounds

15. Renal impairment measured as eGFR < 50 on screening creatinine clearance blood draw

4.6 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible to participate in this study. No exclusion based on race or ethnicity will be used. Although all eligible and consenting women and minorities will be included in this study, the demographic information for the study site suggests that this population is typically 87% male and 13% female. Approximately, 14% are Hispanic or Latino, 1 % American Indian or Native Alaskan, 3 % Asian, 1 % Native Hawaiian or other Pacific Islander, 17% Black or African American and 74 % White.

4.7 Subject Withdrawals

Every effort will be made to ensure that subjects complete the study through the 30 day study period, consistent with provisions of informed consent and good clinical judgment with respect to safety. The following are potential reasons to terminate the participation of a subject in the study:

1. The subject's health would be jeopardized.

2. Lost to follow-up: the subject fails to return to the site for scheduled visits and does not respond to reminders or attempts to contact.

3. Withdrawal of consent: subject decides to stop participation for any reason or is unable to complete the study as described in the protocol. The investigator will make a reasonable effort by person-to-person or telephone interview if allowed by the withdrawn volunteer, to ascertain the subject's reason(s) for withdrawal.

The reason for withdrawal will be captured on the appropriate case report form (CRF) and the medical monitor will be informed of removal or early withdrawal of a subject from the study.

4.8 Subject Replacement

If a subject receives any part of the investigation treatment, and must discontinue treatment for any reason, they will not be replaced.

5.0 STUDY PROCEDURES

5.1 General Guidelines

To be eligible for this study, the DSS Candidate must meet each inclusion criteria listed on the eligibility checklist and none of the exclusion criteria should apply. To be enrolled, the DSS candidate must have the ability to understand and the willingness to sign the informed consent form. An ombudsman must be present during the recruiting and consenting process.

The Schedule of Assessments/Events (Section 12.0) summarizes the frequency and timing of the required study assessments.

5.2 Recruitment Procedures

All DSS Candidates will be invited to participate in the proposed study. An information session will be scheduled at the beginning of each training period to provide an overview of study purpose and procedures and to give Candidates s an opportunity to ask questions. Written informed consent will be obtained prior to initiating any study procedures. Consent will be obtained by study coordinator(s) not affiliated with the DSS program.

Fort Jackson has approximately 16 classes per year for DSS Candidates. These classes start every 2-3 weeks with a maximum enrollment for the year of 2,040 Soldiers or approximately 100 enrollees per class. The M-16 weapons training takes place on study days 4-14, with no training on Saturdays and Sundays. Thus, the weapons training occurs on days 4-8 and days 11-14.

Candidates will attend a recruitment briefing to introduce this research study during the first 14 days of class. The presentation will be conducted by study coordinator(s) not affiliated with the DSS training program. During recruitment briefings to a unit where a percentage of the unit is being recruited to participate as a group, an ombudsman not connected in any way with proposed research or the unit, shall be present to monitor that the voluntary nature of individual participants is adequately stressed and that the information provided about the research is adequate and accurate (DoDD 3216.02, March 25, 2002). Written informed consent will be obtained before any study assessments are performed.

Candidates will be required to use issued personal hearing protection. Every Soldier in training at Ft Jackson is issued physical hearing protection, is trained and required to use them at all times on the

weapons range. Fort Jackson makes great efforts to prevent noise-induced hearing loss by means of physical hearing protection for all Soldiers. According to the Department of Defense Instruction, 6055.12, Hearing Conservation Program (HCP), December 3, 2010, the DoD Components shall issue personal hearing protectors at no cost to all personnel working or training in hazardous noise environments. All DoD Components shall ensure proper initial fitting and supervise the correct use of all hearing protection.

We anticipate that all subjects will be using Battle Plugs provided by the Fort Jackson audiologists. Compliance with hearing protection use will be assessed and recorded each day by the study coordinator(s).

5.3 Screening

Once written informed consent has been obtained, the following assessments will be conducted.

- Review of inclusion/exclusion criteria
- Review of relevant medical history
- Review of current medication history
- Collect demographic data
- Body weight – Body weight obtained by the study coordinator(s) at enrollment will be used to determine dosing of the Study Drug.
- Urine sample to be collected from all subjects for routine urinalysis. A urine pregnancy test will be performed for all women of child bearing potential by the study coordinator(s). Pregnancy test results will be recorded.
- A blood draw for serum creatinine will be conducted prior to enrollment in order to calculate estimated GFR by the Cockroft-Gault equation. Those subjects with an estimated eGFR < 50 will be excluded from enrollment.
- Two additional samples of blood (20-25 ml) will be collected for chemistry tests, complete blood counts, and tests of liver function, and homocysteine level.

5.4 Randomization

Enrolled subjects will be randomly assigned to receive study drug or placebo in a 1:1 ratio. Randomization scheme was developed by study statisticians and provided to KP Pharmaceutical Technology.

5.5 Audiological Assessments: Baseline and End of Study

Subjects will be given oral and written instructions by the study coordinator(s) to avoid noise exposure for at least 24 hours prior to all audiological assessments (hearing tests). Audiological assessments will be performed using the following standardized procedures. CPT Jenny Davis, AuD, a licensed audiologist and Army Hearing Program Chief at Fort Jackson, will be responsible for overseeing all testing and for

maintaining all audiologic data files for all subjects enrolled in the study. Audiological data is collected electronically via Interacoustic 629 clinical audiometers with insert earphones and TDH 39 earphones.. The threshold data will be directly exported to an Excel file and copied into the research chart.

5.5.1 Tinnitus Assessments

All subjects will be asked to complete the questionnaires at baseline and at the end of the study to determine the extent to which tinnitus has been induced by the noise exposure. The tinnitus questionnaire is modified from the Tinnitus Ototoxicity Monitoring Interview (TOMI) and the Tinnitus Handicap Index and the Tinnitus Loudness Index. The Tinnitus Handicap Inventory (THI), the most widely validated assessment tool for tinnitus, will also be used.

The TOMI (Fausti et al., 2007) and THI questionnaires, developed at the Portland, Oregon, Veteran's Administration and the Tinnitus Clinic of the Oregon Hearing Research Center have been documented in several ways. The THI was derived from detailed evaluation of over 2000 patients (Meikle 1992; Meikle et al., 1995). It is a twelve-item scale that quantifies the magnitude of tinnitus-related impairment, disability, and handicap according to guidelines established by the World Health Organization (WHO 2011; Meikle and Griest 2002). Tinnitus Handicap Inventory (THI) is the most widely validated and used tinnitus instrument (Newman et al., 1996, 2008). The THI has high internal consistency and reliability (Cronbach's alpha=.93) and test-retest stability (r=.92) Newman et al 2008).

Subjects will be administered the tinnitus questionnaires at the time of their initial and final audiometric evaluations. Differences in the questionnaire scores will be compared pre- and post-noise in the same subject and between treatment groups. In addition to the THI, an important measure for use in evaluating potential preventive or treatment effects for tinnitus is that of magnitude scaling for the subjective loudness dimension of tinnitus. Subjective ratings for tinnitus, using a scale from 0 – 10, are well documented by a number of investigators (Meikle, 1992; Levine, 1999; Bauman and Jastreboff, 1999; Newman et al., 1996). Such ratings are quickly and easily administered, and can be efficient measures for quantifying tinnitus treatment effects. The subjective loudness rating for tinnitus captures the aversive aspect of tinnitus, in a similar manner to that of pain rating scales, which are widely used by pain experts to quantify the aversive aspect of chronic pain. It has the advantage that it does not require the subject to try and recall his or her tinnitus problems during a preceding time interval (such as a week or more), but instead can be given as an immediate and current rating of the tinnitus magnitude.

5.5.2 Otoscopy and Tympanometric Screening

Prior to each hearing test, each Soldier's ear will be examined with a Welch Allyn 3.5v MacroView otoscope to ensure that the ear canals are clear and the tympanic membranes are normal in appearance. Tympanometry will be screened in each ear using pass/fail criteria of middle ear pressure: (pass is between +110 daPa and -160 daPa) and compliance:(Pass is between .2 and 1.4 ml) using the automated Interacoustics Handheld Middle Ear Analyzer MT10.

5.5.3 Pure Tone Air Conduction Threshold/Bone Conduction Testing

Pure-tone air conduction threshold testing both at baseline and post-treatment (11-15 days after the last day of study drug/placebo administration) will be conducted utilizing the modified Hughson-Westlake procedure. Pure-tone air-conduction testing will be conducted at .5, 1, 2, 3, 4, 6, 8 kHz. Bone conduction testing will be conducted at .5, 1, 2, 3, 4 kHz if the pure tone air-conduction threshold at that frequency is greater than or equal to 15 dB HL. All testing will be conducted using standard Army test procedures using Interacoustic 629 clinical audiometers meeting all Defense Occupational Environmental Health

Readiness System – Hearing Conservation (DOEHRS-HC) and American National Standards Institute (ANSI) criteria. All testing will be conducted in a single -walled sound booth with doors closed, meeting ANSI specifications. Test equipment, test environment, procedures, and personnel will meet all relevant DOEHRS-HC, ASHA, AAA, and ANSI standards and guidelines.

Pure-tone threshold testing will be conducted using the modified Hughson Westlake procedure as follows: Initial descent towards threshold is accomplished in 10-dB steps. Beginning with the first non-response, level is increased by 5-dB for each non-response, and decreased by 10-dB after each correct detection response. Threshold is defined as the lowest level at which two responses are obtained out of three presentations on an ascending run.

Threshold shifts will also be evaluated using DoD significant change criteria and ASHA early detection criteria (1994). Significant noise-induced threshold shifts (STS) in either ear are defined by the DOEHRS-HC as an increase of 10 dB or greater change (decrease in hearing) for the average of 2, 3 AND 4 kHz in either ear. A significant negative STS (improved hearing) is defined as a decrease of 10 dB or greater change (improvement in hearing) for the average of 2, 3 AND 4 kHz in either ear. An early warning shift STS (decrease in hearing) is defined as a 15 dB or greater change at 1, 2, 3 OR 4 kHz in either ear. Subjects with an STS will undergo a final tympanogram screen to rule out middle ear pathology as a cause of the hearing loss. If they pass the tympanogram screen in both ears, they will be referred for a diagnostic audiologic follow-up exam. If they do not pass the tympanogram screen they will be referred for otologic check and then diagnostic audiological assessment.

Experimental design consists of within-subjects serial testing in which baseline standard frequency (.5, 1, 2, 3, 4, 6, and 8 kHz) audiograms are initially acquired. By comparing similar measures obtained at the end of the study period to the relevant pre-exposure measure, reliable noise-induced changes in pure-tone hearing threshold can be identified. Thus, subjects will serve as their own control for identifying hearing change, relative to their baseline evaluation.

6.0 INTERVENTION

6.1 Study Day 1

- Second urine pregnancy test will be obtained by the study coordinator(s) for women of child-bearing potential. Pregnancy test results will be obtained and recorded by the study coordinator(s) immediately prior to study drug administration.
- Assess noise exposure compliance
- Assess for medication compliance/change
- Study Drug to be dispensed and recorded at morning and evening meals by the on-site study coordinator(s).

6.2 Study Days 2-18

- Assess for Adverse Events and complete AE Log
- Assess for medication compliance/change

- Assess for hearing protection compliance (Days 4-14 only)

- Study Drug to be dispensed and recorded at morning and evening meals by on-site study coordinator(s).

- Urine and blood samples will be collected while on treatment (during second week). Two additional samples of blood (20-25 ml) will be collected for chemistry tests, complete blood counts, and tests of liver function, and homocysteine level. Urine sample will be collected for urinalysis.

6.3 Study Days 29-32

At the end of the study, Study Days 29-32, subjects will return to complete post-study audiologic exams including pure-tone thresholds, screening tympanometry and tinnitus questionnaires. Subjects will be contacted by telephone or text message to remind them to avoid noise exposure for at least 24 hours prior to the audiological assessments (hearing tests) by the on-site study coordinator(s). Concomitant medication usage, adverse events and study procedures/ compliance will be reviewed and confirmed.

- Urine and blood samples will also be collected post treatment. Two additional samples of blood (20-25 ml) will be collected for chemistry tests, complete blood counts, and tests of liver function, and homocysteine level. Urine sample will be collected for urinalysis.

7.0 STUDY DRUG

Study drug will be shipped to the study site as blind-labeled, ready-to-use bottles containing D-methionine or placebo ready for administration.

7.1 Formulation, Packaging, and Labeling

Study drug and Placebo will be formulated, shipped and packaged by KP Pharmaceutical Technology (KPT), Bloomington, Indiana. The suspension is provided in small vials at a D-methionine concentration of 200 mg/mL. Study drug will be prepared and labeled for each subject in individual vials by KPT. The formulation is comprised of the common excipients polysorbate 80 (Tween), methylparaben, propylparaben, sorbitol and orange extract added for taste and palatability. The formulation is stable when stored at controlled room temperature for at least 3 months. For longer storage, suspension must be maintained under refrigeration.

The placebo is flavor and color matched and delivered in equivalent volume to the active compound. The test drug D-methionine and the placebo are both formulated according to Good Manufacturing Practice (GMP) standards by KP Pharmaceutical Technology (KPT) as an oral suspension. KPT prepared these same formulations for the ex-US clinical trials for prevention of radiation-induced oral mucositis and cisplatin-induced hearing loss.

7.2 Dispensing Study Drug

Study drug will be dispensed by MACH Pharmacy to on-site study coordinator(s), who are qualified medical personnel (RN or LPN). The study drug will be distributed to the study participants as labeled in accordance with the study protocol. CPT William Grimes, M.D. will supervise the activities of the study coordinator(s) and audiologists.

The formulated drug product (D-methionine) will be provided for clinical use as an oral suspension. Each dose will be packaged in individually labeled vials. Each vial will have a subject identification number on the label (Section 15.1 Master Label). The label will include instructions to shake the suspension prior to administration.

7.3 Preparation, Administration, and Dosage of Study Drug

Subjects will be given two doses per day of an oral orange flavored suspension of either D-methionine or placebo daily starting three days prior to weapons training, for 11 days during weapons training, and an additional 4 days after completion of weapons training for total of 18 days administration. The total daily dose of D-methionine will not exceed 100 mg/kg per day.

Each subject's dose of D-methionine will be individually determined based on actual subject weight. For ease of administration and packaging, dosing is broken down by weight categories with dosing based on the lowest weight in that category as follows:

Body Weight lbs (kg)	**Total Daily Dose (Up to 100 mg/kg/day)**
100-125 lbs (45 - 57 kg)	4.6 grams (4600 mg)
126-150 lbs (57 - 68 kg)	5.6 grams (5600 mg)
151-175 lbs (68 - 80 kg)	6.8 grams (6800 mg)
176-200 lbs (80 - 91 kg)	8.0 grams (8000 mg)
201-225 lbs (91 - 102 kg)	9.2 grams (9200 mg)

For example, if subject's body weight is 155 lbs (70.4 kg) then the subject's total daily dose equals 6.8 grams per day. This dose will be divided into two equal doses (3.4 grams) and administered prior to morning and evening meal (approximately 12 hours apart). Each dose (AM and PM) will be packaged in individually labeled vials by KP Pharmaceuticals.

KPT will assign the appropriate randomization code and prepare individualized doses of study drug prior to shipping to the Moncrief Army Community Hospital (MACH) Pharmacy at FT Jackson.

7.4 Study Drug Storage

All study drug will be stored under conditions consistent with package labeling (15-30 ° C; 59-86 ° F). All study drug will be stored in a secure limited-access area under controlled temperature in accordance with labeled storage requirements. Refrigeration is not required. Room temperature will be monitored and recorded daily, including weekends and holidays.

Shelf life at Ft. Jackson is not an issue because the study drug will arrive one week prior to the administration to each study participant and will only be dispensed over an 18 day period thereafter.

7.5 Study Product Accountability Procedures

Upon receipt of study drug, the MACH Pharmacist will ensure that the information on the packing slips matches exactly with information sent to the site, including content, amount, lot numbers, quantity, and expiration date.

The study coordinator(s) will also maintain accurate records of all study drug. In addition, accurate records will be kept regarding when and how much of each study drug is dispensed and used by each subject. This information will be recorded in each subject's EHR and on their medication log.

7.6 Assessment of Participant Compliance with Study Drug

Subject compliance will be determined by reconciliation of the subject's daily dosing regimen against the medication log.

7.7 Concomitant Medications and Procedures

All other medications taken by the subject during the study period will be recorded. Information will include: drug, dose, route of administration, start and end dates and indication.

7.8 Prohibited Medications and Procedures

Treatment with intravenous (IV) antibiotics, such as Amikacin: Amikin®, Gentamicin, Netilmicin: Netromycin®, Streptomycin, Tobramycin: Nebcin® or any other aminoglycosides and/or vancomycin. Also, any surgical or medical condition that might significantly alter the absorption, distribution, metabolism, or excretion of any drug. Subjects who received treatment for drug or alcohol abuse within the previous 6 months are prohibited from participation in this study.

7.9 Dietary Information (Daily Protein Intake)

Daily dietary information is available from Ft Jackson to ensure that adequate protein is available in the participant's daily diet.

7.10 D-methionine Antidote

The toxicity of methionine can be alleviated by dietary supplementation with glycine (Benevega and Harper 1967; Benevega 1974). However, these studies were conducted with L-methionine in animals.

8.0 ASSESSMENTS OF SAFETY

8.1 Safety Parameters

Safety and tolerability will be determined by evaluating any treatment emergent adverse events or change in baseline concomitant medications.

8.2 Definition of an Adverse Event (AE)

An adverse event (AE) is any unanticipated or unintended medical occurrence or worsening of a sign or symptom (including an abnormal laboratory finding) or disease in a study participant, which does not necessarily have a causal relationship with the study condition, procedures or study agent(s), that occurs after the informed consent is obtained.

Pre-existing conditions or illnesses, which are expected to exacerbate or worsen, are not considered adverse events and will be accounted for in the subject's medical history.

8.3 Definition of a Serious Adverse Event (SAE)

A Serious Adverse Event (SAE) is defined as an AE meeting one of the following outcomes:

- Death during the period of protocol defined surveillance
- Life Threatening Event (defined as a participant at immediate risk of death at the time of the event)
- Inpatient hospitalization or prolongation of existing hospitalization during the period of protocol defined surveillance
- Results in congenital anomaly or birth defect
- Results in a persistent or significant disability/incapacity

Any other important medical event that may not result in one of the above outcomes, may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.4 Methods and Timing for Assessing, Recording, Analyzing, and Managing Safety Parameters

8.4.1 Assessment of Safety

General health status and symptom assessments performed at Baseline/Eligibility Visit and Study Visit 1 will serve as baseline for any new signs or symptoms that arise during the study. All such changes occurring after Study Visit 1 will be evaluated as possible adverse events and appropriately recorded on the Adverse Event Case Report Form (CRF).

Baseline and Study Visit 1 assessments are limited to general health history information, pregnancy testing and laboratory assessment for kidney function. Additional safety assessments have been added to include: laboratory evaluations (hematology, blood chemistry, and urinalysis) at pre-specified time points (e.g., pre-treatment, during treatment, and post-treatment). Expected adverse events include possible gastrointestinal (GI) symptoms (nausea, vomiting, constipation, diarrhea, dysphagia).

Subjects will be questioned prior to receiving each study dose and on the last study day for the presence of GI side effects. *See Estimating Severity Grade and method for classifying GI adverse events.
Other subject reported symptoms that occur between Study Day 1 and Study Day 18, including the follow up visit at Study Day 29 - 32, will be evaluated as a possible adverse event. If a subject experiences a side effect that requires additional medical attention, the subject will seek medical attention through appropriate standard operating sick call procedures and the medical monitor will be contacted.

Adverse events will be evaluated according to the Estimating Severity Grade and will be recorded on the Adverse Event Log and the Adverse Event CRF. Adverse events deemed greater than Grade 2 will be considered a Serious Adverse Event (SAE). All SAEs will be recorded on the SAE CRF and reported according to FDA and institutional requirements (See Section 8.5 – Specific Serious Adverse Event Reporting Requirements).

Compliance and adverse event reports will be obtained each day that study drug is administered (Study Days 1-18) and at the end of the study (Study Day 29 -32) by the Study Coordinator(s). Each adverse event will be graded according to the Table for Estimating Severity Grade.

For all subjects receiving at least one dose of study drug, the number and percentage of subjects reporting adverse events will be tabulated by cohort and overall by severity and body system.

ESTIMATING SEVERITY GRADE	
Grade 1 (Mild)	Event requires minimal or no treatment and does not interfere with the subject's daily activities
Grade 2 (Moderate)	Event results in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
Grade 3 (Severe)	Event interrupts a patient's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.
Grade 4 (Life-Threatening	Any adverse drug experience that places the patient or participant, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.
Grade 5	Death

GASTROINTESTINAL	Grade 1	Grade 2	Grade 3	Grade 4
Nausea	Mild or transient; maintains reasonable intake	Moderate discomfort; intake decreased significantly; some activity limited	No significant intake; requires IV fluids	Hospitalization required
Vomiting	1 episode in 24 hours	2-5 episodes in 24 hours	> 6 episodes in 24 hours or needing IV fluids	Physiological consequences requiring hospitalization or requiring parenteral nutrition
Constipation	Requiring stool softener or dietary modification	Requiring laxatives	Obstipation requiring manual evacuation or enema	Obstruction or toxic megacolon
Diarrhea	Mild or transient; 3-4 loose stools/day or mild diarrhea lasting for less than 1 week	Moderate or persistent; 5-7 loose stools/day or diarrhea lasting > 1 week	> 7 loose stools/day or bloody diarrhea; or orthostatic hypotension or electrolyte imbalance or > 2L IV fluids required	Hypotensive shock or physiological consequences requiring hospitalization
Oral discomfort/dysphagia	Mild discomfort; no difficulty swallowing	Some limits on eating/drinking	Eating/talking very limited; unable to swallow solid foods	Unable to drink fluids; requires IV fluids

All AEs will be reviewed by the Medical Monitor (see Section 8.6) and the Data Safety Monitoring Committee (DSMC). The medical monitor will discuss each adverse event and determine causality in consultation with the Principal Investigator. The degree of certainty about causality will be graded using the categories below:

- **Definitely Related:** There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event occurs in a plausible time relationship to drug administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug should be clinically plausible.
- **Probably Related:** There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event occurs within a reasonable time sequence to administration of the drug, is unlikely to be attributed to concurrent disease or other drugs or chemicals.
- **Possibly Related:** There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g., the subject's clinical condition, other concomitant events). Although an adverse drug event may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
- **Unlikely:** A clinical event whose temporal relationship to drug administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the trial medication) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the subject's clinical condition, other concomitant treatments).
- **Unrelated:** The AE is completely independent of study drug administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.
- **Expected Events Related to Disease Process:** Expectedness refers to the awareness of adverse events previously observed, not on the basis of what might be anticipated from the pharmacological properties of the study agent.

Subjects will be questioned by the on-site study coordinator(s) prior to receiving each study dose and on the last study day for the presence of side effects or adverse experiences. If an event is reported, the following questions will be asked of the subjects and recorded by the on-site study coordinator(s):

- Onset of event
- Duration of event
- Intensity of event
- Relationship to study medication

Each subject's baseline data (pre-noise exposure) will act as their own control. Any side effects reported after receiving the first dose will be recorded (with answers to the aforementioned questions). At study completion, any new occurrence of a side effect or an increase in intensity of a pre-existing event will be recorded. If a subject experiences a side effect that warrants additional medical attention, the subject will seek medical attention through appropriate standard operating sick call procedures and the medical monitor will be contacted and it will be entered on the CRF.

Side effects will be compared within subjects, as well as between treatment groups (D-methionine and placebo) for onset, duration, frequency and relationship to treatment. These comparisons will be made for side effects present at baseline, and for those that occurred during and after completion of treatment.

8.5 Specific Serious Adverse Event Requirements

The Southern Illinois University School of Medicine is the sponsor for the Investigational New Drug application (IND) filed with the U.S. Food and Drug Administration (FDA). In the interest of subject

safety and to fulfill regulatory requirements, all deaths and life-threatening SAEs due to any cause, which occur during the course of the study must be reported to the Institutional Review Board (IRB) and the Regulatory Affairs Representative within 24 hours after the clinical site becomes aware of the event, and all other SAEs must be reported as soon as possible, but no later than 5 business days. Serious adverse events will be reported to: Dwight D. Eisenhower Army Medical Center (DDEAMC) IRB and to Rick Lampe at 609-636-9909. See SAE Reporting procedures, regulatory authority reporting and follow-up under Section 8.6.

In accordance with the FDA Code of Federal Regulations (CFR), the sponsor (Southern Illinois University) must report SAEs that are serious, unexpected, and related to the study intervention to the FDA in the form of a written IND Safety Report. Deaths and life-threatening events with any possible relationship to a study intervention must be reported to the FDA by telephone or fax as soon as possible but within 7 calendar days of IRB awareness. This initial report must be followed by as complete a written report as possible within 8 additional calendar days. All other IND Safety Reports must be submitted to the FDA as soon as possible, but no later than 15 calendar days after IRB is notified of the SAE. SAEs that do not meet the requirements for expedited reporting and all documented adverse events will be reported to the FDA in the IND annual report by the sponsor.

8.6 Safety Monitoring

Role of Research Monitors

For research involving greater than minimal risk to volunteers, the DoD requires that an independent research monitor must be appointed by name. Research monitors can be physicians, dentists, psychologists, nurses, or other healthcare providers capable of overseeing the progress of research protocols, especially issues of individual volunteer/patient management and safety. Research monitors must be independent of the investigative team and must possess sufficient educational and professional experience to serve as the volunteer/patient advocate. Depending on the nature of the study, the research monitor may be asked to assess one or more of the following phases of a research: volunteer recruitment, volunteer enrollment, data collection, or data storage and analysis.

At the discretion of the IRB or the Human Research Protection Office (HRPO), the research monitor may be asked to discuss research progress with the principal investigator, interview volunteers, consult on individual cases, or evaluate adverse event reports for the safety and protection of the volunteers. Research monitors shall promptly report discrepancies or problems to the IRB and the HRPO. They shall have the authority to stop a research study in progress, remove individual volunteers from a study, and take whatever steps are necessary to protect the safety and well-being of research volunteers until the IRB can assess the research monitor's report. At a minimum the HRPO requires that the research monitor provide a written opinion regarding the relationship and outcome of any unanticipated problems related to participation, serious adverse events, and subject deaths.

COL Mark D. Packer MD will serve as the medical monitor and CPT William P. Grimes, MD will serve as the site Principal Investigator and will oversee all study drug administration by the on-site study coordinator(s). Col Packer is Clinical Assistant Professor UTSA School of Medicine. CPT Grimes is a family physician in the Department of Preventive Medicine at Moncrief Army Community Hospital, Fort Jackson. David B. Pavlakovich, PA-C will serve as the independent research monitor on site.

Adverse event data will be reviewed by the research monitor at the end of each class. The research monitor will discuss each adverse event and determine causality in consultation with the Principal Investigator. Study personnel will immediately alert the research monitor to any unanticipated problems

or serious adverse events. Unanticipated problems and adverse events will be reported to the IRB in accordance with institutional policy and to the FDA as required. Data and safety monitoring reports will be provided to the IRB at the time of continuing review per DDEAMC policy.

The investigators, research monitors, coordinator(s) and data manager of this protocol will meet at least once per month via videoconference or teleconference to review the following: study progress (including data quality and timeliness, recruitment, accrual and retention), study procedures (including subject privacy and data confidentiality protection), outcomes of adverse events and new literature reviews to assess change to the risk/benefit ratio and to determine if modifications need to be made or the study terminated. The Regulatory Representative (Rick Lampe) will be included via teleconference to assess the regulatory impact of outcomes of adverse events and new literature reviews to assess change to the risk/benefit ratio and to determine if modifications need to be made or the study terminated..

8.7 Stopping Rules Based on Adverse Events

The safety of individual research subjects is of primary importance in this study as hearing loss does not result in serious morbidity or mortality and the active drug is considered relatively benign. Thus, for this study, the following stopping rules for safety will be utilized:

1. The study will be stopped if any subject dies, if any subject requires emergency surgery, or if any subject suffers a permanent and irreversible disability, unless it can be shown that this event was unrelated to participation in the clinical trial.

2. Should an adverse event occur that is not mentioned in the consent form, then the study will be stopped until both the consent form has been revised to include this adverse event and the institutional review board (IRB) gives re-approval of the study. The adverse event will also be reported to the FDA as it impacts safety.

3. If an adverse event that is not covered under item 1 occurs in more than three subjects, then the risk of the adverse event associated with the active drug group will be compared to the risk of the adverse event associated with the placebo group. If the rate is significantly higher for the active drug group at an unadjusted confidence level of 95% (i.e., alpha level of 0.05), then the data safety monitoring committee (DSMC) will be required to determine, based on the severity of the adverse event, if the trial should be stopped for safety.

8.8 Data Safety Monitoring Committee

The data safety monitoring committee (DSMC) evaluates study data on an ongoing basis to assure participant safety and study integrity. The DSMC will review study data and unanticipated problems and make recommendations based on their reviews. The DSMC will review the protocol prior to study initiation and meet no less than once per year and more frequently if needed to review enrollment and safety data and after the planned interim analysis. Evaluation will also occur if three subjects have met the stopping criteria. If all three are determined to be in the D-methionine-treated group, the DSMC will consider a lower dose as a safe dose with which to continue the study. The DSMC Chair may call an emergency meeting at any time should issues of patient safety arise. The DSMC will be composed of at least four voting members. The current DSMC includes: DSMC Chair (Patrick J. Antonelli, MD, Chair of Otolaryngology, University of Florida, Gainesville, the Medical Monitor for the Sponsor (Mark D. Packer, MD), the Research Monitor, David B. Pavlakovich, PA-C, D. Bradley Welling, MD, PhD, Chair

of Otolaryngology, Harvard University, Boston, the Regulatory Representative (Rick Lampe), and the Yale University epidemiologist/statistician (Carrie Redlich, MD, MPH). Only the Yale University statistician will be unblinded and will endeavor to keep the committee blinded during discussion of the stopping criteria, adverse events, and any study adjustments recommended by the committee.

8.9 Adverse Events (AE) Reporting

We will follow 1996 and 2000 International Conferences on Harmonization, Sections E2 and E6 Good Clinical Practice, HHS and FDA regulations. An adverse event (AE) is defined as any untoward medical occurrence that does not necessarily have a causal relationship with this treatment.

A Serious Adverse Event (SAE) is defined as any untoward medical occurrence that results in death, is life-threatening, requires hospitalization or prolongation of hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly or birth defect.

8.10 Reporting Requirements to the Human Research Protection Office (HRPO)

The HRPO requires approval of protocol amendments, acknowledgement or acceptance of continuing review documents, reporting of protocol deviations, serious adverse advents, and unanticipated problems. The following is a synopsis of what must be reported.

- Major amendments and amendments that increase risk to subjects must be pre-approved.
- All other amendments must be submitted for acceptance with the continuing review report.
- Unanticipated problems involving risks to subjects or others, serious adverse events related to participation, and deaths related to participation must be promptly reported.
- Suspensions, voluntary/involuntary clinical holds, or any terminations of the research must be promptly reported to the HRPO.
- Deviations that affect the safety or right of subjects or the integrity of the study must be promptly reported; exceptions (from approved inclusion/exclusion criteria or stopping criteria) must be pre-approved.
- Continuing Review reports and IRB approval documentation must be submitted as soon as the documentation is available. All amendments that occurred during the reporting period must be submitted at this time.
- The final study report and any supporting documents must be submitted to the HRPO as soon as all documents become available.
- The knowledge of any pending compliance inspection/visit by the FDA, OHRP, or other government agency concerning the DOD supported research, the issuance of Inspection Reports, FDA Form 483, warning letters, or actions taken by any Regulatory Agencies including legal or medial actions and any instances of serious or continuing noncompliance with the regulations or requirements must be reported immediately to the HRPO.
- Accurate and complete study records must be maintained and made available to representatives of

the USAMRMC as a part of their responsibility to protect human subjects in research.

8.11 Reporting to FDA

Reporting to FDA will be accomplished within the regulatory time frame by the Regulatory Representative. For each adverse event observed, the Principal Investigator must decide whether it meets the definition of a "serious" adverse event. The regulatory definition of a serious adverse event is an event that is fatal or life threatening, results in persistent or significant disability, requires intervention to prevent permanent impairment/damage, or an event that results in congenital anomaly, hospital admission or prolongation of hospitalization. These will be reported within 24 hours by phone and by fax to the Regulatory Representative and submitted to FDA within the regulatory timeframes, and followed up in a timely manner. Regulatory Representative contact information is provided below.

Rick Lampe
Phone: 609-636-9909
Email: rick_lampe@comcast.net
rlampe@metarmor.com

The Regulatory Representative will confer with the sponsor's medical monitor prior to submission. The sponsor has not given up the SAE reporting responsibility and will remain responsible for such.

9.0 DATA MANAGEMENT

9.1 Data Quality Assurance

To ensure the integrity of the data collected from study participants several procedures will be implemented. All personnel involved in data collection will be thoroughly trained in the assessment methods thus ensuring consistent applications of procedures and measurement consistency across participants. All required data for this study are to be collected on case report forms (CRFs). Data will be recorded on appropriate CRFs at the time of each assessment or as soon as possible after the results of the hearing tests are available.

Data collection will occur at the study site through the Research Electronic Data Capture (REDCap) program (Harris et al., 2009) created and supported by Vanderbilt University and all study data will be transferred to Martin Slade, MS of the data management unit at Yale University according to standard operating procedures developed for this study. All data will be automatically saved to a computer hard drive and stored on a secure server (i.e., password protected) that is backed up daily. All demographic and medical history forms will be kept secured in locked cabinets. All informed consent documents will be kept at the study site in a separate locked file cabinet with restricted access. Issues related to data integrity will be discussed as a recurring agenda item in the bi-monthly study team meetings.

The data manager is responsible for data security. Data management activities include: source verification of audiology data and monitoring outliers. Data security measures include keeping hard copies of data double-locked at all times with access limited to investigators, the coordinator(s) and data manager, as well as using password protection and secure socket layer for the database.

9.2 Management of Tympanometry Data:

Subjects who do not pass the tympanometry screening on initial hearing testing will be noted and excluded from the study. The results of the tympanometry screen will be recorded for each subject.

Tympanometry data will be imported to the master data base so that subjects with an abnormal final tympanogram can be excluded from the final data analysis since the abnormal tympanometry may indicate that the threshold shift is due to middle ear pathology and not noise damage.

9.3 Management of Pure Tone Audiometric Data:

On days of pure tone air conduction threshold testing, data will be stored for each ear of every subject in the computerized audiometer and will be exported to a centralized Microsoft Access database. These data will also be imported into an Excel spreadsheet for storage and input into a statistical package for offline data analysis. The identifiers in the computer databases will consist solely of the subject identification number (PIN).

10.0 STATISTICAL CONSIDERATIONS

10.1 Sample Size Justification

Based upon the results of the 2004 study at the Marine Corps Recruit Depot (MCRD), it is estimated that the rate of significant threshold shift (STS) will be approximately 38% for the control (placebo) group. A power analysis incorporating a 95% confidence level (alpha = 0.05), 80% power, a 1:1 ratio (placebo to D-methionine), and a detectable difference in threshold shift rates of 30% yields a required sample size of 252 subjects for each of the two arms (placebo and D-methionine) of the study. Thus a total of 504 subjects need to complete the study. Assuming an attrition rate of 20% in each arm over the course of the study, a total of 600 subjects will need to be enrolled.

10.2 Primary Efficacy Analysis

Two groups are planned in the randomized prospective design: the D-met and the placebo groups. The primary objectives, and their associated analyses, for this study are as follows:

1. To determine if there is a significant difference between the D-methionine and placebo treated groups in the average severity of documented side effects where severity is defined as the frequency of side effect multiplied by the intensity of the side effect. Repeated measures analysis of variance (ANOVA) will be utilized to determine significance.

2. To determine if there is a significantly reduced change in threshold hearing in each ear, adjusting for handedness, for the D-methionine group as compared to the placebo group at the tested audiometric frequencies. The tested audiometric frequencies will be 0.5, 1, 2, 3, 4, 6 and 8 kHz. Multi-way (2 groups x 7 frequencies) analysis of variance (ANOVA) with interaction effects and correction for multiple comparisons (Newman-Keuls) will be utilized to determine significance. Determination for early detection will be according to the American Speech-Language Hearing Association (ASHA, 1994) criteria of: 1) greater than or equal to a 20 dB change at any frequency, 2) greater than or equal to a 10 dB change at any 2 adjacent frequencies, or 3) loss of response at 3 consecutive frequencies where responses were obtained at baseline. To be considered significant ototoxicity, these changes must replicate within 24 hours (may be immediate) with no indication of middle ear abnormality. Subjects serve as their own controls for audiometric change, which is computed relative to baseline measures.

3. To determine if there is a significantly lower rate of STS (in either ear) for the D-methionine group as compared to the placebo group where STS is defined according to DOEHRSHC criteria as an increase of 10 dB or greater change (decrease in hearing) for the average of 2, 3 AND 4 kHz in

either ear. A significant negative STS (improved hearing) is defined as a decrease of 10 dB or greater change for the average of 2, 3 AND 4 kHz in either ear. An early warning shift STS (decrease in hearing) is defined as a 15 dB or greater change at 1, 2, 3 OR 4 kHz in either ear. Fisher's exact test will be utilized to determine statistical significance.

Secondary objective of the study is as follows:

1. To determine if there is a significant difference in reported level of tinnitus between the D-methionine and the placebo groups where the level (loudness) of tinnitus will be scored on an eleven point scale with a value of 0 associated with very quiet and a value of 10 associated with very loud and on an eleven point scale where the degree of annoyance secondary to the tinnitus will also be scored on an eleven point scale with a value of zero indicating that it is not bothersome and a value of 10 indicating that it is unbearable. Repeated measures analysis of variance (ANOVA) will be utilized to determine significance.

The 95% confidence intervals will be estimated for all D-methionine vs. placebo differences.

10.3 Interim Analysis

Subjects are required to fire a minimum of 500 rounds of M-16 weapons fire as opposed to the 300 rounds for the Camp Pendleton study over the same period of time. Therefore a NIHL as great or greater in this study population is anticipated as opposed to the Camp Pendleton sample. However, no comparative data exist for NIHL for 500 rounds as opposed to 300 rounds, so this sample size will be adequate.

To determine if the study data indicate greater NIHL for the 500 rounds could potentially support reduction of the sample size, therefore an interim analysis will be conducted once half the subjects have been enrolled. This analysis will be limited to verification of the assumptions made for the determination of the sample size requirements, be handled by an outside statistician with no communication of the data or results to FDA, specifically, on the observed shift rate among the placebo arm. The final sample size will be adjusted based upon the results of this analysis.

Thus, if the shift rate of the placebo arm is greater than originally assumed, then the total number of subjects will be decreased accordingly based on the outside body's statistician's determination. If, on the other hand, the shift rate of the placebo arm is less than originally assumed, then the total number of subjects will be increased, also based on the outside body statistician's pronouncement. No data or results will be communicated to anyone involved in the study; only a determination of the increase or decrease in sample size will be determined and the study enrollment only will be adjusted accordingly.

11.0 ETHICS/PROTECTION OF HUMAN SUBJECTS

11.1 Risks to Human Subjects

a. Human Subjects Involvement and Characteristics

This is a randomized, double-blind, placebo-controlled study to determine the effect of D-methionine on preventing or reducing noise-induced permanent hearing loss (NIHL) before, during, and after military weapons training. The study drug, D-methionine is an oral, liquid suspension to be administered twice daily for 18 days at a dose not to exceed 100 mg/kg/day. Six hundred subjects will be enrolled in this study and will be randomly assigned 1:1 to receive study drug or placebo.

The proposed study includes U.S. Army personnel enrolled in Drill Sergeant School (DSS) at the Combat Training Center of Excellence in Fort Jackson, South Carolina. Approximately 2000 Army personnel participate in 17 DSS training classes each year and each class includes about 100 candidates. The training period is 16 days and includes 9 days of required M-16 weapons training (~500 rounds @ 156 dB SPL). Subjects will be recruited during the first week of training and will be enrolled in the study for up to 30 days.

All Soldiers are required to pass a physical training (PT) class to qualify for DSS training. There are additional restrictions based on weight, height and pregnancy. All Candidates are routinely screened for drug use by urinalysis and are excluded from DSS if any are found. DSS candidates are generally between 21 – 45 years of age, therefore the study sample will include subjects between 21-45 years of age. Approximately 75% of the candidates are male and the study sample is expected to be similar.

Inclusion Criteria:

1. Male or female
2. 21 to 45 years of age
3. Negative pregnancy test confirmed by urine test at enrollment and prior to taking first study drug dose
4. Willing to use an effective method of birth control during the study (Female participants should avoid pregnancy and male participants should avoid fathering children during study). *Subject should either abstain from sexual relations or practice a method of birth control while taking part in the study. Except for surgical removal of the uterus, birth control methods such as condoms, a diaphragm or cervical cap, birth control pills, IUD, or sperm killing products are not totally effective in preventing pregnancy. Male study participants should refrain from fathering babies while enrolled in this study.*
5. Pure tone air conduction threshold average at 0.5, 1 and 2 kHz of no greater than 40 dB HL bilaterally with no air bone gaps greater than 10 dB and normal otoscopy and tympanometry screens
6. Willing to limit the use of nutritional supplements containing or derived from protein to 50 grams of protein per day while participating in this study.
7. Ability to comply with all study requirements

Exclusion Criteria:

Some of these exclusion criteria already apply to DSS Candidates, but also apply in this study:

1. History of allergic or idiosyncratic reaction to methionine, amino acid mixtures, nutritional supplements, egg white or other proteins or food additives
2. Vegetarian (Individual excludes meat and fish from their diet)

3. History of chronic balance disorders

4. Abnormal otoscopic findings, otologic surgery, autoimmune inner ear disease, significant air-bone gaps, abnormal tympanograms, or other indication of middle-ear abnormality, history of fluctuant hearing or asymmetry in hearing worse than 25 dB at any test frequency, or central nervous system disorder that is likely to affect hearing

5. Treatment with intravenous (IV) antibiotics within the past 6 months

6. Any surgical or medical condition which might significantly alter the absorption, distribution, metabolism, or excretion of any drug

7. History of abnormal kidney function or kidney impairment

8. Treatment for alcohol or substance abuse within last 6 months

9. Women of childbearing age who are not using effective contraceptive methods and who may become pregnant during the course of the study

10. Women who are pregnant or breastfeeding

11. National Guardsmen

12. History of psychotic schizophrenia

13. Exposure within the previous 6 months to systemic ototoxic substances including aminoglycosides or vancomycin

14. Body weight exceeding 225 pounds

15. Renal impairment measured as eGFR < 50 on screening creatinine clearance blood draw

b. Source of Materials

1.) Demographic, health and medication history information will be collected at baseline. Weight will be determined from the medical record and pregnancy testing will be performed at baseline. Pregnancy testing is not routinely performed and is considered to be a study-related procedure.

Audiologic assessments such as Threshold Testing, Otoscopy and Tympanometry Testing are routinely performed on all DSS Candidates within 3-6 months of the training period. Repeat audiological assessments will be performed at the study baseline and are considered study-related procedures. Tinnitus assessments will be performed at the study baseline and are considered to be study-related procedures. All audiologic assessments will be performed using the following standardized procedures. CPT Jenny Davis, AuD, a licensed audiologist from the Army Hearing Program at Ft Jackson, will be responsible for overseeing all testing and for maintaining all audiologic data files for all subjects enrolled in the study.

2.) Otoscopy and Tympanometric Screening: Prior to each hearing test each Subject's ear will be examined with a Welch Allyn 3.5v Macroview otoscope to ensure that the ear canals are clear and the

tympanic membranes are normal in appearance. Tympanometry will be screened in each ear using pass-fail criteria of middle ear pressure: (pass is between +110 daPa and -160 daPa) and compliance: (Pass is between .2 and 1.4 ml) using the automated Interacoustics Handheld Middle Ear Analyzer MT10.

3.) Threshold Testing: Pure-tone threshold testing both at baseline and post-test (15-16 days after completion of weapons training) will be conducted utilizing the modified Hughson -Westlake procedure. Pure-tone air-conduction testing will be conducted at .5, 1, 2, 3, 4, 6, 8 kHz. Bone conduction testing will be conducted at 5, 1, 2, 3, 4 kHz if the air-conduction threshold at that frequency is 15 dB HL or greater. Audiological data is collected electronically via Interacoustic 629 clinical audiometers with insert earphones and TDH 39 earphones. Pure-tone threshold testing is conducted by the audiologists using DOEHRSHC standard test procedures using the modified Hughson Westlake procedure as follows: Initial descent towards threshold is accomplished in 10-dB steps. Beginning with the first non-response, level is increased by 5-dB for each non-response, and decreased by 10-dB after each correct detection response. Threshold is defined as the lowest level at which two responses are obtained out of three presentations on an ascending run. Test equipment, test environment, procedures, and personnel will meet all relevant DOEHRS, ASHA, AAA, ANSI and JC standards and guidelines.

4.) Tinnitus Measures: Tinnitus measures: A tinnitus questionnaire modified from the Tinnitus Ototoxicity Monitoring Interview and the Tinnitus Handicap Index and the Tinnitus Loudness Index will be used on each visit to assess tinnitus.

c. Potential Risks

Methionine is a micronutrient, and thus it is not alien to the human system. Both the D- and L-methionine isomers are present in a wide variety of foods. Methionine comprises 26 mg/g high quality protein in the diet (National Academy of Sciences 1980). Methionine is used therapeutically for other purposes and at relatively high doses. Previous studies of D-methionine have had few, minor side effects, such as nausea and vomiting. The recommended dosing is 200-400mg orally three to four times per day.

There is the potential for loss of confidentiality; however safeguards have been made to protect individual the Candidates' identity.

11.2 Adequacy of Protection Against Risks

a. Recruitment and Informed Consent

Recruitment procedures will be finalized during the one year planning period with input from the military to ensure appropriateness and to enhance effectiveness. CPT Jenny Davis, AuD, Army Hearing Program, will provide guidance with study recruitment and orientation procedures. At a minimum, all DSS Candidates will be introduced to the study within the first 14 days of class and given the opportunity to participate. These recruitment briefings will be coordinated and presented by non-military study personnel who will explain the purpose of the study, the procedures, the risks and benefits, voluntary nature of study participation and answer all questions prior to obtaining written informed consent. During recruitment briefings to a unit where a percentage of the unit is being recruited to participate as a group, an ombudsman not connected in any way with proposed research or the unit shall be present to monitor that the voluntary nature of individual participants is adequately stressed and that the information provided about the research is adequate and accurate (DoDD 3216.02, March 25, 2002) Written informed consent will be obtained before any study assessments are performed.

b. Protections Against Risk

All participants will be carefully screened for eligibility. All doses of study drug will be based on subject weight and will be distributed with verbal and written instructions for administration by study personnel. Subjects will be required to use assigned military hearing protection during weapons training. Adverse events will be carefully assessed and monitored throughout this study. Should any subject experience a side effect or event that warrants additional medical attention, he/she will be provided medical care through appropriate standard operating sick call procedures at Fort Jackson.

Every effort will be made to protect the confidentiality of the data obtained from each subject. Each subject will be assigned a study identification code number. The list of subject names and code numbers will be accessible to study personnel only. This list will be kept in a locked file cabinet in the on-site study coordinators' office. This code number will be used on all data collection forms. All of the data will be collated into a database that will be coded so that individual subject identities will be known only to the investigators. A separate code-breaking file will be kept in written form separate from the electronic database. This method will be used to protect individual privacy. Only aggregate data will be reported in publications with no reference to individual subjects.

c. Potential Benefits of the Proposed Research to Human Subjects and Others

No promise of a direct benefit will be made to the participants. There is currently no available treatment or procedure that can fully protect Soldiers against permanent noise-induced hearing loss. Results from this study will improve the understanding of NIHL.

d. Importance of the Knowledge to be Gained

If an oral preparation of D-methionine could prevent noise-induced hearing loss and/or tinnitus, potentially millions of Americans and people world-wide could have an improved quality of life by retaining their hearing. Further the financial impact of preventing permanent NIHL and/or tinnitus could provide tremendous cost savings to the military and to industry.

11.3 Clinical Trial Registration: Registered on April 27, 2011 (NCT01345474), and currently listed as "Recruiting".

12.0 SCHEDULE OF ASSESSMENTS/EVENTS

Study Day	Day --30 to --1		Day 1	Day 2	Day 3	Day 4 –14	Day 15-18	Day 19-28	Day 29-32
Orientation/Recruitment									
Informed Consent									
Screen/Eligibility • Demographic • Health/Medication Survey • Weight • Pregnancy Test • Blood and Urine Samples[g]	**X** **X** **X** **X** **X**		**X**				**X**		**X**
Hearing Tests[a] • Tinnitus Questionnaires • Otoscopy • Tympanometry • Pure tone air conduction threshold[b] • Bone conduction[c]		**X** **X** **X** **X** **X** **X**							**X** **X** **X** **X** **X** **X**
Randomization	**X**								
Oral D-methionine[d]			**X**	**X**	**X**	**X**	**X**		
Weapons training[e]						**X**			
Hearing noise exposure compliance[a]			**X**						**X**
AEs[f]			**X**	**X**	**X**	**X**	**X**		**X**

a = No noise exposure for 24 hours prior to hearing testing
b= Modified Hughson-Westlake procedure (0.5, 1, 2, 3, 4, 6, 8 kHz) re: DOEHRS-HC and ANSI criteria
c= Only if Pure Tone air conduction threshold is ≥ 15 dB HL at any frequency from .5 -4 kHz
d= Total Daily Dose: Up to 100mg/kg/day x 18 days (to be administered twice daily)
e= Weapons training takes place on Training Day 23-32
f= Drug Compliance and Adverse Event Assessment
g= Serum creatinine at baseline; Additional safety assessments at baseline, during and after treatment (Hematology, Chemistry, Homocysteine, Urinalysis)

13.0 STUDY TIMELINE

Up to 16 Drill Sergeant School (DSS) classes are scheduled each year and each class includes approximately 100 candidates. To meet the enrollment goal of 600 subjects it is anticipated that approximately 50 subjects per class will enroll. An estimated 504 subjects are needed to complete all follow-up assessments (252 subjects per group) to detect significant differences for the primary outcome measures. The five year proposed timeline is provided in the table below. Recruitment will begin by the fourth quarter of Year 3. New DSS Training Sessions begin approximately every two-three weeks. Approximately 50 subjects are expected to enroll per session X 13 sessions. Please note that that the protocol includes enrolling the first group and completing all procedures and data collection on that group prior to additional treatment groups. The study team will then review that process before proceeding to the next group. After all procedures are documented to be flowing smoothly recruitment will proceed from each group of DSS students with classes starting every 2-3 weeks and thus, recruitment and data collection will overlap.

	Mar 1 2011				**Mar 1 2012**				**Mar 1 2013**				**Mar 1 2014**				**Mar 1 2015***			
Years	**1**				**2**				**3**				**4**				**5**			
Months	**1**	**3**	**6**	**9**	**12**	**15**	**18**	**21**	**24**	**27**	**30**	**33**	**36**	**39**	**42**	**45**	**48**	**51**	**54**	**57**
Site Visit	x		x			x			x											
Study Team Meetings	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			
Prepare and File IND	x		x	x	x	x	x													
IRB Submission/ Approval						x	x	x	x	x										
Order Study Drug	x																			
Formulate Study Drug	x																			
Finalize Recruitment Plan																				
Hire Study Staff	x								x	x	x									
Site Training										x	x	x								
Finalize MOP									x	x	x									
Pilot											x	x								
Recruit/Enrol l Subjects												x	x	x	x	x	x	x		
Data Collection											x	x	x	x	x	x	x	x	x	
Data Analysis																				
FDA Reports											x				x				x	
Publications / Presentations																			x	x
Final Reports																				x

* Subject Enrollment is continuing beyond March 2015.

14.0 BIBLIOGRAPHY

Abelow, B. (1998). *Understanding acid base*. Baltimore, MD: Williams and Wilkins.

Alberti, P. W. (1998). Noise the most ubiquitous pollutant. *Noise Health, 1*, 1-5.

American Speech Language Hearing Association (1994). American Speech-Language Hearing Association Guidelines for the Audiologic Management of Individuals Receiving Cochleotoxic Drug Therapy. *ASHA*, 36 (March Supp 12), 11-19.

Axelsson, A., Hellstrom, P. A., Altschuler, R., & Miller, J. M. (1991). Inner ear damage from toy cap pistols and firecrackers. *International Journal of Pediatric Otorhinolaryngology, 21*(2), 143-148.

Axelsson, A., Vertes, D., & Miller, J. (1981). Immediate noise effects on cochlear vasculature in the guinea pig. *Acta Otolaryngol, 91*(3-4), 237-246.

Baker, D. H. (1979). Efficacy of the D- and L-isomers of N-acetylmethionine for chicks fed diets containing either crystalline amino acids or intact protein. *The Journal of Nutrition, 109*(6), 970-974.

Baker, D. H. (2006). Comparative species utilization and toxicity of sulfur amino acids. *J Nutr, 136*(6 Suppl), 1670S-1675S.

Baldessarini, R. J., Stramentinoli, G., & Lipinski, J. F. (1979). Methylation hypothesis. *Arch Gen Psychiatry, 36*(3), 303-307.

Barney, R., & Bohnker, B. K. (2006). Hearing thresholds for U.S. Marines: comparison of aviation, combat arms, and other personnel. *Aviation, Space, and Environmental Medicine, 77*(1), 53-56.

Bauman, N., & Jastreboff, P. F. (1999). Real-ear measurement of the sound levels used by patients during TRT. *Proceedings of the sixth international tinnitus seminar*. London: Tinnitus and Hyperacusis Centre.

Benevenga, N. J., & Harper, A. E. (1967). Alleviation of methionine and homocystine toxicity in the rat. *J Nutr, 93*(1), 44-52.

Benevenga, N. J. (1974). Toxicities of methionine and other amino acids. *J Agric Food Chem, 22*(1), 2-9.

Berglund, L., Halldin, C., Lilja, A., Lindberg, B. S., Lundquist, H., Langstrom, B., Malmborg, P., Nagren, K & Stalnacke, C. G. (1984). 11C-methionine kinetics in pregnant rhesus monkeys studied by positron emission tomography: A new approach to feto-maternal metabolism. *Acta Obstet Gynecol Scand, 63*, 641-645.

Bergstrom, M., Lundqvist, H., Ericson, K., Lilja, A., Johnstrom, P., Langstrom, B., von Holst, H., Erikson, L. & Blomquist, G. (1987a). Comparison of the accumulation kinetics of L-(methyl-11C)-methionine and D-(methyl-11-C)-methionine in brain tumors studied with positron emission tomography. *Acta Radiologia, 28*, 225-229.

Bergstrom, M., Muhr, C., Ericson, K., Lundquist, H., Lilja, A., Eriksson, L., Blomquist, G., Langstrom, B. & Johnstrom, P. (1987b). The normal pituitary examined with positron emission tomography and (methyl-11C)-L-methionine. *Neuroradiology, 29*, 221-225.

Bergström, M., Muhr, C., Lundberg, P. O., Bergström, K., Lundqvist, H., & Långström, B. (1986). Amino acid metabolism in pituitary adenomas. *Acta Radiol Suppl, 369*, 412-414.

Bielefeld, E. C., Kopke, R. D., Jackson, R. L., Coleman, J. K., Liu, J., & Henderson, D. (2007). Noise protection with N-acetyl-l-cysteine (NAC) using a variety of noise exposures, NAC doses, and routes of administration. *Acta Otolaryngol, 127*(9).

Block, R. J., & Bolling, D. (1945). *The amino acid composition of proteins and foods*. Springfield, IL: Charles C. Thomas Publishers.

Bobbin, R. P., Fallon, M., LeBlanc, C., & Baber, A. (1995). Evidence that glutathione is the unidentified amine (Unk 2.5) released by high potassium into cochlear fluids. *Hear Res, 87*(1-2), 49-54.

Boggs, R. W., Rotruck, J. T., & Damico, R. A. (1975). Acetylmethionine as a source of methionine for the rat. *J Nutr, 105*(3), 326-330.

Boggs, R. W. (1978). Bioavailability of acetylated derivatives of methionine, threonine, and lysine. *Adv Exp Med Biol, 105*, 571-586.

Bohne, B. A., & Rabbitt, K. D. (1983). Holes in the reticular lamina after noise exposure: implication for continuing damage in the organ of Corti. *Hear Res, 11*(1), 41-53.

Bohnker, B. K., Page, J. C., Rovig, G., Betts, L. S., Muller, J. G., & Sack, D. M. (2002). U.S. Navy and Marine Corps Hearing Conservation Program, 1995-1999: mean hearing thresholds for enlisted personnel by gender and age groups. *Mil Med, 167*(2), 132-135.

Bohnker, B. K., Page, J. C., Rovig, G., Betts, L. S., & Sack, D. M. (2002). Navy Hearing Conservation Program: threshold shifts in enlisted personnel, 1995-1999. *Mil Med, 167*(1), 48-52.

Bohnker, B., Rovig, G., Page, J., Philippi, A., Butler, F., & Sack, D. (2003). Navy hearing conservation program: hearing threshold comparisons to Navy SEALS and divers. *Undersea Hyperb Med, 30*(2), 155-162.

Bohnker, B. K., Page, J. C., Rovig, G. W., Betts, L. S., & Sack, D. M. (2004). Navy Hearing Conservation Program: 1995-1999 retrospective analysis of threshold shifts for age, sex, and officer/enlisted status. *Mil Med, 169*(1), 73-76.

Brachet P, Alvarado F, Puigserver A. Kinetic evidence for separate systems in transport of D- and L-methionine by rat small intestine. *Am J Physiol.* Mar 1987;252(3 Pt 1):G320-324.

Burns, R. A., & Milner, J. A. (1981). Sulfur amino acid requirements of immature Beagle dogs. *J Nutr, 111*(12), 2117-2124.

Campbell, K. C., Rybak, L. P., Meech, R. P., & Hughes, L. (1996). D-methionine provides excellent protection from cisplatin ototoxicity in the rat. *Hear Res, 102*(1-2), 90-98.

Campbell, K. C., Meech, R. P., Rybak, L. P., & Hughes, L. F. (1999). D-methionine protects against cisplatin damage to the stria vascularis. *Hear Res, 138*(1-2), 13-28.

Campbell, K. C. M., Meech, R. P., Jackson, R. L., Hughes, L. F., Rybak, L. P., Coleman, J., K.M., & Kopke, R.

D. (2003). Noise exposure alters cochlear oxidized and reduced glutathione levels as a function of noise exposure duration in the chinchilla. *Abstracts of the Association for Research in Otolaryngology, 26*, 164.

Campbell, K. C., Meech, R. P., Klemens, J. J., Gerberi, M. T., Dyrstad, S. S., Larsen, D. L., Mitchell, D. L., El-Azizi, M., Verhulst, S.J., & Hughes, L. F. (2007). Prevention of noise- and drug-induced hearing loss with D-methionine. *Hear Res, 226*(1-2).

Campbell, K. C. M., Nayar, R., Borgonha, S., Hughes, L. F., Rehemtulla, A., Ross, B., & Sunkara, P. (2009). Oral D-methionine (MRX-1024) significantly protects against cisplatin-induced hearing loss: A phase II study in humans. *Abstracts of the Association for Research in Otolaryngology, 32*, 7.

Campbell, K. C. M., Claussen, A., Meech, R., Seymour, K., Verhulst, S., & Hughes, L. (2010). D-methionine (D-met) provides significant outer hair cell and ABR threshold rescue from noise exposure. *Abstracts of the Association for Research in Otolaryngology, 33*, 239.

Campbell, K., Claussen, A., Meech, R., Verhulst, S., Fox, D., & Hughes, L. (2011). D-methionine (D-met) significantly rescues noise-induced hearing loss: timing studies. *Hear Res, 282*(1-2).

Cheng, P. W., Liu, S. H., Young, Y. H., Hsu, C. J., & Lin-Shiau, S. Y. (2008). Protection from noise-induced temporary threshold shift by D-methionine is associated with preservation of ATPase activities. *Ear Hear, 29*(1), 65-75.

Cho, E. S., Andersen, D. W., Filer, L. J., & Stegink, L. D. (1980). D-methionine utilization in young miniature pigs, adult rabbits, and adult dogs. *JPEN J Parenter Enteral Nutr, 4*(6), 544-547.

Cho, E. S., & Stegink, L. D. (1979). D-methionine utilization during parenteral nutrition in adult rats. *J Nutr, 109*(6), 1086-1093.

Clerici, W. J., DiMartino, D. L., & Prasad, M. R. (1995). Direct effects of reactive oxygen species on cochlear outer hair cell shape in vitro. *Hear Res, 84*(1-2), 30-40.

Clerici, W. J., & Yang, L. (1996). Direct effects of intraperilymphatic reactive oxygen species generation on cochlear function. *Hear Res, 101*(1-2), 14-22.

Cohen, H. P., Choitz, H. C., & Berg, C. P. (1958). Response of rats to diets high in methionine and related compounds. *J Nutr, 64*(4), 555-569.

Coleman, J. K. M., Liu, J., Jackson, R., & Kopke, R. (2002a). Post-noise administration of methionine attenuates noise-induced hearing loss in the chinchilla. *Abstracts of the Association for Research in Otolaryngology, 25*, 226.

Coleman, J. K. M., Liu, J., Wood, K., & Kopke, R. (2002b). Low dose methionine with N-acetyl-L-cysteine reduces noise-induced threshold shift in the chinchilla. *Abstracts of the Association for Research in Otolaryngology, 25*, 226.

Coleman, J. K., Kopke, R. D., Liu, J., Ge, X., Harper, E. A., Jones, G. E., Cater, T. L., &. Jackson, R. L. (2007). Pharmacological rescue of noise-induced hearing loss using N-acetylcysteine and acetyl-L-carnitine. *Hear Res, 226*(1-2), 104-113.

Cottington, E. M., LaMantia, C., Stabler, S. P., Allen, R. H., Tangerman, A., Wagner, C., & Mudd, S. H. (2002). Adverse event associated with methionine loading test: a case report. *Arterioscler Thromb Vasc Biol, 22*(6), 1046-1050.

Daniel, R. G., & Waisman, H. A. (1969). Adaptation of the weanling rat to diets containing excess methionine. *J Nutr, 99*(3), 299-306.

Dessi, P., & Boari, A. (1966). [Fatty acids from hepatic lipids of rats treated with superior homologues of L- and D-methionine]. *Boll Soc Ital Biol Sper, 42*(20), 1390-1393.

Di Rocco, A., Tagliati, M., Danisi, F., Dorfman, D., Moise, J., & Simpson, D. M. (1998). A pilot study of L-methionine for the treatment of AIDS-associated myelopathy. *Neurology, 51*(1), 266-268.

Drug Facts and Comparisons. (1999). St. Louis, MO: J.B. Lippincott Company.

Duan, M., Qiu, J., Laurell, G., Olofsson, A., Counter, S. A., & Borg, E. (2004). Dose and time-dependent protection of the antioxidant N-L-acetylcysteine against impulse noise trauma. *Hear Res, 192*(1-2), 1-9.

Edwards, C. H., Rawalay, S. S., Higginbotham, C., & Edwards, G. A. (1972). Distribution in rat tissues and urine of methionine labeled with carbon-14 in positions 1 2 3 4 or methyl cargon or with sulfur-35. *J. Elisha Mitchell Sci, 88*.

Efron, M. L., McPherson, T. C., Shih, V. E., Welsh, C. F., & MacCready, R. A. (1969). D-methioninuria due to DL-methionine ingestion. An artifact detected by a mass screening program for errors of amino acid metabolism. *Am J Dis Child, 117*(1), 104-107.

Engström, B., Flock, A., & Borg, E. (1983). Ultrastructural studies of stereocilia in noise-exposed rabbits. *Hear Res, 12*(2), 251-264.

Fausti, S. A., Helt, W. J., Gordon, J. S., Reavis, K. M., Phillips, D. S., & Konrad-Martin, D. L. (2007). Audiologic monitoring for ototoxicity and subject management. In K. C. M. Campbell (Ed.), *Pharmacology and ototoxicity for audiologists*. Clifton Park: Thomson Delmar learning.

Fernández-Checa, J. C., Kaplowitz, N., García-Ruiz, C., & Colell, A. (1998). Mitochondrial glutathione: importance and transport. *Semin Liver Dis, 18*(4), 389-401.

Fetoni, A. R., Ralli, M., Sergi, B., Parrilla, C., Troiani, D., & Paludetti, G. (2009). Protective effects of N-acetylcysteine on noise-induced hearing loss in guinea pigs. *Acta Otorhinolaryngol Ital, 29*(2), 70-75.

Friedman, M. (1999). *Absorption and utilization of amino acids*. Boca Raton, FL: CRC Press.

Friedman, M., & Gumbmann, M. R. (1984). The utilization and safety of isomeric sulfur-containing amino acids in mice. *J Nutr, 114*(12), 2301-2310.

Friedman, M., & Gumbmann, M. R. (1988). Nutritional value and safety of methionine derivatives, isomeric dipeptides and hydroxy analogs in mice. *J Nutr, 118*(3), 388-397.

Garlick, P. J. (2006). Toxicity of methionine in humans. *J Nutr, 136*(6 Suppl), 1722S-1725S.

Ghibelli, L., Fanelli, C., Rotilio, G., Lafavia, E., Coppola, S., Colussi, C., & Ciriolo, M. R. (1998). Rescue of

cells from apoptosis by inhibition of active GSH extrusion. *FASEB J, 12*(6), 479-486.

Ghittoni, N. E., & Sellinger, O. Z. (1971). Effect of the convulsant methionine sulfoximine on the in vivo uptake and metabolism of D-methionine in rat brain. *J Neurobiol, 2*(2), 153-168.

Gonzalez-Hernandez JC, Aguilera-Aguirre L, Perez-Vazques V, Ramirez J,Clemente-Guerro M, Cortes- Rojo C. (2003). Effect of D-amino acids on some mitochondrial function in rat liver. *Amino Acids*, 24, 163-9.

Hamernik, R. P., Qiu, W., & Davis, B. (2008). The effectiveness of N-acetyl-L-cysteine (L-NAC) in the prevention of severe noise-induced hearing loss. *Hear Res, 239*(1-2).

Hamernik, R. P., Turrentine, G., & Wright, C. G. (1984). Surface morphology of the inner sulcus and related epithelial cells of the cochlea following acoustic trauma. *Hear Res, 16*(2), 143-160.

Hamstra, D. A., Eisbruch, A., Naidu, M. U., Ramana, G. V., Sunkara, P., Campbell, K. C., Ross, B., & Rehemtulla, A. (2010). Pharmacokinetic analysis and phase 1 study of MRX-1024 in patients treated with radiation therapy with or without cisplatinum for head and neck cancer. *Clin Cancer Res, 16*(9), 2666-2676.

Harris, P. A., Taylor, R., Thielke, R., Payne, J., Gonzalez, N., & Conde, J. G. (2009). Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support *J Biomed Inform* (Vol. 42, pp. 377-381). United States.

Hasegawa, H., Shinohara, Y., Akahane, K., & Hashimoto, T. (2005). Direct detection and evaluation of conversion of D-methionine into L-methionine in rats by stable isotope methodology. *J Nutr, 135*(8), 2001-2005.

Helfer, T. M., Jordan, N. N., & Lee, R. B. (2005). Postdeployment hearing loss in U.S. Army Soldiers seen at audiology clinics from April 1, 2003, through March 31, 2004. *Am J Audiol, 14*(2), 161-168.

Heller, H. (1970). Importance of enzyme determination in the urine for the diagnosis and control of renal diseases. Med Klin, 65(34), 1480-1485.

Hellstrom, P. A., Dengerink, H. A., & Axelsson, A. (1992). Noise levels from toys and recreational articles for children and teenagers. *Br J Audiol, 26*(5), 267-270.

Henderson, D., & Hamernik, R. P. (1995). Biologic bases of noise-induced hearing loss. *Occup Med, 10*(3), 513-534.

Henry, J. A., Dennis, K. C., & Schechter, M. A. (2005). General review of tinnitus: prevalence, mechanisms, effects, and management. *J Speech Lang Hear Res, 48*(5), 1204-1235.

Howard, J., Roberts, W. M., & Hudspeth, A. J. (1988). Mechanoelectrical transduction by hair cells. *Annu Rev Biophys Chem, 17*, 99-124.

Huang, T., Cheng, A. G., Stupak, H., Liu, W., Kim, A., Staecker, H., Lefebvre, P., Malgrange, B., Kopke, R., Moonen, G & Van De Water, T. R. (2000). Oxidative stress-induced apoptosis of cochlear sensory cells: otoprotective strategies. *Int J Dev Neurosci, 18*(2-3), 259-270.

Hyde, G. E., & Rubel, E. W. (1995). Mitochondrial role in hair cell survival after injury. *Otolaryngol Head*

Neck Surg, 113(5), 530-540.

Jervis, E. L., & Smyth, D. H. (1960). The active transfer of D-methionine by the rate in testing in vitro. *J Physiol, 151*, 51-58.

Kaji, H., Saito, N., Hisamura, M., Murao, M., Ishimoto, M., Kondo, H., & Saito, K. (1983). Nutritional aspect of methionine isomers studied by pulmonary exhalation of dimethyl sulfide and urinary excretion of alpha-keto-gamma-methiolbutyrate in humans. *Jpn J Med, 22*(2), 106-111.

Kaji, H., Niioka, T., Kojima, Y., Yoshida, Y., & Kawakami, Y. (1987). Urinary 3-methylthiopropionate excretion and the effect of D- or L-methionine ingestion studied in healthy subjects. *Res Commun Chem Pathol Pharmacol, 56*(1), 101-109.

Kies, C., & Fox, H. M. (1973). Comparison of urea and diammonium citrate as sources of nonspecific nitrogen for human adults. *J Nutr, 103*(5), 664-669.

Kies, C., Fox, H., & Aprahamian, S. (1975). Comparative value of L-, and D-methionine supplementation of an oat-based diet for humans. *J Nutr, 105*(7), 809-814.

Kil, J., Pierce, C., Tran, H., Gu, R., & Lynch, E. D. (2007). Ebselen treatment reduces noise-induced hearing loss via the mimicry and induction of glutathione peroxidase. *Hear Res, 226*(1-2), 44-51.

Kim, K. I., & Bayley, H. S. (1983). Amino acid oxidation by young pigs receiving diets with varying levels of sulphur amino acids. *Br J Nutr, 50*(2), 383-390.

Klavins, J. V., Kinney, T. D., & Kaufman, N. (1963). Histopathologic changes in methionine excess. *Arch Pathol, 75*, 661-673.

Klavins, J. V., & Johansen, P. V. (1965). Pathology of amino acid excess: Effects and interactions of excessive amounts of dietary methionine, homocystine, and serine. *Arch Pathol, 79*, 600-614.

Kleinman, J. G., & Lemann, J. R. (1987). Acid production in clinical disorders of fluid and electrolyte metabolism. In M. H. Maxwell, C. R. Kleeman & R. G. Narins (Eds.), *Clinical disorders of fluid and electrolyte metabolism*. New York: McGraw Hill.

Konno, R., Isobe, K., Niwa, A., & Yasumura, Y. (1988). Excessive urinary excretion of methionine in mutant mice lacking D-amino-acid oxidase activity. *Metabolism, 37*(12), 1139-1142.

Kopke, R. D., Liu, W., Gabaizadeh, R., Jacono, A., Fehali, J., Spray, D., & Van De Water, T. R. (1997). Use of organotypic cultures of Corti's organ to study the protective effects of antioxidant molecules on cisplatin-induced damage of auditory hair cells. *American Journal of Otology, 18*, 559-571.

Kopke, R. D., Weisskopf, P. A., Boone, J. L., Jackson, R. L., Wester, D. C., Hoffer, M. E., & McBride, D. (2000). Reduction of noise-induced hearing loss using L-NAC and salicylate in the chinchilla. *Hear Res, 149*(1-2), 138-146.

Kopke, R. D., Bielefeld, E., Liu, J., Zheng, J., Jackson, R., Henderson, D., & Coleman, J. K. (2004). N-acetylcysteine (NAC) and Acetyl-L-Carnitine (ALCAR) show different effects in protecting the cochlea from noise in the chinchilla. *Abstracts of the Association for Research in Otolaryngology, 27*, 231.

Kopke, R., Bielefeld, E., Liu, J., Zheng, J., Jackson, R., Henderson, D., & Coleman, J. K. (2005a). Prevention of impulse noise-induced hearing loss with antioxidants. *Acta Otolaryngol, 125*(3), 235-243.

Kopke, R. D., Coleman, J. K., Liu, J., Campbell, K. C., & Riffenburgh, R. H. (2002). Candidate's thesis: enhancing intrinsic cochlear stress defenses to reduce noise-induced hearing loss. *Laryngoscope, 112*(9), 1515-1532.

Kopke, R. D., Jackson, R. L., Coleman, J. K., Liu, J., Bielefeld, E. C., & Balough, B. J. (2007). NAC for noise: from the bench top to the clinic. *Hear Res, 226*(1-2), 114-125.

Kramer, S., Dreisbach, L., Lockwood, J., Baldwin, K., Kopke, R., Scranton, S., & O'Leary, M. (2006). Efficacy of the antioxidant N-acetylcysteine (NAC) in protecting ears exposed to loud music. *J Am Acad Audiol, 17*(4), 265-278.

Kuzmicky, D. D., Kohler, G. O., Walker, H. G., & Mackey, B. E. (1977). Availability of oxidized sulfur amino acids for the growing chick. *Poult Sci, 56*(5), 1560-1565.

Lang, L. (1994). Environmental impact on hearing: is anyone listening. *Environ Health Perspect, 102*(11), 924-929.

Lauenstein, L., Meyer, G. J., Sewing, K. F., Schober, O., & Hundeshagen, H. (1987). Uptake kinetics of 14C L-leucine and 14C L- and 14C D-methionine in rat brain and incorporation into protein. *Neurosurg Rev, 10*(2), 147-150.

Le Prell, C. G., Hughes, L. F., & Miller, J. M. (2007a). Free radical scavengers vitamins A, C, and E plus magnesium reduce noise trauma. *Free Radical Biology and Medicine, 42*(9), 1454-1463.

Le Prell, C. G., Yamashita, D., Minami, S. B., Yamasoba, T., & Miller, J. M. (2007b). Mechanisms of noise-induced hearing loss indicate multiple methods of prevention. *Hear Res, 226*(1-2), 22-43.

Le Prell, C. G., Ohlemiller, K. K., Gagnon, P. M., & Bennett, D. C. (2009a). Reduction in permanent noise-induced threshold deficits in mice fed a combination of dietary agents. *Abstracts of the Association for Research in Otolaryngology, 32*, 280.

Le Prell, C. G., Schmitt, J., Dolan, D. F., Boxer, P. A., Prieskorn, D. M., DeRemer, S., & Goodson, M. (2009b). prevention of temporary noise-induced threshold deficits using dietary agents. *Abstracts of the Association for Research in Otolaryngology, 32*, 280.

Letowski, T. (2003). *Bone conduction communication: Applications and limitations*. Paper presented at the 28th Annual Conference of the National Hearing Conservation Association, Dallas, TX.

Levine, R. A. (1999). *Somatic modulation appears to be a fundamental attribute of tinnitus.* Paper presented at the Sixth International Tinnitus Seminar, London.

Liberman, M. C. (1987). Chronic ultrastructural changes in acoustic trauma: serial-section reconstruction of stereocilia and cuticular plates. *Hear Res, 26*(1), 65-88.

Liberman, M. C., & Dodds, L. W. (1987). Acute ultrastructural changes in acoustic trauma: serial-section reconstruction of stereocilia and cuticular plates. *Hear Res, 26*(1), 45-64.

Lieberman, M. (2007). *Mark's essential medical biochemistry*. Philadelphia: Lippincott Williams & Wilkins.

Lim, D. J. (1986). Effects of noise and ototoxic drugs at the cellular level in the cochlea: a review. *Am J Otolaryngol, 7*(2), 73-99.

Linder, M. D. (1985). Nutrition and metabolism of proteins. In M. C. Linder (Ed.), *Nutritional biochemistry and metabolism with clinical applications*. New York: Elsevier.

Lockwood, D. S., Ding, D. L., Wang, J., & Salvi, R. J. (2000). D-Methionine attenuates inner hair cell loss in carboplatin-treated chinchillas. *Audiol Neurootol, 5*(5), 263-266.

Lorito, G., Giordano, P., Petruccelli, J., Martini, A., & Hatzopoulos, S. (2008). Different strategies in treating noise-induced hearing loss with N-acetylcysteine. *Med Sci Monit, 14*(8), BR159-164.

Lu, S. C. (1998). Regulation of hepatic glutathione synthesis. *Semin Liver Dis, 18*(4), 331-343.

Lynch, E. D., Gu, R., Pierce, C., & Kil, J. (2004). Ebselen-mediated protection from single and repeated noise exposure in rat. *Laryngoscope, 114*(2), 333-337.

Lynch, E. D., & Kil, J. (2005). Compounds for the prevention and treatment of noise-induced hearing loss. *Drug Discov Today, 10*(19), 1291-1298.

Maenz, D. D., & Engele-Schaan, C. M. (1996). Methionine and 2-hydroxy-4-methylthiobutanoic acid are transported by distinct Na(+)-dependent and H(+)-dependent systems in the brush border membrane of the chick intestinal epithelium. *J Nutr, 126*(2), 529-536.

Meikle, M. B. (1992). *Methods for evaluation of tinnitus relief procedures.* Paper presented at the Fourth International Tinnitus Seminar, Amsterdam.

Meikle, M. B., Griest, S. E., Stewart, B. J., & Press, L. S. (1995). Measuring the negative impact of tinnitus: A brief severity index. *Abstracts of the Association for Research in Otolaryngology*(167).

Meikle, M. B., & Griest, S. E. (2002). *Tinnitus severity and disability: Prospective efforts to develop a core set of measures.* Paper presented at the Seventh International Tinnitus Seminar, Perth, Australia.

Metternich, F. U., & Brusis, T. (1999). [Acute hearing loss and tinnitus caused by amplified recreational music]. *Laryngorhinootologie, 78*(11), 614-619.

Meyer, G. J., Schober, O., & Hundeshagen, H. (1985). Uptake of 11C-L- and D-methionine in brain tumors. *Eur J Nucl Med, 10*(7-8), 373-376.

Miller, J. M., Ren, T. Y., Dengerink, H. A., & Nuttall, A. L. (1996). Cochlear blood flow changes with short sound stimulation. In A. Axelsson (Ed.), *Scientific basis for noise-induced hearing loss*. New York: Thieme.

Monteagudo, F. S., Straughan, J. L., & van der Merwe, L. P. (1986). The choice between intravenous N-acetylcysteine and oral methionine in paracetamol poisoning. *S Afr Med J, 69*(5), 279.

Muramatsu, K., Odagiri, H., Morishita, S., & Takeuchi, H. (1971). Effect of excess levels of individual amino acids on growth of rats fed casein diets. *J Nutr, 101*(9), 1117-1125.

National Academy of Sciences (1980). Recommended dietary allowances. Washington, D.C.: National Academy of Sciences.

Newman, C. W., Jacobson, G. P., & Spitzer, J. B. (1996). Development of the Tinnitus Handicap Inventory. *Arch Otolaryngol Head Neck Surg, 122*(2), 143-148.

Newman, C. W., Sandridge, S. A., & Bolek, L. (2008). Development and psychometric adequacy of the screening version of the tinnitus handicap inventory. *Otol Neurotol, 29*(3), 276-281.

Ohinata, Y., Miller, J. M., & Schacht, J. (2003). Protection from noise-induced lipid peroxidation and hair cell loss in the cochlea. *Brain Res, 966*(2), 265-273.

Ohlemiller, K. K., Wright, J. S., & Dugan, L. L. (1999). Early elevation of cochlear reactive oxygen species following noise exposure. *Audiol Neurootol, 4*(5), 229-236.

Pickles, J. O., Osborne, M. P., & Comis, S. D. (1987). Vulnerability of tip links between stereocilia to acoustic trauma in the guinea pig. *Hear Res, 25*(2-3), 173-183.

Pourbakht, A., & Yamasoba, T. (2003). Ebselen attenuates cochlear damage caused by acoustic trauma. *Hear Res, 181*(1-2), 100-108.

Printen, K. J., Brummel, M. C., Cho, E. S., & Stegink, L. D. (1979). Utilization of D-methionine during total parenteral nutrition in postsurgical patients. *Am J Clin Nutr, 32*(6), 1200-1205.

Puel, J. L., d'Aldin, G., Saffiedine, S., Eybalin, M., & Pujol, R. (1996). Excitotoxicity and plasticity of IHS-auditory nerve contributes to both temporary and permanent threshold shift. In A. Axelsson (Ed.), *Scientific basis of noise-induced hearing loss*. New York: Thieme Publishers.

Quirk, W. S., Avinash, G., Nuttall, A. L., & Miller, J. M. (1992). The influence of loud sound on red blood cell velocity and blood vessel diameter in the cochlea. *Hear Res, 63*(1-2), 102-107.

Rabinowitz, P. M. (2000). Noise-induced hearing loss. *Am Fam Physician, 61*(9), 2749-2756, 2759-2760.

Raphael, Y., & Altschuler, R. A. (1992). Early microfilament reorganization in injured auditory epithelia. *Exp Neurol, 115*(1), 32-36.

Reser, D., Rho, M., Dewan, D., Herbst, L., Li, G., Stupak, H., Zur, K., Romaine, J., Frenz, D., Goldblum, L., Kopke, R., Arezzo, J. & Van De Water, T. (1999). L- and D- methionine provide equivalent long term protection against CDDP-induced ototoxicity in vivo, with partial in vitro and in vivo retention of antineoplastic activity. *Neurotoxicology, 20*(5), 731-748.

Ritter, D. C., & Perkins, J. L. (2001). Noise-induced hearing loss among U.S. Air Force cryptolinguists. *Aviat Space Environ Med, 72*(6), 546-552.

Roberts, W. M., Howard, J., & Hudspeth, A. J. (1988). Hair cells: transduction, tuning, and transmission in the inner ear. *Annu Rev Cell Biol, 4*, 63-92.

Robertson, D. (1983). Functional significance of dendritic swelling after loud sounds in the guinea pig cochlea. *Hear Res, 9*(3), 263-278.

Rovig, G. W., Bohnker, B. K., & Page, J. C. (2004). hearing health risk in a population of aircraft carrier flight deck personnel. *Military Medicine, 169*(6), 429-432.

Samson, J., Wiktorek-Smagur, A., Politanski, P., Rajkowska, E., Pawlaczyk-Luszczynska, M., Dudarewicz, A., Sha, S. H., Schacht, J., &. Sliwinska-Kowalska, M. (2008). Noise-induced time-dependent changes in oxidative stress in the mouse cochlea and attenuation by D-methionine. *Neuroscience, 152*(1), 146-150.

Sasamura, T., Matsuda, A., & Kokuba, Y. (1998). Tumor growth inhibition and nutritional effect of D-amino acid solution in AH109A hepatoma-bearing rats. *J Nutr Sci Vitaminol (Tokyo), 44*(1), 79-87.

Sauberlich, H. E. (1961). Studies on the toxicity and antagonism of amino acids for weaning rats. *J Nutr, 75*, 61-72.

Saunders, J. C., Cohen, Y. E., & Szymko, Y. M. (1991). The structural and functional consequences of acoustic injury in the cochlea and peripheral auditory system: a five year update. *J Acoust Soc Am, 90*(1), 136-146.

Saunderson, C. L. (1985). Comparative metabolism of L-methionine, DL-methionine and DL-2-hydroxy 4-methylthiobutanoic acid by broiler chicks. *Br J Nutr, 54*(3), 621-633.

Schober, O., Meyer, G. J., Stolke, d., & Hundeshagen, H. (1985). Non-selective transport of [11C-methyl]-L and D-methionine into a malignant glioma. *Journal of Nuclear Medicine, 13*, 103-105.

Schober, O., Duden, C., Meyer, G. J., Muller, J. A., & Hundeshagen, H. (1987). Non-selective transport of [11 C-methyl]-L and D-methionine into a malignant glioma. *European Journal of Nuclear Medicine, 13*, 103-105.

Segal, S., Eviatar, E., Lapinsky, J., Shlamkovitch, N., & Kessler, A. (2003). Inner ear damage in children due to noise exposure from toy cap pistols and firecrackers: a retrospective review of 53 cases. *Noise Health, 5*(18), 13-18.

Seidman, M. D., Shivapuja, B. G., & Quirk, W. S. (1993). The protective effects of allopurinol and superoxide dismutase on noise-induced cochlear damage. *Otolaryngol Head Neck Surg, 109*(6), 1052-1056.

Sell, D. R., Rogler, J. C., & Featherston, W. R. (1980). Influence of dietary cystine on intestinal absorption and tissue distribution of methionine in the chick. *Poult Sci, 59*(8), 1885-1891.

Sha, S. H., & Schacht, J. (2000). Antioxidants attenuate gentamicin-induced free radical formation in vitro and ototoxicity in vivo: D-methionine is a potential protectant. *Hear Res, 142*(1-2), 34-40.

Slepecky, N. (1986). Overview of mechanical damage to the inner ear: noise as a tool to probe cochlear function. *Hear Res, 22*, 307-321.

Smith, A. W. (1998). The World Health Organisation and the prevention of deafness and hearing impairment caused by noise. *Noise Health, 1*(1), 6-12.

Stach, B. (2003). *Comprehensive dictionary of audiology illustrated* (2nd ed.). Chicago: Thompson Learning.

Stegink, L. D., Schmitt, J. L., Meyer, P. D., & Kain, P. H. (1971). Effect of diets fortified with DL-methionine

on urinary and plasma methionine levels in young infants. *J Pediatr, 79*(4), 648-655.

Stegink, L. D., Moss, J., Printen, K. J., & Cho, E. S. (1980). D-methionine utilization in adult monkeys fed diets containing DL-methionine. *J Nutr, 110*(6), 1240-1246.

Stegink, L. D., Bell, E. F., Filer, L. J., Ziegler, E. E., Andersen, D. W., & Seligson, F. H. (1986). Effects of equimolar doses of L-methionine, D-methionine and L-methionine-dl-sulfoxide on plasma and urinary amino acid levels in normal adult humans. *J Nutr, 116*(7), 1185-1192.

Stekol, J. A., & Szran, J. (1962). Pathological effects of excessive methionine in the diet of growing rats. *The Journal of Nutrition, 77*, 81-90.

Stipanuk, M. H., & Watford, M. (2000). Amino acid metabolism. In M. H. Stipanuk (Ed.), *Biochemical and physiological aspects of human nutrition*. Philadelphia: Saunders.

Sugiyama, K., Kushima, Y., & Muramatsu, K. (1987). Effect of dietary glycine on methionine metabolism in rats fed a high-methionine diet. *J Nutr Sci Vitaminol (Tokyo), 33*(3), 195-205.

Taggart, R. T., Wolgemuth, K. S., Williams, E. A., Smith, s., Marshall, L., Kopke, R. D. (2001). Genetic variation in the connexin 26 gene (GJB2) associated with susceptibility and resistance to noise-induced hearing loss.

Thorne, P. R., Duncan, C. E., & Gavin, J. B. (1986). The pathogenesis of stereocilia abnormalities in acoustic trauma. *Hear Res, 21*(1), 41-49.

Toppila, E., Starck, J., Pyykko, I., & Miller, J. M. (2002). *Protection against acute noise with antioxidants*. Paper presented at the Nordic Noise: An International Symposium on Noise and Health, in Nobel Forum, Karolinska Institute, Stockholm, Sweden.

Vertes, D., Axelsson, A., & Lipscomb, D. M. (1979). Some vascular effects of noise exposure in the chinchilla cochlea. *Acta Otolaryngol, 88*(1-2), 47-55.

Vogt, W. (1995). Oxidation of methionyl residues in proteins: tools, targets, and reversal. *Free Radic Biol Med, 18*(1), 93-105.

Vuyyuri, S. B., Hamstra, D. A., Khanna, D., Hamilton, C. A., Markwart, S. M., Campbell, K. C., Sunkara, P., Ross, B., & Rehemtulla, A. (2008). Evaluation of D-methionine as a novel oral radiation protector for prevention of mucositis. *Clin Cancer Res, 14*(7), 2161-2170.

World Health Organization (WHO) (1997). *Expert committee on the use of essential drugs*. World Health Organization: Geneva, Switzerland

World Health Organization (WHO) (2011). *Expert committee on the selection and use of essential medicines*. World Health Organization: New York, New York.

Wolgemuth, K. s., Luttrell, W. e., Kahmi, A. G., & Wark, D. J. (1995). The effectiveness of the Navy's hearing conservation program. *Military Medicine, 160*(5), 219-222

Yamasoba, T., Harris, C., Shoji, F., Lee, R. J., Nuttall, A. L., & Miller, J. M. (1998a). Influence of intense sound exposure on glutathione synthesis in the cochlea. *Brain Res, 804*(1), 72-78.

Yamasoba, T., Nuttall, A. L., Harris, C., Raphael, Y., & Miller, J. M. (1998b). Role of glutathione in protection against noise-induced hearing loss. *Brain Res, 784*(1-2), 82-90.

Yamasoba, T., Pourbakht, A., Sakamoto, T., & Suzuki, M. (2005). Ebselen prevents noise-induced excitotoxicity and temporary threshold shift. *Neurosci Lett, 380*(3), 234-238.

Yoshida, Y. (1985). [Analysis of methionine metabolism studied by the gas chromatographic determination of 3-methylthiopropionate in urine and its clinical application]. *Hokkaido Igaku Zasshi, 60*(2), 183-194.

Yu, N., Li, X., & Hu, B. (1999). [The effects of salicylate on noise-induced hearing loss in the guinea pig]. *Zhonghua Er Bi Yan Hou Ke Za Zhi, 34*(6), 344-346.

Zezulka, A. Y., & Calloway, D. H. (1976). Nitrogen retention in men fed isolated soybean protein supplemented with L-methionine, D-methionine, N-acetyl_L-methionine, or inorganic sulfate. *Journal of Nutrition, 106*, 1286-1291.

Zheng, L., Chen, J., Zhu, Y., Yang, H., & Hu, M. (1994). Comparison of the transport characteristics of D- and L-methionine in a human intestinal epithelial model (Caco-2) and a perfused rat intestinal model. *Pharmaceutical Research, 11*, 1771-1776.

15.0 SIGNATURES

15.1 "As the Principal Investigator, I confirm that I have read and will comply with the DDEAMC Human Research Protection Program (HRPP), Chapter 13, Investigator Responsibilities as available at, http://www.ddeamc.amedd.army.mil/clinical/investigation/documents/HRPP%20Version%20Date%20August%202010.pdf. The protection of research subjects is the shared responsibility of Principal Investigator (PI), Associate Investigators (AIs), members of the research team, and the DDEAMC Institutional Review Board (IRB). However, the ultimate responsibility for the safety and welfare of research subjects lies with the PI."

15.2 "I am aware that I am not authorized to accept any funds or other form of compensation for conducting this research."

15.3 Date prepared: 23 March 2016

_____

PRINCIPAL INVESTIGATOR:

CPT William P. Grimes, M.D.
Department of Preventive Medicine
Moncrief Army Community Hospital

_____

COL Traci E. Crawford, Commander
Moncrief Army Community Hospital

Section	Version 6.1 17 February 2016	Version 6.2 23 March 2016
Title Page	Version 6.1 17 February 2016	Version 6.2 23 March 2016
Page iii	17 February 2016 Version 6.1	23 March 2016 Version 6.2
Page v	TBA (Data Programmer)	Meredith Stowe, PhD Yale
		Corrected Table of Contents Page Numbers
Page xv	Removed "Outer hair cell quantification" in Fig 20 label	Corrected List of Figures Page Numbers Corrected Fig 20 label to " ABR Threshold Shifts"
Page 8	Recent Microsoft WORD Software upgrades caused formatting and image errors in newer versions of the protocol. Several adjustments have been made to align the tables, images and figures.	Adjusted Table 1 formatting
Page 13	Same as above	Adjusted spacing around Fig 6
Page 14	Same as above	Adjusted spacing around Fig 7
Page 18	Same as above	Adjusted spacing between Fig 11 and 12
Page 20	Same as above	Adjusted Fig 13
Page 21	Same as above	Adjusted Fig 14
Page 22	Same as above	Adjusted Fig 15
Page 23	Same as above	Adjusted Fig 16 and 17
Page 25	Same as above	Adjusted Fig 19
Page 25	284 kHz	2, 4, 8 kHz
Page 25		Corrected Figure 19 and 20 alignment
Page 26	Removed Figure 20 label "Outer hair cell quantification…."	Corrected Fig 20 label to "ABR Threshold Shifts"
Page 27		Adjusted Fig 21
Page 51		Adjusted Schedule of Assessments/Events Table
Page 52		Adjusted Timeline formatting and added asterisk next to date. Subject enrollment is continuing beyond March 2015.
Page 65		17 February 2016 CPT William P. Grimes, MD Department of Preventive Medicine

Section	Version 6.0 15 December 2015	Version 6.1 17 February 2016
Title Page	MAJ Christopher Wilson, MD, MPH Chief, Preventive Medicine 15 December 2015	CPT William P. Grimes, MD Department of Preventive Medicine 17 February 2016
Page iii	15 December 2015 Version 6.0	17 February 2016 Version 6.1
Page iv	MAJ Christopher Wilson, MD, MPH MAJ Christopher Wilson, MD, MPH Chief, Preventive Medicine	CPT William P. Grimes, MD CPT William P. Grimes, MD Department of Preventive Medicine
Page ix	MAJ Christopher Wilson, MD, MPH	CPT William P. Grimes, MD
Page 33	MAJ Christopher Wilson, MD, MPH	CPT William P. Grimes, MD
Page 39	MAJ Christopher Wilson, MD, MPH MAJ Wilson is Chief of Preventive Medicine	CPT William P. Grimes, MD CPT Grimes is a Family Physician in the Department of Preventive Medicine
Page 63	15 December 2015 MAJ Christopher Wilson, MD, MPH Chief, Preventive Medicine	17 February 2016 CPT William P. Grimes, MD Department of Preventive Medicine

Section	Version 5.1 15 July 2015	Version 6.0 15 December 2015
Title Page	5 July 2015	December 2015
Page iii		Version 6.0, 15 December 2015
Page iv	Removed: Jill Anderson, PhD, AUD, Off-site research coordinator	Add: CPT Eric Bunnell, AUD, Deputy Chief, Fort Jackson Army Hearing Program, Responsible for performing audiology testing Add: Daniel Fox, PhD, MPH, Off-site research coordinator
Page v	Judi Weissinger, PhD/Weissinger Solutions, Inc.	Rick Lampe
Page v		Add: Roosevelt Barnwell, Ombudsmen
Page ix.		Insert Exclusion Criterion: 7. History of abnormal kidney function or kidney impairment
Page x	12 hours	approximately 12 hours
Page x	Two medical monitors will be assigned to this protocol. One off site (SIU School of Medicine) and one on site to be named.	An on-site research monitor and an off-site medical monitor are assigned to this protocol.
Section 1.1 Page 1	11-12 days (typo)	11-15 days
Section 4.1 Page 26	fire 500 rounds of M-16 weapons fire (156 dB SPL) within a 9-11 day period. Spent magazines	each fire approximately 500 rounds of M-16 weapons fire (156 dB SPL) within a 9-11 day period. Spent cartridges
Section 4.5 Page 27		Insert Exclusion Criterion: 7. History of abnormal kidney function or kidney impairment
Section 5.2 Page 29	Combat plugs	Battle plugs
Section 5.2 Page 29	Deleted: a 50% study enrollment is anticipated	
Section 5.3 Page 30	A urine pregnancy test will be performed for all women of child bearing potential by the study coordinator(s). Pregnancy test results will be recorded.	Screening Assessments - Urine sample to be collected from all subjects for routine urinalysis. A urine pregnancy test will be performed for all women of child bearing potential by the study coordinator(s). Pregnancy test results will be recorded. • Two additional samples of blood (20-25 ml) will be collected for chemistry tests, complete blood counts, and tests of liver function, and homocysteine level.
Section 6.2 Page 33		• Urine and blood samples will be collected while on treatment (during second week). Two additional samples of blood (20-25 ml) will be collected for chemistry tests, complete blood counts, and tests of liver function, and homocysteine level. Urine sample will be collected for urinalysis.

Section 6.3 Page 33		• Urine and blood samples will also be collected post-treatment. Two additional samples of blood (20-25 ml) will be collected for chemistry tests, complete blood counts, and tests of liver function, and homocysteine level. Urine sample will be collected for urinalysis.
Section 8.4 Page 36		Additional safety assessments have been added to include: laboratory evaluations (hematology, blood chemistry, and urinalysis) at pre-specified time points (e.g., pre-treatment, during treatment, and post-treatment).
Section 8.6 Page 40	Judi Weissinger, PhD	Rick Lampe
Section 8.8 Page 40-41	CDR Royce Clifford MD,	Revised DSMC Roster: The DSMC will be composed of at least four voting members. The current DSMC includes: DSMC Chair (Patrick J. Antonelli, MD, Chair of Otolaryngology, University of Florida, Gainesville, the Medical Monitor for the Sponsor (Mark D. Packer, MD), the Research Monitor, David B. Pavlakovich, PA-C, D. Bradley Welling, MD, PhD, Chair of Otolaryngology, Harvard University, Boston, the Regulatory Representative (Rick Lampe),
Section 8.11 Page 42	Judi Weissinger, PhD	Rick Lampe
Section 10.3 Page 44	Removed following statement that was inadvertently included in par 3: 95% confidence intervals will be used to estimate D-methionine vs. placebo differences	
Section 11.0 Page 46		Insert Exclusion Criterion: 7. History of abnormal kidney function or kidney impairment
Section 12.0 Page 49	Schedule of Assessments Table	Added additional urine and blood testing to the Schedule of Assessments Table
Section 15.0 Page 63	15 July 2015	15 December 2015; Removed reference to IRBNet (no longer being used by DDEAMC IRB)

Section	**Version 5.0 30 March 2015**	**Version 5.1 15 July 2015**
Title Page	30 March 2015	15 July 2015
Page iii		Version 5.1, 15 July 2015, add associate investigator, minor edits
Page iv		Demarcus F. Bush, AUD Responsible for performing audiologic testing.
Page v		Add/remove ombudsmen, minor edits

Section	**Version 4.4 June 27, 2014**	**Version 5.0 March 30 2015**
Title Page	Protocol Version 4.4 Serial #0003 LTC William Bimson, D.O. Deputy Commander for Clinical Services 27 June 2014	Protocol Version 5.0 Serial #0005 MAJ Christopher E. Wilson, MD, MPH, Chief, Preventive Medicine 30 March 2015
iii	Version #: 4.4	Version #: 5.0
iii	Version Date: 27 June 2014	Version Date: 30 March 2015
iii		Site PI and other personnel changes
iv	CPT Rebecca Ludwig, AUD	CPT Jenny Davis
v	MAJ Christopher Wilson MD, MPH	David B. Pavlakovich, PA-C
	Linda Poole, Jeffrey Bullock	Lin Wright
5.5	CPT Rebecca Ludwig AUD	CPT Jenny Davis AUD
7.2	LTC William Bimson, D.O.	Major Christopher Wilson, M.D.
8.6	LTC William Bimson D.O. MAJ Christopher Wilson, MD, MPH	MAJ Christopher Wilson, MD, MPH David B. Pavlakovich, PA-C

8.8	MAJ Christopher Wilson, MD, MPH	David B. Pavlakovich, PA-C
11.1	CPT Rebecca Ludwig, AUD, Chief	CPT Jenny Davis, AUD
11.2	CPT Rebecca Ludwig AUD, Chief	CPT Jenny Davis AUD
16.0	June 27, 2014	March 30, 2015
16.0	LTC William Bimson, D.O.	MAJ Christopher E. Wilson MD, MPH

Section	**Version 4.3 February 25, 2014**	**Version 4.4 June 27, 2014**
Title Page	Version 4.3 February 25, 2014	Version 4.4 June 27, 2014
iii	Version #: 4.3	Version #: 4.4
iii	Version Date: 25 February 2014	Version Date: 27 June 2014
iii		Fort Jackson requested changes, personnel changes
iv	CPT Virginia Bailey	CPT Jenny Davis
v	MAJ Matthew Hanna, PA-C	MAJ Christopher Wilson, M.D.
v	Marilynn Bailey, Yvette Bennett, Jeffrey Bullock, Kendra Neely	Jeffrey Bullock, Ciera Nicholson, Sarah Sims, Gregory Bullock
x	10. Army Reservists or National Guardsmen	10. National Guardsmen
4.3	. . .exactly 500 rounds . . . to meet the 500 round requirements .	. . .approximately 500 rounds . . . to meet training requirements.
4.4	2. 21 to 40 years of age.	2. 21 to 45 years of age.
4.4	6. Willingness to refrain from using nutritional supplements containing or derived from protein while participating in this study.	6. Willing to limit the use of nutritional supplements containing or derived from protein to 50 grams per day while participating in this study
4.5	10. Army Reservists or National Guardsman	10. National Guardsmen
8.6	MAJ Matthew Hanna, PA-C, MAPS	MAJ Christopher Wilson, M.D.
8.8	MAJ Matthew Hanna PA-C, MAPS	MAJ Christopher Wilson M.D.
11.1	. . . generally between 21 - 40 years of age.	. . .generally between 21- 45 years of age.
11.1	.. . subjects between 21-40 years of age.	. . . subjects between 21 - 45 years of age.
11.1	2. 21 to 40 years of age	2. 21 to 45 years of age
11.1	6. Willingness to refrain from taking nutritional supplements containing or derived from protein while participating in this study.	6. Willing to limit the use of supplements containing or derived from protein to 50 grams per day while participating in this study.

Section	**Version 4.2 January 8, 2014**	**Version 4.3 February 25, 2014**
15.0		Added Signature Page: As the Principal Investigator, I confirm that I have read and will comply with the DDEAMC Human Research Protection Program (HRPP), Chapter 13, Investigator Responsibilities as available at, http://www.ddeamc.amedd.army.mil/clinical/investigation/documents/HRPP%20Version%20Date%20August%202010.pdf . The protection of research subjects is the shared responsibility of Principal Investigator (PI), Associate Investigators (AIs), Careline/Department Chief, members of the research team, and the DDEAMC Institutional Review Board (IRB). However, the ultimate responsibility for the safety and welfare of research subjects lies with the PI." 15.2 " I am aware that I am not authorized to accept any funds or other form of compensation for conducting this research. 16.0 Date prepared: 25 February 2014 PRINCIPAL INVESTIGATOR: LTC William Bimson, D.O. Deputy Commander of Clinical Services Moncrief Army Medical Center DEPT CHIEF COL Mark L. Higdon, Commander

Section	Version 4.1 August 28, 2013	Version 4.2 January 8, 2014
Title page	Leonard Rybak, M.D., Ph.D Professor of Surgery Southern Illinois University Medical School	Col Mark D. Packer, MD Executive Director, DoD/VA hearing Center of Excellence Clinical Assistant Professor, UTSA school of Medicine Chief of Neurology & Cranial Base Surgery
Title page	Protocol Version 4.1 28 August 2013	Protocol Version 4.2 8 January 2014
Page iii		Added version change, date change and personnel change
Page iv	Leonard Rybak, MD, PhD	Col Mark D. Packer, MD
Page v		Added: Research Monitor- MAJ Matthew Hanna, PA-C, MAPS
Page v		Added: Marilynn Bailey, Yvette Bennett, Jeffrey Bullock and Kendra Neely as Ombudsmen
Page ix	6. Willing to refrain from using multivitamins, herbals or other nutritional supplements while participating in this study	6. Willing to refrain from using supplements containing or derived from protein while participating in this study
Page xi		Modified schema to reflect Post audiological assessments on Study Days 29-32
Page 1	(Monday-Friday and Monday-Thursday over a 2 week period), and 4 days after weapons training for a total of 18 days of dosing. Tinnitus questionnaires will be assessed before weapons training and 15-16 days after completion of weapons training (i.e. 11days	(over a 2 week period), and 4 days after weapons training for a total of 18 days of dosing. Tinnitus questionnaires will be completed before weapons training begins (i.e. 11-15days
Page 26	The first primary endpoint is to confirm safety and tolerability of administering up to 100 mg/kg daily, given in divided doses, 12 hours apart	The first primary endpoint is to confirm safety and tolerability of administering up to 100 mg/kg daily, given in divided doses, twice daily (with morning and evening meal).
Page 27	6. Willingness to refrain from taking multivitamins, herbals, or other nutritional supplements while participating in this study.	6. Willingness to refrain from using nutritional supplements containing or derived from protein while participating in this study.
Page 33	Study Days 28-30	Study Days 29-32
Page 29		Deleted: with weapons training occurring Monday through Friday of the first week and Monday through Thursday of the second week.
Page 29	Candidates will be invited, but not required, to attend a recruitment briefing to introduce this research study during the first 14 days of class	Candidates will attend a recruitment briefing to introduce this research study during the first 14 days of class
		Deleted: The type of personal protection will be captured on the Candidate's Reference Audiogram, Form DD2215 and Hearing Conservation, Form DD 2216 that is completed after noise exposure.
Page 30	We anticipate that all subjects will be using Combat Plugs provided by the Ft Jackson audiologists. Compliance with hearing protection use will be assessed each day by the study coordinator(s). and recorded on the Case Report Form (CRF).	We anticipate that all subjects will be using Combat Plugs provided by the Ft Jackson audiologists. Compliance with hearing protection use will be assessed and recorded each day by the study coordinator(s).
Page 30	Audiological data will be recorded on the standard military audiologic assessment collection form, Department of Defense (DD) Form 2215/2216 modified to include 8 kHz data.	Deleted: Audiological data will be recorded on the standard military audiologic assessment collection form, Department of Defense (DD) Form 2215/2216 modified to include 8 kHz data. The threshold data will also be directly exported to an Excel file and copied into the research chart. onto case report forms.

Page 32	The threshold data will also be directly exported to an Excel file and copied onto case report forms.	The threshold data will also be directly exported to an Excel file and copied into the research chart.
Page 32		Deleted: The computerized database will be configured so that when the post-noise audiogram is entered in the database, criteria for hearing change will automatically be calculated. and flagged, so that the examiner can recheck the frequencies in question
Page 33	Evening formations	Evening meals
Page 33	Study Day 28- 30	Study Day 29 - 32
Page 33	Subjects will be contacted by to remind them to avoid noise exposure for at least 24 hours prior to the audiological assessments (hearing tests) by the on-site study coordinator(s).	Subjects will be contacted by telephone or text message to remind them to avoid noise exposure for at least 24 hours prior to the audiological assessments (hearing tests) by the on-site study coordinator(s).
Page 36	Baseline and Study Visit 1 assessments are limited to general health history information and pregnancy testing.	Baseline and Study Visit 1 assessments are limited to general health history information, pregnancy testing and laboratory assessment for kidney function.
Page 37	(Study Day 29 or 30)	(Study Day 29 -32)
Page 39	Dr. Leonard Rybak	Col Mark D. packer
Page 39	An on-site medical monitor will be identified prior to study enrollment	Deleted
Page 40		Added MAJ Matthew Hanna, PA-C, MAPS will serve as the independent research monitor on site
Page 40	The monitor will review data after each cohort has completed the study because all of the investigators are blind to the study arm assignment	Sentence Deleted
Page 41	Leonard Rybak, MD, PhD	Mark D. Packer, MD
Page 41		Added: the Research Monitor, MAJ Matthew Hanna PA-C, MAPS
Page 43	Data management activities include: using double data entry and monitoring outliers	Data management activities include: source verification of audiology data and monitoring outliers
Page 46	6. Willingness to refrain from taking multivitamins, herbals, or other nutritional supplements while participating in this study.	6. Willing to refrain from using supplements containing or derived from protein while participating in this study
Page 49	Day -14 to -10 and Day 29-30	Day -30—10 and Day 29-32

Section	**Version 4.0 August 1, 2013**	**Version 4.1 August 28, 2013**
Title page		Changed serial number, Protocol Version, Date and Principal Investigator
Page iii		Changed Version, Date Added Version, Date and Reason for Revision
Page iv		Changed Principal Investigator
Page v		Corrected name of Study Coordinator, added second Study Coordinator
Page ix	3. History of balance disorders	Changed Principal Investigator 3. History of chronic balance disorders

Section 4.1	This is a prospective, randomized, double-blind, placebo-controlled study to evaluate the effect of D-methionine (D-met) on permanent NIHL after required weapons training. The study will include 600 Army personnel enrolled in Drill Sergeant School (DSS) scheduled to undergo 9 days of weapons training over an 11 day period at Ft Jackson, South Carolina. This training requires that they fire 500 rounds of M-16 weapon fire (156 dB SPL) in a 9-11 day period. Spent magazines are collected and tallied by DSS leaders to document rounds fired. All subjects will be randomized to two equal arms; one to oral D-methionine (test drug) and the other to flavor-matched placebo. In both arms, there will be no other change in scheduled treatment. This facility trains 1600- 2040 drill sergeant instructors annually.	This is a prospective, randomized, double-blind, placebo-controlled study to evaluate the effect of D-methionine (D-met) on permanent NIHL after required weapons training. The study will include 600 Army personnel enrolled in Drill Sergeant School (DSS) scheduled to undergo 9 days of weapons training over an 11 day period at Ft Jackson, South Carolina. This training requires that they fire 500 rounds of M-16 weapon fire (156 dB SPL) within a 9-11 day period. Spent magazines are collected and tallied by DSS leaders to document rounds fired. All subjects will be randomized to two equal arms; one to oral D-methionine (test drug) and the other to flavor-matched placebo. In both arms, there will be no other change in scheduled treatment. This facility trains approximately 2000 drill sergeant instructors annually.
Section 4.5	3. History of balance disorders	3. History of chronic balance disorders
Section 5.2	Candidates will be invited, but not required, to attend a recruitment briefing to introduce this research study during the first week of class. (Days 1 - 5)	Candidates will be invited, but not required, to attend a recruitment briefing to introduce this research study during the first 14 days of class.
Section 7.2		Changed Principal Investigator
Section 8.6		Changed Principal Investigator
Section 8.8	The DSMC will meet prior to study initiation- no less than once per year and more frequently if needed to review enrollment and safety data and after the planned interim analysis.	The DSMC will review the protocol prior to study initiation and meet no less than once per year and more frequently if needed to review enrollment and safety data and after the planned interim analysis.
Section 11.1 Exclusion Criteria	3. History of balance disorders.	3. History of chronic balance disorders
Section 11.2	a. Recruitment and Informed Consent Recruitment procedures will be finalized during the one year planning period with input from the military to ensure appropriateness and to enhance effectiveness. CPT Rebecca Ludwig, AuD, Army Hearing Program Manager, will provide guidance with study recruitment and orientation procedures. At a minimum, all DSS Candidates will be introduced to the study within the first 1-4 days on class Day 11 of training and given the opportunity to participate.	a. Recruitment and Informed Consent Recruitment procedures will be finalized during the one year planning period with input from the military to ensure appropriateness and to enhance effectiveness. CPT Rebecca Ludwig, AuD, Army Hearing Program Manager, will provide guidance with study recruitment and orientation procedures. At a minimum, all DSS Candidates will be introduced to the study within the first 14 days of class and given the opportunity to participate.
Section 13.0 Study Timeline		Dates added to reflect current status. Extended to 57 months.

Page 63 Product Label	"Placebo to D-methionine"	Removed and submitted as "Revised Product Label." "D-Methionine/Placebo"
Pages 64 - 91	Case Report Forms listed	Removed and submitted as Case Report Forms (separate docs)_
Section	**Version 3.5 May 1, 2013**	**Version 4.0 August 1, 2013**
Page iv and throughout the document	PI: LTC Neil Page, M.D.	PI: LTC William Bimson, M.D.
Page vi	On site Study Coordinator: TBA	Beth Bullock, R.N. : On-site Study Coordinator
Page 47	within the first 1-4 days	on class Day 11
Page 49	19-23 and 26-29	23-32
Section	**Version 3.4 March 7, 2013**	**Version 3.5 May 1, 2013**
Cover Page	Protocol Version 3.4	Protocol Version 3.5
Cover Page	April, 2013, 2012	May 1, 2013
DIAGNOSIS AND KEY SUBJECT SELECTION CRITERIA		Added: 14. Renal impairment measured as eGFR < 50 on screening creatinine clearance blood draw Deleted: History of renal impairment
Section 4.5		Added: 14. Renal impairment measured as eGFR < 50 on screening creatinine clearance blood draw Deleted: History of renal impairment
Section 5.3		Added: A blood draw for serum creatinine will be conducted prior to enrollment in order to calculate estimated GFR by the Cockroft-Gault equation. Those subjects with an estimated eGFR < 50 will be excluded from enrollment.
Section 11.1		Added: A blood draw for serum creatinine will be conducted prior to enrollment in order to calculate estimated GFR by the Cockroft-Gault equation. Those subjects with an estimated eGFR < 50 will be excluded from enrollment Deleted: History of kidney impairment
Section 12.0		Added: Serum Creatinine
Section	**Version 3.3 March 7, 2013**	**Version 3.4 March 7, 2013**
Cover Page	Protocol Version 3.3	Protocol Version 3.4
Cover Page	March 7, 2013, 2012	April 2013
Page v		Added: Marilynn Bailey, Sarah Dishon, and Deb Dunkelberg---Ombudsmen---Will serve as an independent, neutral and impartial mediator of the Soldiers in order to ensure that no coercion is observed for the recruiting and consenting study activities.
Section 7.3	The formulated drug product (D-methionine) will be provided for clinical use as an oral suspension. Each dose will be packaged in individually labeled vials. The label will include instructions to shake the suspension prior to administration.	The formulated drug product (D-methionine) will be provided for clinical use as an oral suspension. Each dose will be packaged in individually labeled vials. Each vial will have a subject identification number on the label (Section 15.1 Master Label). The label will include instructions to shake the suspension prior to administration.
Section 7.4		Added: Refrigeration is not required. Room temperature will be monitored and recorded daily, including weekends and holidays.
Section 15.1		Replaced Master Label

<table>
<tr><th>Section</th><th>November 30, 2012 Version 3.2</th><th>Version 3.3 March 7, 2013</th></tr>
<tr><td>Cover Page</td><td>Protocol Version 3.2</td><td>Protocol Version 3.3</td></tr>
<tr><td>Cover Page</td><td>November 30, 2012</td><td>March 07 2013</td></tr>
<tr><td>Page iii</td><td>Version # 3.2</td><td>Version # 3.3</td></tr>
<tr><td>Page iii</td><td>Version Date: 30November2012</td><td>Version Date: 07March2012</td></tr>
<tr><td>Page v</td><td>TBA</td><td>Marilyn Bailey and staff</td></tr>
<tr><td>DIAGNOSIS AND KEY SUBJECT SELECTION CRITERIA (Exclusion criteria)</td><td></td><td>Added: 12. History of psychotic schizophrenia
13. Exposure within the previous 6 months to systemic ototoxic substances including aminoglycosides or vancomycin
14 Body weight exceeding 225 pounds</td></tr>
<tr><td>Section 4.5</td><td></td><td>Added: 12. History of psychotic schizophrenia
13. Exposure within the previous 6 months to systemic ototoxic substances including aminoglycosides or vancomycin
14. Body weight exceeding 225 pounds</td></tr>
<tr><td>Section 7.2</td><td>Study drug will be dispensed by on-site study coordinator(s), who are qualified medical personnel (RN or LPN). The study drug will be dispensed as labeled in accordance with the study protocol. LTC Neil Page, M.D., will supervise the activities of the study coordinator(s) and audiologists.</td><td>Study drug will be dispensed by MACH Pharmacy to on-site study coordinator(s), who are qualified medical personnel (RN or LPN). The study drug will be distributed to the study participants as labeled in accordance with the study protocol. LTC Neil Page, M.D., will supervise the activities of the study coordinator(s) and audiologists.</td></tr>
<tr><td>Section 7.3</td><td>Each subject's dose of D-methionine will be individually determined based on actual subject weight and assigned dose level. For ease of administration and packaging, dosing is broken down by weight categories with dosing based on the lowest weight in that category as follows:</td><td>Each subject's dose of D-methionine will be individually determined based on actual subject weight. For ease of administration and packaging, dosing is broken down by weight categories with dosing based on the lowest weight in that category as follows:</td></tr>
<tr><td>Section 7.3</td><td><table><tr><th>Body Weight lbs (kg)</th><th>Total Daily Dose (Up to 100 mg/kg/day)</th></tr><tr><td>100-125 lbs (45 - 57 kg)</td><td>4.6 grams (4600 mg)</td></tr><tr><td>126-150 lbs (57 - 68 kg)</td><td>5.6 grams (5600 mg)</td></tr><tr><td>151-175 lbs (68 - 80 kg)</td><td>6.8 grams (6800 mg)</td></tr><tr><td>176-200 lbs (80 - 91 kg)</td><td>8.0 grams (8000 mg)</td></tr><tr><td>201-225 lbs (91 - 102 kg)</td><td>9.2 grams (9200 mg)</td></tr><tr><td>226-250 lbs (103 -114 kg)</td><td>10.3 grams (10,300 mg)</td></tr></table></td><td><table><tr><th>Body Weight lbs (kg)</th><th>Total Daily Dose (Up to 100 mg/kg/day)</th></tr><tr><td>100-125 lbs (45 - 57 kg)</td><td>4.6 grams (4600 mg)</td></tr><tr><td>126-150 lbs (57 - 68 kg)</td><td>5.6 grams (5600 mg)</td></tr><tr><td>151-175 lbs (68 - 80 kg)</td><td>6.8 grams (6800 mg)</td></tr><tr><td>176-200 lbs (80 - 91 kg)</td><td>8.0 grams (8000 mg)</td></tr><tr><td>201-225 lbs (91 - 102 kg)</td><td>9.2 grams (9200 mg)</td></tr></table></td></tr>
<tr><td>Section 7.3</td><td>For example, if subject's weight is 155 lbs (70.4 kg) at baseline, then the subject will receive 6.8 grams of study drug per day. This dose will be divided into two equal doses (3.4 grams) and administered prior to morning and evening meal (approximately 12 hours apart).</td><td>For example, if subject's body weight is 155 lbs (70.4 kg) then the subject's total daily dose equals 6.8 grams per day. This dose will be divided into two equal doses (3.4 grams) and administered prior to morning and evening meal (approximately 12 hours apart). Each dose (AM and PM) will be packaged in individually labeled vials by KP Pharmaceuticals.</td></tr>
<tr><td>Section 7.4</td><td>All study drug will be stored under conditions consistent with package labeling. All study drug will be stored in a secure limited-access area under controlled temperature in accordance with labeled storage requirements. Room temperature will be monitored and recorded daily</td><td>All study drug will be stored under conditions consistent with package labeling. All study drug will be stored in a secure limited-access area under controlled temperature in accordance with labeled storage requirements. Room temperature will be monitored and recorded daily including weekends and holidays.</td></tr>
</table>

Section 7.8	Treatment with intravenous (IV) antibiotics, such as Amikacin: Amikin®, Gentamicin, Netilmicin: Netromycin®, Streptomycin, Tobramycin: Nebcin® or any surgical or medical condition which might significantly alter the absorption, distribution, metabolism, or excretion of any drug. Subjects who received treatment for drug or alcohol abuse within the previous 6 months are prohibited from participation in this study.	Treatment with intravenous (IV) antibiotics, such as Amikacin: Amikin®, Gentamicin, Netilmicin: Netromycin®, Streptomycin, Tobramycin: Nebcin® or any other aminoglycosides and/or vancomycin. Also, any surgical or medical condition which might significantly alter the absorption, distribution, metabolism, or excretion of any drug. Subjects who received treatment for drug or alcohol abuse within the previous 6 months are prohibited from participation in this study.
Section 11.1 Exclusion Criteria		Added: 12. History of psychotic schizophrenia 13. Exposure within the previous 6 months to systemic ototoxic substances including aminoglycosides or vancomycin
Section	**November 5, 2012 Version 3.1**	**November 30, 2012 Version 3.2**
Cover Page	Protocol Version 3.1	Protocol Version 3.2
Page iii	Version # 3.1	Version # 3.2
Page iii	Version Date: 5November2012	Version Date: 30November2012
Page 28	5. Treatment with intravenous (IV) antibiotics within the past 6 months	5. Treatment with intravenous (IV) antibiotics or Carboplatin/Cisplatin within the past 6 months
Section 5.1		Added: An ombudsman must be present during the recruiting and consenting process.
Section 5.2		Added: Consent will be obtained by study coordinator(s) not affiliated with the DSS program.
Section 8.6	The Regulatory Representative will be included via teleconference to assess the regulatory impact of outcomes of adverse events and new literature reviews to assess change to the risk/benefit ratio and to determine if modifications need to be made or the study terminated	The Regulatory Representative (Dr. Weissinger) will be included via teleconference to assess the regulatory impact of outcomes of adverse events and new literature reviews to assess change to the risk/benefit ratio and to determine if modifications need to be made or the study terminated
Section 8.8	The data safety monitoring committee (DSMC) evaluates study data on an ongoing basis to assure participant safety and study integrity. The DSMC will review study data and unanticipated problems and make recommendations based on their reviews. The DSMC will meet prior to study initiation, no less than once per year and more frequently if needed to review enrollment and safety data and after the planned interim analysis. Evaluation will also occur if three subjects have met the stopping criteria. If all three are determined to be in the D-methionine-treated group, the DSMC will consider a lower dose as a safe dose with which to continue the study. The DSMC Chair may call an emergency meeting at any time should issues of patient safety arise. The DSMC will be composed of at least three voting members. The current DSMC includes: DSMC Chair (CDR Royce Clifford, MD, Officer-in-charge, Marine Air Group 39 Medical Clinic Camp Pendleton, CA), the medical monitor for the Sponsor (Leonard Rybak, MD, PhD), the Regulatory Representative (Judi Weissinger, PhD), and the Yale University epidemiologist/statistician (Carrie Redlich, MD, MPH). Only the Yale University statistician will be unblinded and will endeavor to keep the committee blinded during discussion of the stopping criteria, adverse events, and any study adjustments recommended by the committee.	The data safety monitoring committee (DSMC) evaluates study data on an ongoing basis to assure participant safety and study integrity. The DSMC will review study data and unanticipated problems and make recommendations based on their reviews. The DSMC will meet prior to study initiation, no less than once per year and more frequently if needed to review enrollment and safety data and after the planned interim analysis. Evaluation will also occur if three subjects have met the stopping criteria. If all three are determined to be in the D-methionine-treated group, the DSMC will consider a lower dose as a safe dose with which to continue the study. The DSMC Chair may call an emergency meeting at any time should issues of patient safety arise. The DSMC will be composed of at least three voting members. The current DSMC includes: DSMC Chair (CDR Royce Clifford, MD, Officer-in-charge, Marine Air Group 39 Medical Clinic Camp Pendleton, CA), the medical monitor for the Sponsor (Leonard Rybak, MD, PhD), the Regulatory Representative (Judi Weissinger, PhD), and the Yale University epidemiologist/statistician (Carrie Redlich, MD, MPH). Only the Yale University statistician will be unblinded and will endeavor to keep the committee blinded during discussion of the stopping criteria, adverse events, and any study adjustments recommended by the committee.

Section 11.1	3. Negative pregnancy test confirmed by urine test at enrollment and prior to taking first study drug dose	3. Negative pregnancy test confirmed by urine dipstick test at enrollment and prior to taking first study drug dose
Section	**October 5, 2012 Version 3.0**	**November 5, 2012 Version 3.1**
Section 4.4		Added an Inclusion Criterion: Willingness to refrain from taking multivitamins, herbals, or other nutritional supplements while participating in this study.
Section 4.5	Exclusion Criterion #5:Exposure in the previous 6 months to systemic ototoxic substances including aminoglycoside antibiotics, or chemotherapy with carboplatin, cisplatin, vincristine, vinblastine or difluoromethylornithine	Exclusion Criterion #5: Treatment with intravenous (IV) antibiotics or Carboplatin/Cisplatin within the past 6 months
Section 7.8	Exposure in the previous 6 months to systemic ototoxic substances including aminoglycoside antibiotics, or chemotherapy with carboplatin, cisplatin, vincristine, vinblastine or difluoromethylornithine will exclude a subject. In addition any surgical or medical condition which might significantly alter the absorption, distribution, metabolism, or excretion of any drug and a recent history of drug or alcohol abuse are prohibitive of participation in this study.	Treatment with intravenous (IV) antibiotics, such as Amikacin: Amikin®, Gentamicin, Netilmicin: Netromycin®, Streptomycin, Tobramycin: Nebcin® or any surgical or medical condition which might significantly alter the absorption, distribution, metabolism, or excretion of any drug. Subjects who received treatment for drug or alcohol abuse within the previous 6 months are prohibited from participation in this study.

Section 11.3	April 27, 2001	April 27, 2011
Section	**August 10, 2012 Version 2.2**	**October 5, 2012 Version 3.0**
Title Page		Added IND #, changed serial number, Protocol Version and Principal Investigator
Page iii		Added IND# , changed version date and version#
Roles and Responsibilities for Clinical Study	The Principal Investigator for this IND application is CPT William G. Callis, M.D., M.P.H., Preventive Medicine Physician CPT Rebecca Ludwig- Supervising Audiologist 1LT Virginia Best Bailey, Audiologist	The Principal Investigator for this IND application is, LTC Neil Page, MD, Deputy Commander for Clinical Services CPT Rebecca Ludwig, Co-Investigatory, Supervising Audiologist CPT Virginia Best Bailey, Co-Investigator, Audiologist

Roles and Responsibilities for Clinical Study	Jose Cruz, PAC, MPAS/FT Jackson TBA (non-military) On-site Study Coordinator	DELETED (2) TBA (non-military) (2) On-site Study Coordinators Added: Marilyn Bailey Responsible for assigning an ombudsman for the Army Corp Coordinator study-related recruiting activities Added: TBA Ombudsman Will serve as an independent neutral and impartial mediator of the Soldiers in order to ensure that no coercion is observed for the recruiting and consenting study activities.
Table of Contents	Study Day 33-36	Study Day 29-30
Table of Contents	15.3 CRF-Follow-Up-Study Visit 2 (19-22 Days Post Weapons Training	15.3 CRF – Follow Up – Study Visit 2 (15-16 Days Post Weapons Training)
Clinical Synopsis		Changed Phase 2 to Phase 3 Clinical Trials, updated PI, changed trainees to Candidates
Study Design Schema	Study Day 36	Study Days 29-30
List of Abbreviations		Added: MACH and SOP
Section 1.0	19-22 days Drill Sergeant Instructor Training School	15-16 days Drill Sergeant School (DSS) Added: … commandant COL Mark Higdon, MD, of Moncrief Army Medical Center (MACH) and 2nd Commandant CSM Michael McCoy, DSS.
Section 3.6	Phase II	Phase 3
Section 4.3	This training requires that each Soldier fire exactly 500 rounds of M-16 weapons fire (156 dB SPL) in a 9 day period of weapons training with a possible additional makeup day. Every Soldier Drug logs will be kept for every subject, recording every dose taken or missed. Pre and post training tinnitus assessments are not routinely done and are a required assessment for this study which will be conducted by the audiologist.	This training requires that each Soldier fire exactly 500 rounds of M-16 weapons fire (156 dB SPL) within a 9-11 day period of weapons training with a possible additional makeup day if necessary to meet the 500 round requirements Every DSS Candidate Drug logs will be kept for every subject, recording every dose taken or missed or whether or not battle plugs were used. Tinnitus assessments are not routinely done and are a required assessment for this study which will be conducted by the audiologist and/or study coordinators.
Section 4.7	…the 36 day study period	…the 30 day study period
Section 5.0 Section 5.1 Section 5.2	 Ft Jackson has 17 classes per year for Drill Sergeant Instructor Trainees.	All instances of "Trainees" changed to "Candidates" Added: An ombudsman must be present during the recruiting and consenting process. Ft Jackson has 16 classes per year for DSS Candidates

Section 5.3 Section 5.5	Body weight and height- Weight will be measured on a calibrated scale with the subject wearing usual military clothing and no shoes by the study coordinators (s) Audiological data will be recorded on the standard military audiologic assessment collection form, Department of Defense (DD) Form 2215/2216 modified to include 8 kHz data. The thresholds will also be copied onto case report forms.	Body weight – Body weight obtained at DSS entry and recorded in the candidate electronic health record will be used to determine dosing of the Investigational Drug. Audiological data is collected electronically via XXXX audiometers and recorded on the standard military audiologic assessment collection form, Department of Defense (DD) Form 2215/2216 modified to include 8 kHz data. The thresholds will also be copied onto case report forms.
Section 5.5.3	Pure-tone air conduction threshold testing both at baseline and post-test (19-22 days after completion of weapons training and 15-18 days after the last day of study drug/placebo administration) will be conducted utilizing the modified Hughson-Westlake procedure.19-22 days	Pure-tone air conduction threshold testing both at baseline and post-test (15-16 days after completion of weapons training and 11-12 days after the last day of study drug/placebo administration) will be conducted utilizing the modified Hughson-Westlake procedure.
Section 6.2	Study Days 1-18	Added: Assess for hearing protection compliance Study Days 2-18
Section 6.3	Study Days 33-36	Study Days 28-30
Section 7.1	bottles	Small vials
Section 7.2	CPT William C. Callis M.D., M.P.H. will supervise the activities of the study coordinator(s).	LTC Neil Page, M.D., will supervise the activities of the study coordinator(s) and audiologists.
Section 7.3	The study coordinator(s) will be responsible for recording the weight of each Soldier.	DELETED
Section 7.3	KPT will assign the appropriate randomization code and prepare individualized doses of study drug prior to shipping.	KPT will assign the appropriate randomization code and prepare individualized doses of study drug prior to shipping to the Moncrief Army Community Hospital (MACH) Pharmacy at FT Jackson
Section 7.5	Upon receipt of study drug, the MACH Pharmacist will ensure that the information on the packing slips matches exactly with information sent to the site, including content, amount, lot numbers, quantity and expiration date.	Upon receipt of study drug, the MACH Pharmacist will ensure that the information on the packing slips matches exactly with information sent to the site, including content, amount, lot numbers, quantity and expiration date. Added: This information will be recorded in each subject's EHR and on their medication log (CRF).
Section 7.6	All doses of study drug will be recorded on each subject's medication log.	DELETED
Section 8.4.1	Study Day 36	Study Day 29 or 30
Section 8.6	Two medical monitors will be assigned to this study. Dr. Leonard Rybak will	Dr. Leonard Rybak will serve as the off-site medical monitor and LTC Neil Page, MD will serve as the Principal Investigator and will oversee all study drug administration by the on-site study coordinator(s). Dr. Rybak is Professor of Surgery and a licensed

	serve as the off-site medical monitor and Jose Cruz, PA C, MPAS will serve as the onsite medical monitor. CPT William G. Callis, MD, MPH will serve as the Principal Investigator and will oversee all study drug administration by the on-site study coordinator(s). Dr. Rybak is Professor of Surgery and a licensed otolaryngologist at SIU School of Medicine. Dr. Callis is Preventative Medicine Physician at Moncrief Army Community Hospital, Fort Jackson.	otolaryngologist at SIU School of Medicine. Dr. Page is the Deputy Commander for Clinical Services at Moncrief Army Community Hospital, Fort Jackson.
Section 9.1	Data collection will occur at the study site and all study data will be transferred to the data management unit at Yale University according to standard operating procedures developed for this study.	Data collection will occur at the study site through the Research Electronic Data Capture (REDCap) program created and supported by Vanderbilt University and all study data will be transferred to Martin Slade, MS of the data management unit at Yale University according to standard operating procedures developed for this study.
Section 9.3	Subject name and social security number will not appear in the computer databases	DELETED
Section 10.3	…handled by and outside body	…handled by an outside statistician
Section 11.1	The training period is 55 days… …will be enrolled in the study for up to 36 days Audiologic assessments such as Threshold Testing, Otoscopy and Tympanometry Testing are routinely performed on all DSS trainees. 3)….(19-22 days)	The training period is 16 days …will be enrolled in the study for up to 30 days Audiologic assessments such as Threshold Testing, Otoscopy and Tympanometry Testing are routinely performed on all DSS candidates within 3-6 months of the training period. Repeat audiological assessments will be performed at the study baseline and are considered study-related procedures. 3)
Section 12.0 Schedule of Assessments	Weight & Height Day 19-32 Day 33-36	Weight Day 29-30 Day 29-30

Section 13.0	…17 Drill Sergeant School classes …every three weeks	…16 Drill Sergeant School classes ….every two-three weeks
Appendices	Version 30March2012 Phase 2 15.1 CASE REPORT FORMS 15.1 CASE REPORT FORMS	Version 15August2012 DELETED 15.1 MASTER LABEL 15.2 CASE REPORT FORMS
Section	**April 19. 2012 Version 2.1**	**August 10, 2012 Version 2.2**
	Minor typographical errors, date errors and personnel changes	Minor typographical, date and personnel name corrections
Section	**April 18, 2012 Version 2.0**	**April 19th, 2012 Version 2.1**
	Minor typographical errors	Minor typographical errors corrected
Section	**December 21, 2011 Version 1.0**	**April 18th, 2012 Version 2.0**
2.7.1	**TABLE 1. Relative utilization on sulfur amino acid isomers, analogs, and precursors**	**TABLE 1. Relative utilization on sulfur amino acid isomers, analogs, and precursors (Baker 2006)**
2.7.3	**Figures 3 & 4. Effects of experimental diets on the growth (A) and food consumption (B) of rats is followed by incorrect diagrams**	**Figures 3 & 4. Effects of experimental diets on the growth (A) and food consumption (B) of rats, is followed by the correct diagrams**
4.4	4. Willing to use an effective method of birth control during the study (Both male and female participants should avoid pregnancy during study). *Subject should either abstain from sexual relations or practice a method of birth control while taking part in the study. Except for surgical removal of the uterus, birth control methods such as condoms, a diaphragm or cervical cap, birth control pills, IUD, or sperm killing products are not totally effective in preventing pregnancy. Male study participants should refrain from fathering babies while enrolled in this study.*	4. Willing to use an effective method of birth control during the study (Female participants should avoid pregnancy and male participants should avoid fathering children during the study.) *Subject should either abstain from sexual relations or practice a method of birth control while taking part in the study. Except for surgical removal of the uterus, birth control methods such as condoms, a diaphragm or cervical cap, birth control pills, IUD, or sperm killing products are not totally effective in preventing pregnancy. Male study participants should refrain from fathering babies while enrolled in this study.*
5.3	• Body weight and height - Weight will be recorded and used for ordering study drug for each individual subject.	• Body weight and height - Weight will be measured on a calibrated scale with the subject wearing usual military clothing and no shoes by the study coordinator(s). Screening weight will be recorded on the Case Report Form by the study coordinator(s) and used to determine dosing of the Investigational Drug.

8.4.1

Compliance and adverse event reports will be obtained each day that study drug is administered (Study Days 1-18) and at the end of the study (Study Day 36). Each adverse event will be graded according to the Table for Grading Severity of Adverse Events (AEs). All other laboratory and clinical AEs that occur in a subject will be assessed for severity and classified into one the categories below.

- **Grade 1 (Mild)**: event requires minimal or no treatment and do not interfere with the patient's daily activities.
- **Grade 2 (Moderate)**: event results in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Grade 3 (Severe)**: event interrupts a patient's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.
- **Grade 4 (Life threatening)**: Any adverse drug experience that places the patient or participant, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.
- **Grade 5 (Death)**

8.4.1 Assessment of Safety

General health status and symptom assessments performed at Baseline/Eligibility Visit and Study Visit 1 will serve as baseline for any new signs or symptoms that arise during the study. All such changes occurring after Study Visit 1 will be evaluated as possible adverse events and appropriately recorded on the Adverse Event Case Report Form (CRF).

Baseline and Study Visit 1 assessments are limited to general health history information and pregnancy testing. No additional physical exam, clinical and/or laboratory assessments are included in the protocol. Expected adverse events include possible gastrointestinal (GI) symptoms (nausea, vomiting, constipation, diarrhea, dysphagia).

Subjects will be questioned prior to receiving each study dose and on the last study day for the presence of GI side effects. *See Estimating Severity Grade and method for classifying GI adverse events.

Other subject reported symptoms that occur between Study Day 1 and Study Day 18, including the follow up visit at Study Day 36, will be evaluated as a possible adverse event. If a subject experiences a side effect that requires additional medical attention, the subject will seek medical attention through appropriate standard operating sick call procedures and the medical monitor will be contacted.

Adverse events will be evaluated according to the Estimating Severity Grade and will be recorded on the Adverse Event Log and the Adverse Event CRF. Adverse events deemed greater than Grade 2 will be considered a Serious Adverse Event (SAE). All SAEs will be recorded on the SAE CRF and reported according to FDA and institutional requirements (See Section 8.5 – Specific Serious Adverse Event Reporting Requirements).

Compliance and adverse event reports will be obtained each day that study drug is administered (Study Days 1-18) and at the end of the study (Study Day 36) by the Study Coordinator(s). Each adverse event will be graded according to the Table for Estimating Severity Grade.

For all subjects receiving at least one dose of study drug, the number and percentage of subjects reporting adverse events will be tabulated by cohort and overall by severity and body system.

ESTIMATING SEVERITY GRADE	
Grade 1 (Mild)	Event requires minimal or no treatment and does not interfere with the subject's daily activities
Grade 2 (Moderate)	Event results in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
Grade 3 (Severe)	Event interrupts a patient's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.
Grade 4 (Life Threatening)	Any adverse drug experience that places the patient or participant, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.
Grade 5	Death

GASTROINTESTINAL				
	Grade 1	Grade 2	Grade 3	Grade 4
Nausea	Mild or transient; maintains reasonable intake	Moderate discomfort; intake decreased significantly; some activity limited	No significant intake; requires IV fluids	Hospitalization required
Vomiting	1 episode in 24 hours	2-5 episodes in 24 hours	> 6 episodes in 24 hours or needing IV fluids	Physiological consequences requiring hospitalization or requiring parenteral nutrition

		Constipation: Requiring stool softener or dietary modification; Requiring laxatives; Obstipation requiring manual evacuation or enema; Obstruction or toxic megacolon Diarrhea: Mild or transient; 3-4 loose stools/day or mild diarrhea lasting for less than 1 week; Moderate or persistent; 5-7 loose stools/day or diarrhea lasting > 1 week; > 7 loose stools/day or bloody diarrhea; or orthostatic hypotension or electrolyte imbalance or > 2L IV fluids required; Hypotensive shock or physiological consequences requiring hospitalization Oral discomfort/dysphagia: Mild discomfort; no difficulty swallowing; Some limits on eating/drinking; Eating/talking very limited; unable to swallow solid foods; Unable to drink fluids; requires IV fluids
Bibliography List	None	Added pages 50-61 listing references
Appendices list	None	Added 6 CRFs including Baseline Eligibility Visit, Study Visit 1, Study Visit 2, Medication Log/AE Assessment, Adverse Events, Serious Adverse Events
Baseline eligibility protocol	Inadvertently left out page 4 of 5 in Baseline Eligibility Visit	Page 4 of 5 in Baseline Eligibility Visit inserted