CS RIOT CONTROL AGENT EXPOSURE IN US ARMY MASK CONFIDENCE TRAINING: ASSOCIATION BETWEEN EXPOSURE TO O-CHLOROBENZYLIDENE MALONONITRILE AND URINARY METABOLITE 2-CHLOROHIPPURIC ACID

by

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DEDICATION

To my daughter, Perri. Born July 3, 2015.

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20 May 2016

ABSTRACT

CS Riot Control Agent Exposure in US Army Mask Confidence Training: Association between Exposure to o-Chlorobenzylidene Malononitrile (CS) and Urinary Metabolite 2-Chlorohippuric Acid

Lieutenant Maccon A. Buchanan, Masters of Science in Public Health, 2016

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Abstract

This study was conducted among US Army trainees at Fort Sam Houston, Texas to evaluate the association between exposure to 2-chlorobenzylidene malononitrile (CS riot control agent) and metabolite 2-chlorohippuric acid (CHA) measured in urine of test subjects (n=87) after completion of the Mask Confidence Training chamber exercise. This is the first study to apply personal monitoring and the CDC's CLIA approved CS Metabolites method (Code ERB-253) for sensitive analysis of CHA to explore the association between exposure and metabolite in a prospective, observational cohort. Exposure assessment was conducted using OSHA modified P&CAM 304. GC/ECD was used to quantify CS exposure concentrations. Solid phase extraction and HPLC/MS was used to quantify CHA metabolite in urine at pre-exposure, 2, 8, 24, and 30-hour post-

exposure time intervals. Urine samples were creatinine corrected to reduce variation in subject Glomerular filtration rates. CS exposure concentrations ranged from 0.086 – 4.900 mg/m³ ($\bar{x}$=2.741 mg/m³). Correcting CHA levels for creatinine at the 2-hour time interval resulted in a range of 94.6 – 1121.6 µg/g-cr ($\bar{x}$=389.46 µg/g-cr). Correcting CHA levels for creatinine at the 8-hour time interval resulted in a range of 15.80 – 1170.20 µg/g-cr ($\bar{x}$=341.13 µg/g-cr). Correcting CHA levels for creatinine at the 24-hour time interval resulted in a range of 4.00 – 53.1 µg/g-cr ($\bar{x}$=19.3 µg/g-cr). Correcting CHA levels for creatinine at the 30-hour time interval resulted in a range of 1.99 – 28.4 µg/g-cr ($\bar{x}$=10.63 µg/g-cr). Based on a skewed distribution, all CHA levels were natural log transformed for statistical analysis. Utilizing time as a continuous variable, Spearman's correlation revealed lnCHA (corrected) levels were strongly correlated with time sampled ($r = -0.748$, $p<0.01$) and weakly correlated with CS concentration ($r = 0.270$, $p<0.01$). A linear relationship was observed between lnCHA, CS concentration, and time of urine sample according to the following regression equation: ln(CHA, µg/g-cr) = 5.423 + 0.316 (CS conc., mg/m³) – 0.002 (time sampled), ($R = 0.910$, $R^2 = 0.827$, $p<0.01$). This relationship suggests that CHA has the potential to be an effective retrospective predictor of CS exposure in future biomarker developments.

Keywords CS riot control agent, o-chlorobenzylidene malononitrile, CS, CS gas, tear gas, chlorohippuric acid, sensitive method, retrospective analysis, high performance liquid chromatography, HPLC, gas chromatography, mass spectrometry, GCMS

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CHAPTER 1: Introduction

BACKGROUND

O-chlorobenzylidene malononitrile (CS), commonly referred to as OCBM, CS gas, CS riot control agent (RCA), or tear gas, is the most common RCA used by military and law enforcement around the world (17). Its popularity of use over other RCAs is attributed to its potency, ease of manufacture, pyrotechnic dissemination, and its quick rate of action (ability to cause immediate incapacitating effects) (37). Law enforcement's use of CS to disperse crowds during violent or destructive protests has garnered more attention during recent civil uprising such as the 2014 – 2015 Ferguson, Missouri unrest, the 2015 Baltimore, Maryland protests, as well as overseas such as in the 2014 Hong Kong protests and the 2014 Kurdish riots in Turkey. During investigations of CS deployment cases, governmental agencies have been limited in analytical methods for retrospective quantification through urinary metabolites, which could provide evidence to support allegations of its use during wartime operations.

Over the past half century, extensive research through laboratory analysis and animal testing, as well as clinical observations of CS exposure effects on human health, has contributed extensively to the data existing in literature. There is no evidence to date that supports causality between CS exposure and chronic illness, cancer, reproductive effects, or death (40). However, research has shown that exposure to CS increases relative risk of acute respiratory illnesses during US Army Basic Combat Training in the week following exposure when compared to the week preceding exposure (21). Health effects of CS are commonly associated with symptoms of skin erythema, coughing,

mucosal irritation, runny nose, itchy eyes, and sensation of burning lungs in the majority of exposed populations (16). Dimitroglou et al. (2015) provides a comprehensive, systematic review of literature associated with potential health effects from exposure to CS (16).

Other studies were conducted to advance the forensic science and the ability to determine the use of CS in international warfare, a violation of the 1993 Chemical Warfare Convention (CWC) (42). The use of CS during wartime settings has created controversy over the years. The CWC recognizes that RCAs may be used in domestic law enforcement for riot control purposes (42). The US stance on using RCAs in wartime settings follows Executive Order 11850, signed by President Ford in 1975 and still in effect today. This order allows the US military to use RCAs in specific defensive military modes, such as the control of rioting prisoners of war, in situations where civilians are being used by the enemy as a screen, rescue missions, or to protect convoys from civil disturbances, terrorists, or paramilitary organizations (18).

In the US Armed Forces, the requirement for personnel to complete the Mask Confidence Training (MCT) exercise, either in basic training or during preparations for deployment, is common. In this event, soldiers, sailors, marines and airmen experience CS exposure with a goal of garnering trust in chemical warfare protective equipment issued to them for use in the event of chemical, biological, radiological, or nuclear attack. US Army soldiers, often attached to deployable units, are also required to complete this training annually and prior to deployment.

The Centers for Disease Control and Prevention (CDC) Division of Laboratory Science developed a method of quantification (Method Code ERB-2537) of 2-

chlorohippuric acid (CHA), the primary metabolite of CS in the body (8). This study will utilize Method ERB-2537 for quantification of CHA levels in exposed test subjects. This method successfully used solid phase extraction combined with high performance liquid chromatography (HPLC) and mass spectrometry (MS) to measure CHA and determined the lower level of detection (LOD). The LOD was determined by calculating the standard deviation at each standard concentration following repeated measurements of the four low concentration standards in urine (8). The CDCs method has been effective in animal testing, however, no accessibility to exposed groups in controlled situations as well as limitations on testing human subjects have resulted in the lack of a comprehensive analysis in human specimens. The CDC, in association with the Army Medical Department Center and School (AMEDD C&S), has solicited research assistance from the Uniformed Services University of the Health Science's (USUHS) Department of Preventive Medicine and Biostatistics in an attempt to advance the science in sensitive analytical methods for urinary metabolites of CS. Completing this study at AMEDD Basic Officers Leadership Course (BOLC) provides a target of opportunity in a controlled training environment that can be monitored throughout the MCT event and would be accessible for urine specimen collection in the days following exposure to provide to the CDC for subsequent analysis.

STUDY OVERVIEW

This observational, prospective cohort study sampled individual exposure to CS and measured urinary metabolite levels from a selected population of US Army personnel during regularly scheduled MCT events of the BOLC held at Joint Base San Antonio (Fort Sam Houston/Camp Bullis), TX. The overarching goal of this study was to assess the relationship

between CS exposure and CHA found in urine to test the following hypothesis: a statistically significant correlation exists between CHA metabolite level in urine and CS exposure concentration in an observational US Army training cohort.

The objectives of this study were to:

1) Determine CS exposure concentrations during US Army BOLC MCT exercise.

2) Determine CHA metabolite levels in urine of test subjects.

3) Assess the association between CS concentration and CHA metabolite concentration.

This study required sample analysis of volunteers enrolled in the three-day BOLC MCT event scheduled for 13-16 July 2015. At no time did the researchers attempt to alter the standing MCT exercise protocol. Regularly assigned BOLC active duty cadre and Department of Defense (DOD) civilian instructors implemented Army approved MCT procedures for completion of the event. Investigators of this study were present only to sample CS concentration from a fixed point inside the chamber, place sample pumps on each test subject, observe the MCT exercise, record out-of-mask and stay-times (time in chamber), and collect urine samples. Personally Identifiable Information (PII) from study volunteers was obtained to track subjects through the urine collection process and was destroyed upon completion of this study. The USU Office of Research deemed this study as testing on human research subjects and forwarded this study's protocol to the Institutional Review Board (IRB) for evaluation. The USU IRB approved this study on July 2, 2015 citing this study to be "No More Than Minimal Risk" human subjects' research and assigned protocol no. TO-87-3516. Funding for this project was awarded through the Henry M. Jackson Foundation for the Advancement of Military Research.

A sample size calculation determined that 85 personnel would be sufficient to find significant statistical results in this study. Test subjects provided a urine sample prior to CS exposure to establish urinary metabolic baselines and at three time intervals upon completion of the MCT event. If 85 subjects enrolled in the study, completed the MCT chamber exercise, and provided all four urine samples, a total of 340 specimens would have been submitted to the CDC for analysis.

The test subject's CS exposure concentrations were assessed using Occupational Safety and Health Administration (OSHA) modified National Institute of Occupational Safety and Health (NIOSH) Physical and Chemical Analytical Method (P&CAM) 304 with laboratory analysis completed by the US Navy Comprehensive Industrial Hygiene Laboratory (CHIL) in Norfolk, Virginia. Laboratory analysis of CHA metabolite and creatinine levels were completed by the CDC Division of Laboratory Sciences in Atlanta, Georgia using CS Metabolites CILA (method code: ERB-2537) and Enzymatic Urinary Creatinine Assay (method code: 1003).

Laboratory analysis for creatinine levels in urine samples was important for completion of urine creatinine corrections. Creatinine is the metabolite of creatine, a nitrogenous organic acid and metabolic intermediate that serves as a source of high energy in skeletal muscle and the brain (38). Creatine is produced in the body, can be consumed through foods such as fish and meat, or can be taken as a supplement produced in a laboratory. Biosynthesis of creatine produces the metabolite creatinine, which is excreted in the urine. Creatinine levels in the urine can be used to represent glomerular filtration rate as excretion occurs almost exclusively in the kidneys (13). Variation in renal efficiency is attributed to a variety of factors, such as hydration level and fluid balance, in test subjects

and could alter CHA metabolite levels after exposure to CS. Therefore, creatinine corrections are performed by dividing the concentration of analyte by the concentration of creatinine in the specimen. This provides a standardization between test subjects to account for variation in renal efficiency.

This project utilized IBM Statistical Package for the Social Sciences (SPSS) software and Microsoft Excel to analyze the data. Materials and methods are discussed in detail in Chapter 3.

APPLICATION

This research aimed to determine the association of CS exposure and CHA during US Army MCT exercises to provide future researchers data, statistical results, and interpretations to help advance the science in sensitive analytical methods and, possibly, the future development of a biomarker to investigate alleged exposures to CS. The public health significance is that contributions from this project to the development of biomarker for CS exposure could minimize the likelihood of an organization deploying CS in wartime operations.

The use of human subjects as research volunteers is imperative to provide the CDC a large sample pool for validating the method for sensitive analysis of CHA levels from CS. A validated urinary biomarker could assist medical, occupational health, emergency response, forensic science, and law enforcement professionals in better performing their duties in CS exposure cases.

CHAPTER 2: Literature Review

O-CHLOROBENZYLIDENE MALONONITRILE

Background

CS was first synthesized by chemists Ben Corson and Roger Stoughton at Middlebury College while working with the RCA bromobenzylcyanide (CA) in the 1920's (30). It was not until after World War II, however, that CS saw much use in riot control events or other law enforcement situations. The molecular formula for CS is $C_{10}H_5ClN_2$ and has a molecular mass of 188.6 g/mol. At standard conditions, CS is a solid, appearing as a white crystalline power with a melting point of 93°C and a boiling point of 310°C. It has a pepper-like odor, is insoluble in water, and converts into a vapor and particulates when burned (26).

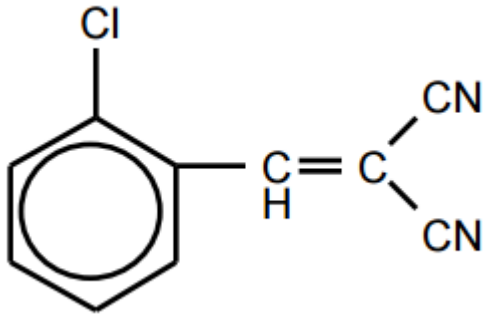

Figure 1. Molecular structure of CS (41)

CS is considered a lachrymatory agent or lacrimator (derived from the Latin word "lacrima" which translates to the English word "tear") that is widely referred to as "tear gas" and is the most commonly used RCA worldwide (28). CS became the RCA of

choice due to its rapid time of onset of effects (seconds to several minutes), a relatively brief duration of effects (15-30 min) once the victim has escaped the contaminated atmosphere, and a high safety ratio (the ratio of the lethal dose [estimated] to the effective dose) compared to its predecessor chloroacetophenone (CN) (41). The irritancy threshold for CS is 0.004 mg/m³ (19). This is also the point at which symptomatic health effects can be sensed, beginning with itchy, watery eyes, and a stinging sensation in the mucous membranes. The intolerable concentration is estimated to be 3.6 mg/m³ based on a study of exposure to military trainees (6). The minimal lethal concentration to humans is estimated to be 2,500 mg/m³ based on animal studies, a concentration many times higher than the estimated incapacitating level (30).

Toxicology

The toxicity for CS is generally regarded as low. CS is sparingly soluble in water and will absorb into most porous surfaces (30). In its raw form at room temperature, the crystalline powder form of CS is often packaged in pill-sized capsules for ease of handling. For the purposes of the Army's MCT exercise, CS capsules are heated on either a hot plate or on a combination of coffee can over a candle or, more commonly, "canned heat" (jellied alcohol fuel in an aluminum can) such as a Sterno® used in heating chaffing dishes (3). Heating releases CS in to the MCT chamber in vapor form, creating a CS rich atmosphere which condenses to form an aerosol, a colloidal suspension of particulates in air. Trainees exposed to CS intake the substance through the dermal route of exposure as well as through inhalation during the mask removal portion of the exercise.

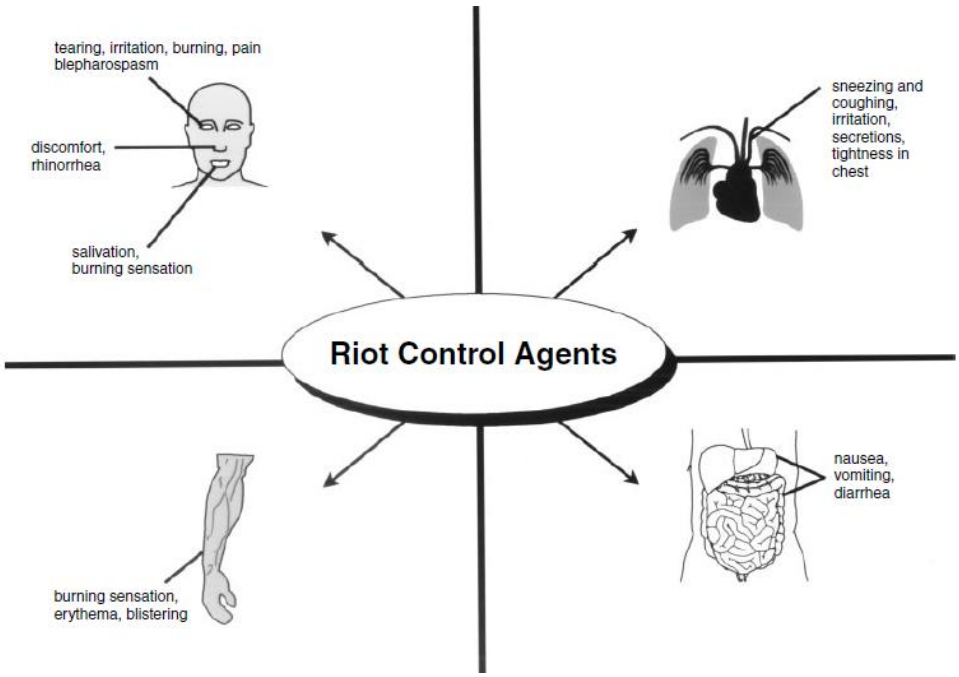

Figure 2. Acute site-specific toxicity of riot control agents (30)

CS is also absorbed through the eyes, which causes an immediate itching and stinging sensation as well as blepharospasms (uncontrollable blinking). Animal studies and observations in exposure human groups provide no evidence for ocular injury involving the cornea (30). Exposure to the powder, vapor, or aerosol form of CS results in some dermal absorption with possible erythema, vesiculation, and skin lesions at high concentration levels (30). Typically, trainees are not exposed to CS during the MCT exercise for more than a few minutes and may not experience persistent erythema or skin lesions. CS vapor especially targets soft mucous membranes and moist areas of the body.

Aerosolized CS is absorbed primarily through the respiratory tract then distributed throughout the body via the blood. Biotransformation occurs mostly in the blood and to a small extent, in the liver. CS can be swallowed inadvertently or in a few cases, large amounts were purposefully ingested in suicide attempts. Health effects associated with

CS ingestion have been reported to be abdominal cramping, diarrhea, and vomiting, however, these symptoms could have also been attributed to medical treatment. No deaths have been reported to have occurred from ingestion of solid form of CS. Evidence from animal studies have shown that at lethal concentrations, inhalation of CS causes damage to lungs, leading to death from asphyxiation or failure of the circulatory system (30). Past research has suggested that mortality in CS-caused animal deaths were attributed to metabolic production of cyanide, a result of CS hydrolysis to malononitrile. However, post mortem examination shows lung damage was adequate to cause death and, in addition, the time of death was not consistent with cyanide poisoning (41).

CS is metabolized primarily in the blood and predominantly excreted in urine at a rate of 82-95% within 96 hours of exposure (8). Results from exposure studies to rodent species determined that CS is metabolized to 2-chlorobenzyl malononitrile (CSH2) and 2-chlorobenzaldehyde (*o*CB) (30). Further bioconversion through glycine conjugation or reduction yielded 2-chlorobenzyl alcohol and 2-chlorobenzyl acetyl cysteine or 1-o-2-chlorobenzyl glucuronic acid (30). Finally, the principal urinary metabolites of CS were found to be 2-chlorohippuric acid, glucuronic acid, 2-chlorobenzyl cysteine, and 2-chlorobenzonic acid (30). Findings from animal studies indicate that the majority of the administered CS dose is eliminated through urine. Elimination of CS follows first-order kinetics as rate of enzyme reaction is proportional to the concentration of CS absorbed in the body (19).

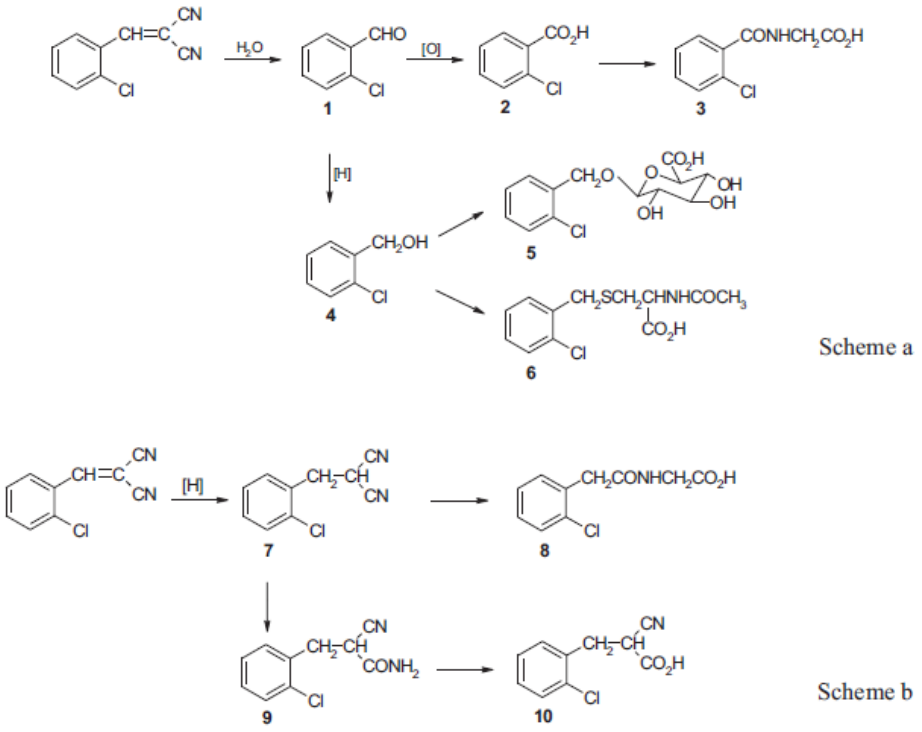

Figure 3. Major and minor metabolic pathway of CS in the body (37)

1) 2-chlorobenzaldehyde
2) 2-chlorobenzoic acid
3) 2-chlorohippuric acid
4) 2-chlorobenzyl alcohol
5) glucuronide
6) N-acetylcystenine conjugate
7) dihydro-CS
8) glycine conjugate
9) carboxamide
10) carboxylic acid

Exposure Limits

The American Conference of Governmental Industrial Hygienists (ACGIH) develops and publishes exposure limit guidelines based on scientific research in the form of Threshold Limit Values (TLVs). These values are intended for use in the practice of industrial hygiene to assist in the control of workplace health hazards (2). They are not intended to be used as legal standards, however, ACGIH recognizes that some local, state or federal agencies may implement them into occupational safety and health programs. The TLV Time Weighted Average (TWA) of a chemical substance is the maximum average airborne concentration that a healthy adult can be exposed to working 8 hours per

day, 40 hours per week over a lifetime without experiencing significant adverse health effects (2). The TLV-ceiling (C) value is the concentration of a hazardous substance in air the ACGIH recommends should not be exceeded at any time during the workday. The ACGIH TLV-C[skin] for CS is 0.39 mg/m³. OSHA, a branch of the US Department of Labor, in collaboration with NIOSH, a branch of the US Department of Health and Human Services, release Permissible Exposure Limits (PEL) and Recommended Exposure Limits (REL) for exposure to CS respectively. The current OSHA PEL is 0.4 mg/m³ as an 8-hour time weighted average concentration (36). This limit legally mandates an employer to ensure their worker's average airborne exposure to CS in any 8-hour workshift of a 40-hour workweek is not exceeded. Concurrently, the NIOSH REL for exposure to CS is also 0.39 mg/m³ (36). This value, based on best available human and/or animal health effect data, is a maximum recommended exposure from NIOSH to employers to maintain a safe and healthy working environment for all employees.

ACGIH TLV-C and NIOSH REL include skin notations in their exposure limits for CS. Skin notations are included to signify that a potential significant contribution of overall exposure is by the cutaneous route, including mucous membranes and eyes, from airborne exposure to gases, vapor, or liquid or by direct skin contact. In addition, dermal application studies show significant absorption or systemic effects and acute animal toxicity studies show low dermal lethal dose 50 (LD_{50}) < 1000mg/kg (24).

As defined by OSHA and NIOSH, the Immediately Dangerous to Life or Health (IDLH) value for exposure to CS is 2.0 mg/m³ (11). This limit was based on a 1961 US Army report of a study of a 2-minute CS exposure to 15 human volunteers at concentrations between 2 and 10 mg/m³ (11). Six of the 15 subjects reported this range to

be “intolerable” (11). IDLH levels are set to values of toxic substances that would be likely to cause death or immediate or delayed permanent adverse health effects or prevent escape from such environment (27). OSHA defines IDLH as “an atmosphere that poses an immediate threat to life, would cause irreversible adverse health effects, or would impair an individual’s ability to escape from a dangerous atmosphere” (33). The US Army has determined that the more stringent OSHA or ACGIH TLVs shall apply to its occupational environments. In the absence of limits set forth by OSHA and ACGIH, the US Army applies OEL guidance including American Industrial Hygiene Association (AIHA) Workplace Emergency Exposure Levels (WEEL) and NIOSH RELs (4).

Sampling CS

This study will utilize OSHA protocol for sampling and quantification of CS exposure. In 1979, the CDC published method number P&CAM 304, developed by NIOSH’s Measurements Research Branch as an analytical method for sampling and quantification of airborne o-chlorobenzylidene malononitrile. This method prescribed use of filter/sorbent collection and extraction with 20% methylene chloride in hexane using HPLC (9). The method was calibrated to a range of 0.147 – 0.82 mg/m^3 at a precision of 0.102. The method also called for use of a polytetrafluoroethylene membrane filter followed by a Tenax-GC sorbent tube to gather vapors and particulates present in the sample environment. In order to draw the sample into the filter and sorbent tube, this method recommended use of a common industrial hygiene sampling pump with an accuracy of ±5%, drawing 90 liters of air at recommended flowrate of 1.5 liters per minute (9). P&CAM 304 is the operational basis for CS sampling and quantification used by US Army industrial hygiene workers and US Navy industrial hygiene laboratories.

In recent years, OSHA updated recommendations in monitoring methods for this particular compound. Specifically, Primary Laboratory Sampling/Analytical Method (SLC1) modified NIOSH P&CAM 304 with the use of a new sampling media (31). This update prescribed the use of the OSHA Versatile Sampler (OVS-Tenax), a 13 mm tube with two sorbent layers and enclosed glass fiber filter. This sampler provides collection of vapor and particulate in one tube, making it easier for technicians to handle the sampling media within the sampling train (apparatus of personal sampling pump, sample tubing, and sample media).

Figure 4. OSHA Versatile Sampler (OVS-Tenax) (22)

Quantification of CS Concentration

In 2008, the Navy Comprehensive Industrial Hygiene Laboratory (CIHL) approved the internal use of a modification to the quantitative analysis method for CS concentrations. In their protocol (document number: GC-55), NIOSH P&CAM 304 was

modified with alternative desorption and chromatography techniques (12). Desorption of CS within the sample media using 20% methylene chloride/hexane in P&CAM 304 was substituted with toluene in the GC-55 method and high pressure liquid chromatography in P&CAM 304 was substituted with gas chromatography (GC) combined with an electron capture detector (ECD) (12).

PREVIOUS MCT SAMPLING STUDY

Hout, et al. (2013) quantified CS exposures of over 6,000 trainees and seven chamber instructors during US Army basic combat training at Fort Jackson, South Carolina in August-September of 2012. The exposure assessment was determined by taking area samples from fixed locations to represent exposure to multiple trainees (21). This study also implemented personal sampling equipment on the seven chamber instructors. Results indicated that CS concentrations in the chamber ranged from 0.4 to 53.3 mg/m³ (mean = 10.4 mg/m³). Analysis concluded that trainees received an average of 9.9 mg/m³ with exposure durations ranging from 5.0 – 15.0 minutes. 98% of trainee's exposure exceeded CS IDLH level (2.0 mg/m³). All trainees' exposure exceeded the ACGIH TLV-C[skin] (0.39 mg/m³). 11% of trainees were exposed to levels exceeding OSHA PEL (0.4 mg/m³). Analysis of personal air sampling determined that chamber instructors received an average of 10.3 mg/m³ (longer duration in chamber but outfitted with Mission Oriented Protective Posture (MOPP) level 4 equipment). All instructors were exposed to levels exceeding both IDLH and ACGIH TLV-C[skin] while 32 of 33 samples exceeded the OSHA PEL (21). This study concluded that it is plausible that CS concentrations experienced during basic training may have caused damage to respiratory epithelium and increased risk of acute respiratory infections by 2.44. It also found that CS

distributes even in the MCT chamber, which allows for estimation of individual exposure by sampling from a general area or fixed point inside the chamber (21).

Based on the results, All Army Activities (ALARACT) message 051/2013 was released as an attempt to minimize exposure at MCT events Army-wide (29). It lowered the number of CS capsules used to establish initial concentration, reduced out-of-mask times to a maximum of 15 seconds, mandated semiannual industrial hygiene surveys of all MCT chambers, and called for periodic wet cleaning of MCT chambers (29). A follow-up study completed by Hout et al. (2014) evaluated the ALARACT 051/2013 intervention to reduce tear gas exposures and associated acute respiratory illnesses in a US Army basic combat training cohort. The data indicated a ten-fold reduction ($p<0.01$) in CS exposure concentrations (21). This study revealed a successful decrease in the percentage of trainees and chamber instructors exposed to CS concentrations greater than IDLH levels and a reduction in ARI rates from 2.44 to 1.79. None of the trainees or instructors during this follow-up study were exposed to CS levels exceeding 8-hour OSHA PEL (21).

PREVIOUS BIOMARKER STUDY

The Journal of Chromatography B published a study completed by Riches, et al. in 2012 in the United Kingdom that attempted to develop an analytical method for urinary metabolites of CS riot control agent. The researchers understood the importance of developing an analytical method for retrospective identification of exposure as evidence in cases of alleged use of chemical warfare agents (CWA) on the battlefield (37). The Riches et al. study evaluated the analysis method for two suspected urinary metabolites of CS riot control agent: 2-chlorohippuric acid and 2-chlorobenzyl-N-

acetylcysteine (37). Lab analysis utilized liquid chromatography/mass spectrometry (LC/MS) to identify 2-chlorophippuric acid (CHA) in all two-hour post-exposure samples from a set of urine samples taken from army recruits exposed to thermally dispersed CS. The metabolite 2-chlorobenzyl-N-acetylcysteine was not found in any of the urine samples (37).

The study determined the lower limit of detection (LOD) to be 1.0 ng/ml and detected CHA in 89% of the samples 20 hours after exposure. Results from analysis of the urine samples revealed a CHA concentration range from 3 – 135 ng/ml with a mean of 29 ng/ml and a median of 12 ng/ml (n=19) (37). Objectives of this study focused more on development of an analytical method of CHA metabolites than validation of a biomarker. This study did not include active or passive CS air particulate or vapor sampling and analysis nor correlation of personal CS exposure to CHA concentration in urine samples. This study also had a particularly small sample size (n=19). During the study, the concentration in the chamber was not controlled and there was no attempt to sample the atmosphere. CS exposure was estimated based on the chamber volume at 55 m^3 to be between 5 and 15 mg/m^3 (37). The details of how this range of concentration was estimated was not presented in the article.

CHAPTER 3: Methodology

RESEARCH GOAL

The goal of this study was to evaluate the association between CHA metabolite and personal exposure to CS riot control agent in US Army soldiers during the MCT exercise at the BOLC in Fort Sam Houston, TX. This study was performed in collaboration with a separate study being completed by a USU graduate student researcher that further followed this cohort to investigate CS exposure and subsequent acute respiratory outcomes (P.I - CPT M. Holuta, USA. Protocol number: TO-87-3564). Both studies utilized the same sampling and exposure assessment methodology and shared exposure concentration results.

There have been numerous studies researching CS exposure concentrations, acute and chronic health effects, MCT training protocol, and urinary metabolite analysis. Unlike previous studies, however, this research project intends to obtain a pre-exposure CHA metabolite baseline, measure individual CS exposure concentration through personal air sampling, analyze post-exposure urinary metabolite levels, and perform data analysis to find the significance of this association. A better understanding of the relationship between exposure and excreted metabolite would assist medical, occupational health, emergency response, forensic science, and law enforcement professionals to more effectively perform their duties in CS exposure cases.

HYPOTHESES

This research will test the following hypotheses:

1) Personal CS exposures exceed ACGIH TLV-C[skin] during MCT post ALARACT 051/2013 implementation
2) Personal CS exposures exceed NIOSH IDLH during MCT using post ALARACT 051/2013 implementation
3) A statistically significant relationship exists between exposure to CS and concentration of CHA biomarker metabolite excreted in urine after US Army MCT exercises

RESEARCH OBJECTIVES

1) Determine CS exposure concentrations during US Army BOLC MCT exercises.

2) Determine CHA metabolite levels in urine of test subjects.

3) Explore the association between CS concentration and CHA metabolite

SPECIFIC AIMS

1) Sample for CS vapor and particulate concentration inside MCT chamber using personal sampling pumps and fix-point general area sampling apparatus.

2) Sample and quantify individual CS exposure levels for MCT trainees.

3) Obtain urine samples from subjects prior to exposure for baseline analysis of CHA.

4) Obtain urine samples from subjects at intervals of 2, 8, and 24 hours post-exposure.

5) Quantify CHA metabolite levels at pre-exposure and post-exposure intervals.

6) Correct CHA metabolite levels for creatinine (an indicator of renal efficiency) using a mathematical equation to reduce variability in urine output.

7) Compare CHA metabolite levels of this study cohort to that of a randomly selected convenience sample population with no known CS exposure

8) Determine association between CS exposure concentration and CHA metabolite levels in this US Army trainee cohort.

STUDY POPULATION

The population for this study was a male and female cohort of US Army trainees enrolled at the AMEDD BOLC titled *HPSP-Basic Officer Leader* (course no. 6-8-C20B). This course included 486 students attending various medical education institutions throughout the country under the Health Professional Scholarship Program (HSPS) as well as 60 students enrolled in medical programs at USU. This iteration of BOLC was scheduled from 12 June – 25 July 2015, with the MCT portion scheduled for 14-17 July during the two-week field phase at Camp Bullis, TX. BOLC staff divided trainees into one of four companies, A through D, with approximately 140 students per company. Researchers solicited volunteers for this study from both groups at the same time, one day before the MCT exercises began.

Utilizing techniques from Designing Clinical Research, 3rd edition by Hulley and Cummings and Biostatistics, 8th edition by Daniels, sample size testing estimated that enrollment of 85 volunteers in this study would provide 80% power to detect correlation of 0.3 or greater at $\alpha = 0.05$ level of significance. This would allow the estimation of a mean with a margin of error of 0.2 standard deviations based on a 95% confidence interval. Calculations also revealed that as few as 50 volunteers would provide significant results. Solicitation efforts attempted to enroll 120 volunteers in the study to allow the removal of a small number of subjects from the study who dropped on request, experienced an adverse event, failed to complete the MCT exercise, or were lost during follow-up.

During the solicitation and enrollment period on 13 July, volunteers were given further information on the study and signed an IRB approved consent form (Appendix C). Researchers also asked volunteers to complete a pre-exposure questionnaire, which asked for current health status and basic demographic information (Appendix D). Upon receiving consent, test subjects were issued a "unique study identification (ID) number" which was free of any PII. Researchers affixed this ID number to each volunteer's uniform prior to entering the MCT chamber and used it to track and record chamber stay-times, out-of-mask times, and urine specimen collection. After the enrollment period, 91 test subjects volunteered for this study, signed a consent form and completed the pre-exposure survey for demographics and current health status.

BASE AND CHAMBER CHARACTERISTICS

Camp Bullis provides over 27,000 acres of base operations support and training support to Joint Base San Antonio mission partners in order to sustain their operational and institutional training requirements. The camp also offers the armed services state-of-the-art training facilities including firing ranges, simulation facilities, maneuvering lands, and other training support services. For the two-week field phase of BOLC, trainees inhabited a cordoned off camp area which was used as a simulation for overseas operations in a forward operating base (FOB). The FOB is a six-acre, fenced-in compound, outfitted with tents for sleeping, training shelters, modular office space, portable toilets and showers, a tent with a gym, and mobile trailers housing BOLC instructors and staff.

The BOLC MCT chamber is a stand-alone, painted cinder block structure in a remote area of Camp Bullis, approximately two miles away from the FOB. A picture of

the chamber is provided in Figure 5. The area adjacent to the chamber has outdoor bleachers covered with a canopy and portable toilets for staff and student use during the MCT event. The chamber dimensions are 15 ft x 10 ft x 11 ft with a total volume of 1,650 ft^3 (46.72 m^3). The chamber has one entrance and one exit on opposite sides of the structure. There is a ventilation fan vent (approx. 1 ft x 1 ft in size) located just under the peak of the roof at one end of the structure, however, the associated fan was not operational during the MCT event and airflow through the vent was negligible. The interior of the structure is a bare concrete floor and painted cinder block walls. There is no furniture or other items in the chamber other than one folding chair used by the MCT instructor, a fire extinguisher, and an improvised CS generator. The CS generator consisted of a combination of tin can over "canned heat" (jellied alcohol fuel in a can) such as a Sterno® used in heating chaffing dishes (Figure 6).

Figure 5. Camp Bullis MCT chamber

Figure 6. MCT CS heating operation

MCT TRAINING

The MCT exercise for BOLC trainees was conducted in accordance with US Army guidance and locally generated operational orders. This event was intended to allow participants the opportunity to have a hands-on experience donning and doffing the M40 full-face chemical protective mask, as well as to experience the mask's reliability in a hazardous atmosphere. Entering the CS-rich chamber provided the trainee immediate warning of mask leaks. This experience was designed to allow the student to gain trust and confidence in his/her chemical protective gear. For the MCT event, trainees wore their general issue Army Combat Uniform (ACU) with addition of the M40 mask and the C2A1 filter canister. Participants were not issued any chemical protective garments. This resulted in completion of the exercises with skin exposed at the wrist, hands, neck, and head.

Instructors divided trainees into seven or eight exposure groups per day over the three days of the BOLC MCT with no more than twenty trainees per group. Students

were provided an extensive training brief and assembled in the staging area in two rows of ten trainees each. Exposure groups were instructed to don their M40 masks and enter the chamber as a unit. Once inside, the two rows were diverted to the left and right of the entrance and stopped once all trainees in the group were inside of the chamber. This progression created a circle-like formation of trainees, with the two chamber instructors in the middle of the circle. One instructor, seated in the lone chair in center of the room, was responsible for heating CS capsules on the overturned coffee can to create a CS-rich atmosphere. A chamber diagram with trainees, instructors, CS generation, and fixed-sampling apparatus is included in Figure 7.

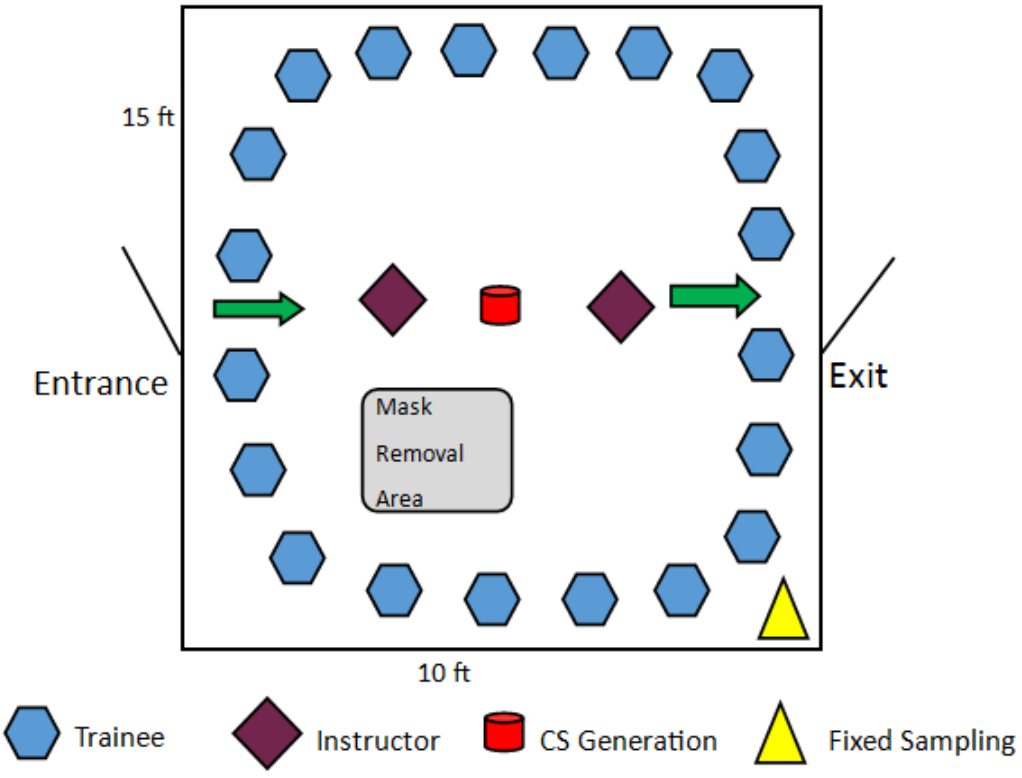

Figure 7. BOLC MCT chamber layout.

Upon heating, the CS was converted from a powder to vapor and particulates, which ascended in to the chamber atmosphere. Periodically, the instructor wafted the CS plume toward each side of the room in an attempt to more evenly distribute CS. Meanwhile, the second chamber instructor led trainees through a series of movements and exercises to test the fit of their M40 mask. If any trainee experienced respiratory effects of CS from an improper seal or defective mask, they exited the chamber immediately on their own accord or were instructed to exit the chamber, thus concluding the individual's MCT exercise without repercussion or reprocessing. To complete the exercise as prescribed, MCT instructors directed trainees (typically in groups of two) to remove masks, state their names and ranks, and provide a random identification number. Once completed with this task, instructors motioned for the pair of trainees to exit the chamber, concluding their event.

EXPOSURE ASSESSMENT

Exposure assessment was conducted in accordance with OSHA modified NIOSH P&CAM 304. A single fixed-point sampling train for general area concentration and a sampling train for personal monitoring characterized exposure during the BOLC MCT exercises. Both techniques are considered active sampling as they are means of collecting an airborne substance that employs a mechanical device such as an air sampling pump to draw the air/contaminant mixture into or through the sampling media (5). This study utilized the OVS, described extensively in Chapter 2. The OVS tube combines a particulate filter with vapor sorbent media within one device for easier handling and lab analysis. For fixed-point sampling, two sampling trains were fastened to one tripod at a height of 1.32 m and a distance of 3.15 m from the source and placed in a corner of the

room as to not disrupt the training event. One of the fixed-point sampling trains was designated as “long-area” sample and the other was designated as “short-area” sample. At the start of the MCT training each day, the long-area sample OVS was uncapped and, within seconds, pump was activated and was allowed to run for the entire event. The short-area samples included an OVS for each exposure group that entered the chamber. OVS for the short-area samples were changed each time a new exposure group entered the chamber (except for the last day due to exhaustion of OVS tubes). Long and short-area samples were obtained for backup and comparison to personal monitoring samples.

Each study volunteer was equipped with a sampling train while staged at the entrance to the chamber with their respective exposure group, here on referred to as a similar exposure group (SEG). Researchers assigned each SEG a two digit number (XY), X representing the day of their event (1, 2, or 3) and Y representing the number of the group that completed the event chronologically (1 - 8). (For example, if a test subject was assigned SEG 23, he/she completed the event on the second day and was in the third group to enter the chamber that day). Personal sampling trains consisted of a waist mounted AirCheck pump (XR5000 or 224-44XR, SKC Inc.) calibrated to 1.5 liters per minute (L/min), 1 meter of ¼ inch Tygon® sample tubing, and the OVS media clipped within 6-8 inches of the individual’s breathing zone. On the morning of the MCT, technicians started all sampling pumps, allowed them to run for a ten-minute warm-up period, and calibrated them using a BIOS Defender Drycal. When the MCT began, technicians activated pumps and uncapped OVS tubes within approximately 10-15 seconds preceding each SEG’s entry into the MCT chamber. As test subjects completed

the event, technicians deactivated pumps within 10-15 seconds, capped and individually packaged OVS tubes, and verified pump flowrates using the aforementioned calibrator.

The chamber exposure assessment phase of this study took three days and required a team eight individuals to complete the evolution; two researchers and six industrial hygiene technicians. Two individuals served as pump calibrators, one as a sampling train assembler, two as sampling train outfitters, one as a recorder inside the chamber, one as a recorder outside the chamber, and one as a pump deactivator near the exit of the chamber. Upon completion of the MCT exercise, researchers capped, labeled, individually packaged, and shipped all sample media to the CIHL in Norfolk, VA. Nine field blanks and six media blanks were included in the shipment.

Laboratory equipment and processes were calibrated in advance in preparation for laboratory analysis. Recovery analysis and creation of a calibration curve using MS/ECD was completed at the CIHL on 20 May 2015 using 5 grams of CS (CAS: 2698-41-1) obtained from Santa Cruz Biotechnology, INC. Recovery analysis concluded that an average of 92% of the CS was desorbed from the OVS media. Calibration found a correlation of 0.99934 (R^2) and a curve equation of $y = 198487.45213x - 5012.47625$ (figure 8).

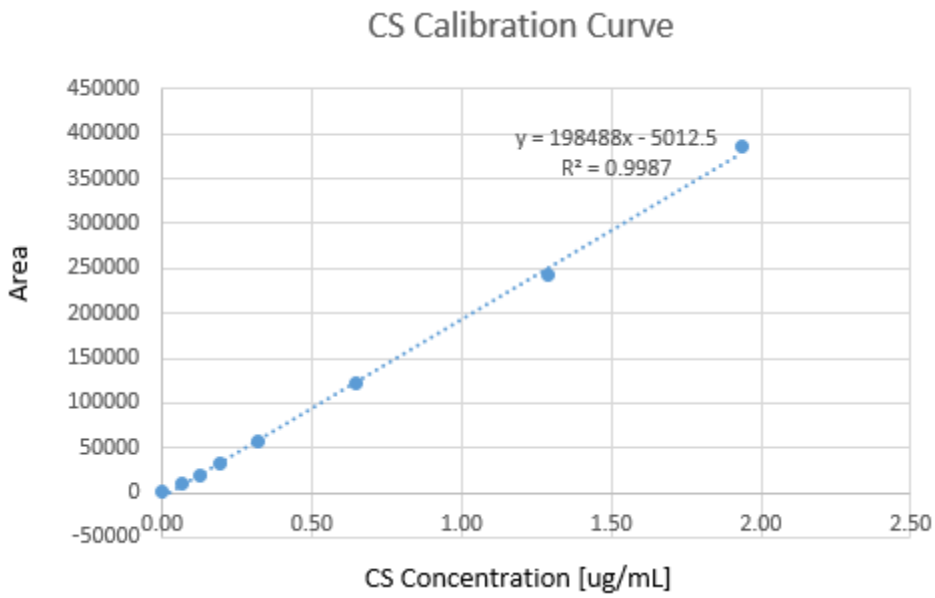

Figure 8. MS/ECD calibration curve for CS analysis

URINE COLLECTION

The CDC provided guidance for urine specimen collection, packaging, and shipment in accordance with *Shipping Instructions for Specimens Collected from People Who May Have Been Exposed to Chemical-Terrorism Agents* (10). Urine collection for study volunteers was completed in four phases: pre-exposure (within 24 hours of their scheduled MCT exercise) and at time intervals of approximately 2, 8, and 24 hours post-exposure. These times were used only as guidelines, as participants were not encouraged to hold or provide urine if they were not naturally ready to do so. Subjects were given a 50 mL urine bottle and asked to provide at least 25 mL of urine without supervision in the designated portable toilet facility located on the FOB or near the MCT chamber (for the two-hour samples). Once urine specimens were received, researchers recorded sample times, labeled bottles with study ID numbers and time, and placed bottles in large shipping coolers with 10-15 lbs of dry ice. All samples froze within two hours of

excretion and all specimens were subsequently shipped to the CDC for laboratory analysis.

URINALYSIS

All urine specimens were analyzed by the CDC Division of Laboratory Sciences in Atlanta, GA. The CDC utilized their recently developed procedures for quantification of o-chlorohippuric acid, CLIA protocol 2537 CS Metabolites (8). This method employed LC/MS/MS for analyte separation and detection. General procedures included the following: technicians began by adding 25 µL of CHA internal standard, 100 µL of urine, and 100 µL of formic acid to a 96-well Nunc plate. The Nunc plate was centrifuged to collect all liquid then vortexed to ensure mixing of all components. Samples were then placed in a Turbovap® concentration evaporator, dried down, and reconstituted with methanol/water in the sample plate. Once sealed with foil, samples were ran through LC, detector, and values were displayed on computer software chromatograms (8).

The CDC laboratories also completed quantification of creatinine levels in all urine specimens submitted for analysis of CHA. The CDC utilized Creatinine Plus testing procedures, an assay for the quantitative determination of creatinine in human urine on a Roche automated clinical chemistry analyzer. This method was based on the conversion of creatinine with the aid of creatininase, creatinase, and sarcosine oxidase to glycine, formaldehyde and hydrogen peroxide. The liberated hydrogen peroxide reacted with aminophenazone to form a quinon imine chromogen, whose color intensity was directly proportional to the creatine concentration in the reaction mixture (1).

STATISTICAL ANALYSIS

All statistical analyses were performed using IBM SPSS software using a two-tailed significance level of 0.05. The correlation among exposure categories and CHA was tested using Pearson and Spearman coefficients. Because of the highly skewed distributions of CHA, the following analysis was carried out using natural logarithmic transformation. Multiple regression was conducted to evaluate the impact of gender, BMI and age on CHA levels after adjusting for CS exposure and time sampled. Due to potential lack of independence among repeated measurements on the same subjects, a mixed-model was used to fit separate slopes and intercepts over time for each subject. This model showed no within-subject correlation, therefore, multiple linear regression was sufficient for all statistical analyses.

CHAPTER 4: Results

GENERAL RESULTS

Of the 91 test subjects who enrolled in the study and signed a consent form, 87 subjects completed the MCT exercise and provided at least one post-exposure urine specimen. The gender distribution of volunteers who completed this study was 39 male and 48 female. The sample as a whole was relatively young with a non-normal distribution range of 20 to 47 years of age ($\bar{x}$=26.4 years). The Body Mass Index (BMI) of this cohort of US Army trainees ranged from 18.24 – 32.69 kg/m² ($\bar{x}$=24.37 kg/m²). None of the study subjects identified themselves as a current smoker. A demographic summary is provided in Table 1. Chamber stay-times for study participants (n=87) ranged from 23 – 441 s (0.38 – 7.35 min) ($\bar{x}$=340.5 s (5.68 min), 95% CI [332, 349]). Four of the 87 test subjects left the chamber before the 200 s mark due to apparent mask seal leaks. Subject out-of-mask times ranged from 4 – 19 s ($\bar{x}$=8 s; 95% CI [8, 9]).

Table 1. Demographics of Study Sample

	Mean ± SD	Number	Percent
Total Enrolled		91	
Completed Study		87	95.6
Age (years)	26.4 ± 5.22		
20-22		8	8.8
23-25		48	52.7
26-30		19	20.9
31-35		4	4.4
35-39		5	5.5
40-49		3	3.3
Gender			
Male		39	42.9
Female		48	52.7
BMI	24.37 ± 2.82		
< 18.5		1	1.1
18.5-24.9		50	54.9
25.0-29.9		32	35.2
>30.0		4	4.4
Male	25.51 ± 2.90		
Female	23.44 ± 2.41		
Smokers		0	

Exposure Assessment and CS Concentration Results

CS exposure assessment utilized two methods for sampling: general (fixed) area monitoring and personal monitoring. Both methods utilized OVS tubes and sampling pumps set at 1.5 L/min. Fixed-area samples were further segregated into long-area samples and short-area samples. Long-area samples were drawn from one OVS tube at a fixed location in the corner of the chamber, activated to sample over the entire duration of a day's MCT exercise (all 7-8 SEGs for that day). Short-area samples were drawn from one OVS tube at the same fixed location which was replaced for each SEG (except for day three exercises due to exhaustion of OVS tubes). Short and long-area sample results

were not included in the statistical analysis for this project. They were taken as a back-up to personal monitoring and used only as a reference for comparison.

All OVS tubes were analyzed by the CIHL in Norfolk, VA. The laboratory utilized protocol Document no. GC-55: Analysis of o-Chlorobenzylidene Malononitrile (OCBM). CS was desorbed from the OVS filter and sorbent layer using Toluene and sent through a HP-1 5m x 530µm x 2.65µm film thickness separation column. The analyte then entered the electron capture detector and peak areas were displayed on a chromatogram for quantification. An example chromatogram of CS concentration is included in Figure 9, recorded during standardization and development of the calibration curve.

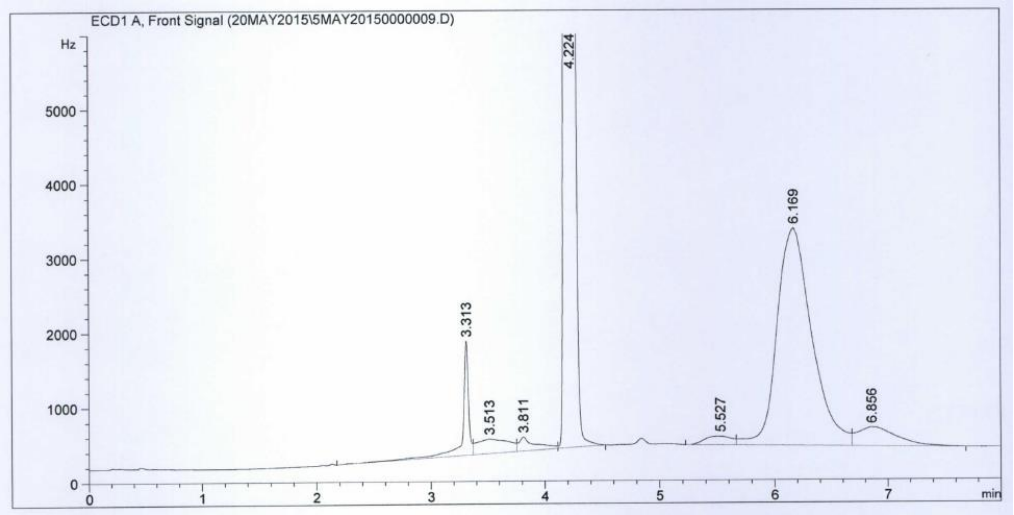

Figure 9. Example chromatogram of CS quantification utilizing GC/ECD

Lab analysis calculated CS concentrations for the long-area sample of each of the three day sampling events: MCT Day 1 – 2.088 mg/m³, Day 2 – 3.617 mg/m³, and Day 3 – 5.209 mg/m³. Short-area samples for CS concentration exposure to SEGs 11-27 (Day 1 and 2) (n=14) ranged from 1.104 – 4.773 mg/m³ ($\bar{x}$=2.779 mg/m³). The Shapiro-

Wilk test indicated that short-area sample data were normally distributed (p=0.074). Table 2 provides a summary of long and short-area samples and, for comparison, personal monitoring SEG means.

Table 2. CS Concentration Exposure Assessment

Day of MCT	Similar Exposure Group (SEG)	Long-area CS Concentration (mg/m^3)	Short-area CS Concentration (mg/m^3)	Short-area Mean (mg/m^3)	Personal Monitoring (Mean of SEG, mg/m^3)	Mean of SEG Means (mg/m^3)
1	11	2.088	1.104	1.886	1.060	1.757
	12		1.536		1.655	
	13		2.137		2.181	
	14		2.132		2.039	
	15		2.237		1.773	
	16		2.052		1.809	
	17		2.003		1.783	
2	21	3.617	2.651	3.671	3.366	2.875
	22		4.773		3.058	
	23		1.897		1.884	
	24		3.786		2.842	
	25		3.445		3.387	
	26		4.773		2.315	
	27		4.373		3.275	
3	31	5.209	--	--	2.193	3.536
	32		--		3.781	
	33		--		4.277	
	34		--		3.28	
	35		--		3.792	
	36		--		3.486	
	37		--		4.653	
	38		--		2.886	

-- Samples not taken due to exhaustion of OVS tubes

Concentration calculations for individual CS exposure from personal monitoring were based on total mass of CS desorbed from OVS tube (µg) divided by the total air volume (m^3) sampled during an individual's time in the chamber (min). Total sampling time was measured from the time the subject entered the chamber to the time they exited

the chamber. This project assumed that CS concentration during lead and lag times from starting/stopping pumps during time entering/exiting chamber and deactivating pumps was negligible due to the subject being outside, in an open-air atmosphere. Total air volume (m^3) sampled was calculated by multiplying total sampling time (min) by the average flow rate (L/min) of pre and post-exposure pump flowrate readings.

Personal monitoring results for CS concentration to subjects from Day 1 MCT exercises (n=25) ranged from 0.960 – 2.463 mg/m^3 ($\bar{x}$=1.763 mg/m^3). The Shapiro-Wilk test indicated that the data were normally distributed (p=0.383) and allowed for parametric analysis. CS concentrations from Day 2 MCT exercises (n=36) ranged from 0.086 – 3.792 mg/m^3 ($\bar{x}$=2.833 mg/m^3). The Shapiro-Wilk test indicated that the data were not normally distributed (p<0.01) and required non-parametric analysis. CS Concentrations from Day 3 MCT exercises (n=26) ranged from 1.953 – 4.900 mg/m^3 ($\bar{x}$=3.553 mg/m^3). The Shapiro-Wilk test indicated that the data were normally distributed (p=0.121) and allowed for parametric analysis. The total of CS concentrations from all three MCT exercise days (n=87) ranged from 0.086 – 4.900 mg/m^3 ($\bar{x}$=2.741 mg/m^3, 95% CI [2.66, 2.87]); the Shapiro-Wilk test indicated that all personal monitoring data were normally distributed (p=0.403) and allowed for parametric analysis. Due to the normality of the CS exposure data overall, logarithmic conversion of CS concentrations was not necessary for statistical analysis. Personal monitoring results in the form of box and whisker plots over the three-day exercise period is provided in Figure 10 with the IDLH limit shown at 2.0 mg/m^3 and TLV-C[skin] shown at 0.4 mg/m^3. Study number 59 in Figure 10 had a low CS concentration exposure, consistent with a chamber stay-time of

only 23 s, due to an apparent mask seal leak and immediate evacuation of the chamber. A table of all individual personal monitoring results is included in Appendix A.

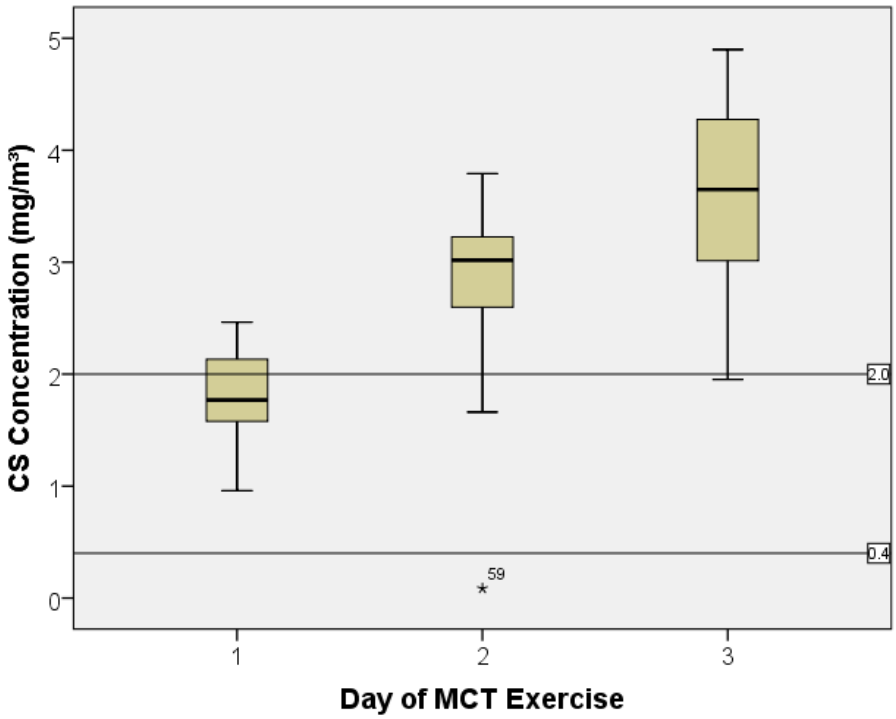

Figure 10. Personal monitoring of CS concentration for three-day MCT event

CHA Metabolite Analysis and CS Exposure Correlations

Analysis of all urine samples for creatinine and CHA were completed by the CDC Division of Laboratory Science in Atlanta, GA. An example chromatogram is provided in Figure 11. A convenience sample taken from the Tennessee Blood Service was analyzed for CHA for use as randomly selected, reference sample group. These individuals (n=108) should not have been exposed to CS. Laboratory analysis revealed the presence of CHA above the lowest calibrator (LOD = 1.00 ng/mL) in 23 out of the 108 samples (21%) in the comparison group. The baseline CHA values above LOD ranged from 1.36

to 32.5 ng/mL. The convenience sample from Tennessee was used only for a reference of comparison and not included in the statistical analysis of the BOLC trainee cohort.

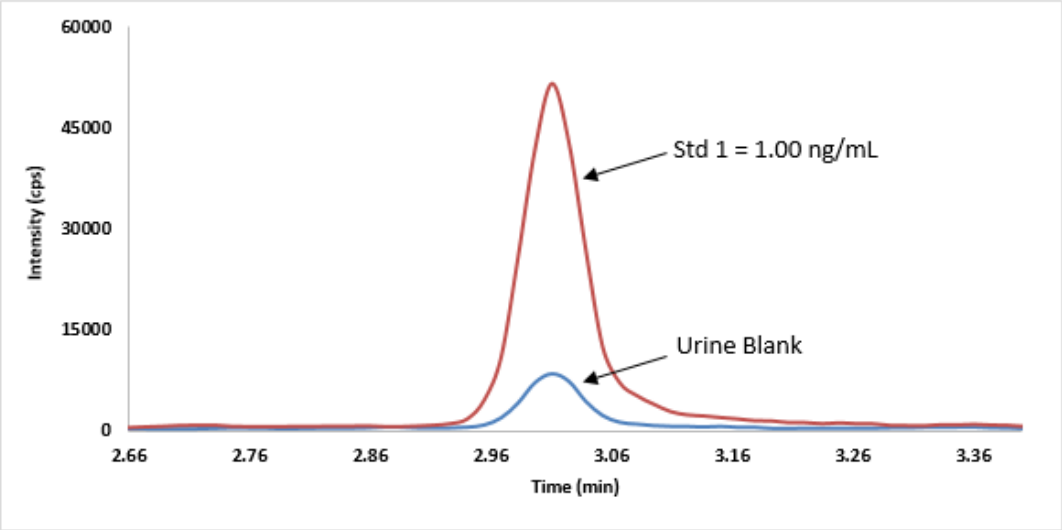

Figure 11. Example chromatogram of CHA LC/MS analysis

Note: Column: Acquity UPLC PFP 1.8 µm, 2.1 x 50 mm; Inj. 15 µL; Flow rate: 200 µL/min; Solvent A: Water, 0.1% Formic acid, Solvent B: Acetonitrile, 0.1% Formic acid, Gradient: 90/10 A/B to 60/40 A/B over five minutes, return to 90/10 A/B for another 5 minutes to re-equilibrate the column; MS: Positive mode MRM transitions CHA 214.2 -> 138.7, CHA_C 214.2 -> 111.1, CHA IS 217.2 -> 140

Of the 91 subjects enrolled in this study, 72 subjects provided a pre-exposure urine specimen for analysis of baseline CHA levels no greater than 24 hours prior to the individual's MCT exercise. Of the 72 individuals who provided pre-exposure urine, 60 subjects had CHA levels below the limit of detection (<LOD, 1 ng/mL) and 12 subjects (17%) had CHA levels ranging from 1.02 – 8.27 ng/mL ($\bar{x}$=3.56 ng/mL), which was within four percent of those with baseline CHA levels in the convenience sample from the Tennessee Blood Service. The summary of CHA levels for pre-exposed and exposed

test subjects in this study over all sampling periods is provided in Figure 12. A summary of CHA levels corrected for creatinine is provided in Figure 13. Creatinine corrections were conducted by dividing CHA in the specimen by creatinine concentration in the same specimen. A summary of natural log transformed (lnCHA) levels is provided in Figure 14.

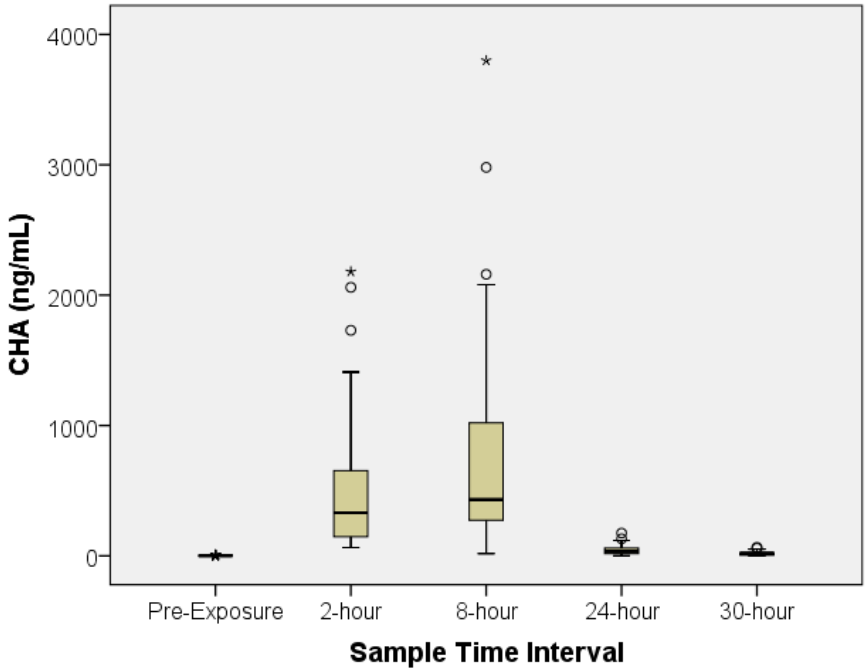

Figure 12. CHA concentrations box-whisker plots for all study subjects

Note: The original design of this project included urine specimen draws at approximately 2, 8, and 24-hours post-exposure. However, the sample times of the 76 samples provided at the 24-hour post0expousre were highly skewed, with 22 subjects submitting a specimen after 30 hours post-exposure. Based on the highly skewed distribution of these data, two sample time intervals were created from the 24-hour samples. Specimens in this range were placed in either the 24-hour (n=46) or 30-hour (n=30) sample time intervals. The arrangement of data points in this manner allowed for normal distributions around the sample time means at the 24 and 30-hour interval.

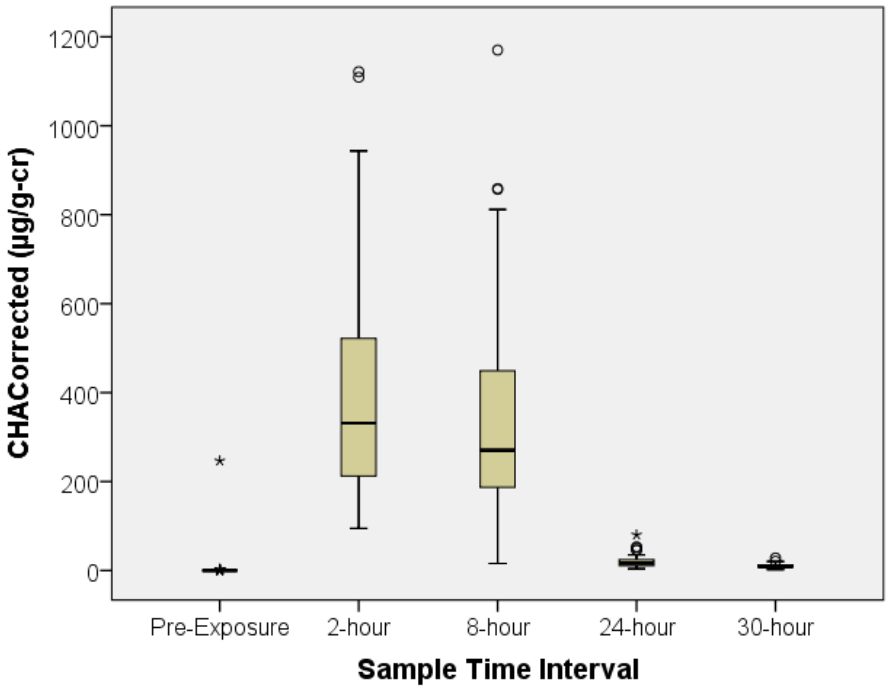

Figure 13. CHA levels corrected for creatinine versus time collected

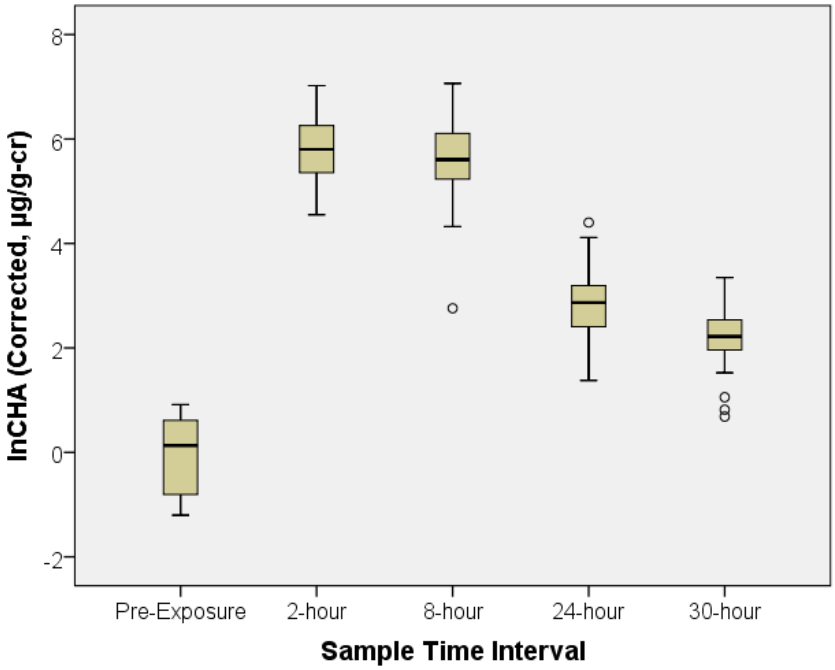

Figure 14. Natural log transformed CHA levels corrected for creatinine

2-hour Time Interval

Of the 87 subjects who provided at least one post-exposure urine specimen, 78 subjects provided a sample that fell to the two-hour time interval. Utilizing the outlier identification testing procedure developed by Tukey and updated by Hoaglin in 1986, for two-hour sample times, ten samples were removed from the study, six from the high end and four from the low end (20). The outlier testing procedure created an upper bounds and lower bounds using the 75^{th} and 25^{th} percentile, based on the following equations: Upper bounds = $Q_3 + 2.2(Q_3 - Q_1)$, Lower bounds = $Q_1 - 2.2(Q_3 - Q_1)$.

The two-hour sample times (n=68) ranged from 71 – 168 min ($\bar{x}$=125 min). The Shapiro-Wilk test for normality indicated that two-hour sample times were normally distributed (p=0.666) and allowed for parametric analysis. Laboratory analysis of CHA metabolite levels in two-hour specimens ranged from 63.4 – 2180 ng/mL ($\bar{x}$=509.33 ng/mL). Creatinine correction was completed by dividing the concentration of analyte (wt/vol) by the concentration of creatinine (wt/vol) measured from the sample urine specimen (13). Correcting CHA levels for creatinine at this time interval resulted in a range of 94.6 – 1121.6 µg/g-cr ($\bar{x}$=389.46 µg/g-cr). The Shapiro-Wilk test for normality indicated that CHA (corrected) data was not normally distributed ($p<0.01$). Based on these findings, natural log transformation of CHA (corrected) levels was necessary for statistical analysis. Natural log (ln) transformation resulted in a CHA (corrected) range of 4.550 – 7.023 µg/g-cr ($\bar{x}$=5.803 µg/g-cr). The Shapiro-Wilk test of normality revealed a normal data distribution (p=0.704) and allowed for parametric analysis.

Pearson correlation coefficient showed lnCHA (corrected) was significantly correlated to CS exposure, $r = 0.361$ ($p< 0.01$) and not significantly correlated with time

of sample (p>0.05). The scatter plot of two-hour lnCHA (corrected) versus CS concentration is shown in Figure 15. Simple linear regression revealed the following relationship:

$$\ln(\text{CHA-2hr, } \mu g/g\text{-cr}) = 5.182 + 0.219\ (\text{CS conc., } mg/m^3)$$

Multiple regression analysis showed that age and gender did not significantly affect the levels of lnCHA (corrected) (p>0.05). BMI was significantly correlated with levels of lnCHA (corrected) (p<0.05), however, adjusting for CS exposure and BMI, there was no significant correlation (p=0.669) between BMI and lnCHA (corrected) at this time interval.

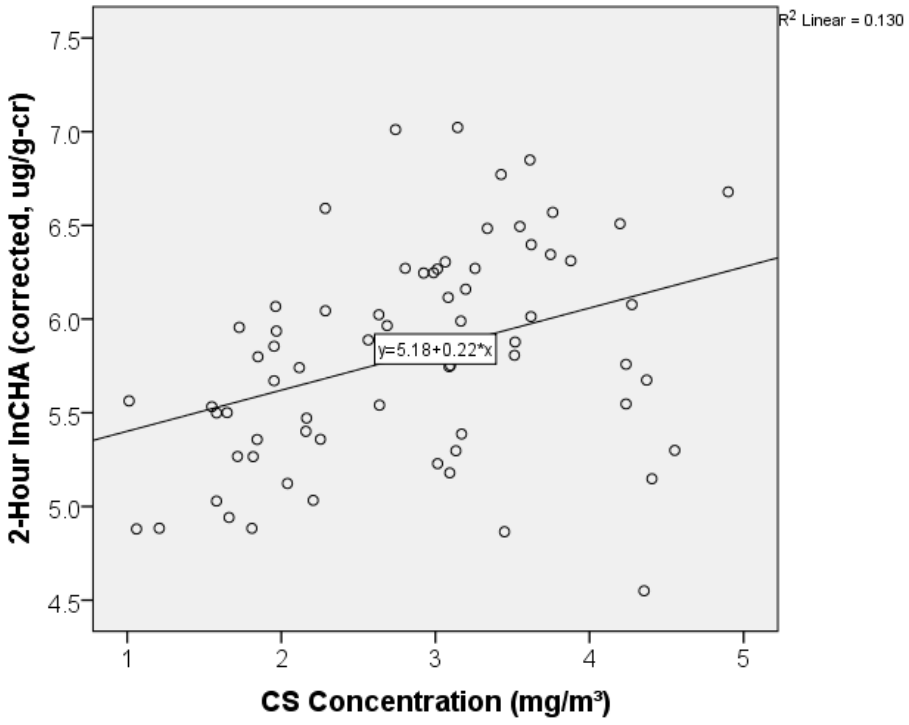

Figure 15. Relationship between CS exposure (mg/m³) and CHA levels corrected for creatinine (µg/g-cr) at the two-hour sample interval

8-hour Time Interval

Of the 87 subjects who provided at least one post-exposure urine specimen, 69 subjects provided at approximately 8 hours post-exposure. Utilizing the outlier testing procedure for eight-hour sample times, no samples were removed from the study. The eight-hour sample times ranged from 363 – 606 min ($\bar{x}$=486 min (8.1 hrs)). The Shapiro-Wilk test indicated that eight-hour sample times were normally distributed (p=0.686) and allowed for parametric analysis. Laboratory analysis of CHA metabolite levels in eight-hour specimens ranged from 17.20 - 3800 ng/mL ($\bar{x}$=709.98 ng/mL). Correcting CHA levels for creatinine at this time interval resulted in a range of 15.80 – 1170.20 µg/g-cr ($\bar{x}$=341.13 µg/g-cr). The Shapiro-Wilk test for normality indicated that CHA (corrected) data was not normally distributed (p<0.01). Based on these findings, natural log transformation of CHA (corrected) levels was necessary for statistical analysis. Natural log transformation resulted in an lnCHA (corrected) range of 2.760 – 7.065 µg/g-cr ($\bar{x}$=5.619 µg/g-cr). The Shapiro-Wilk test of normality revealed a non-normal data distribution (p=0.014) and required non-parametric analysis.

Spearman's rho correlation coefficient revealed that lnCHA (corrected) was significantly correlated with CS concentration, r = 0.360 (p<0.01). Spearman's rho also showed that lnCHA (corrected) was significantly correlated with time sampled, r=0.442 (p<0.01) The scatter plot of eight-hour lnCHA (corrected) versus CS concentration is shown in Figure 16. Simple linear regression revealed the following relationship:

$$\ln(\text{CHA-8hr, µg/g-cr}) = 4.695 + 0.328\ (\text{CS conc., mg/m}^3)$$

Multiple regression analysis showed that age, gender, or BMI did not significantly ($p>0.05$) affect the levels of CHA (corrected) among test subjects at this time interval.

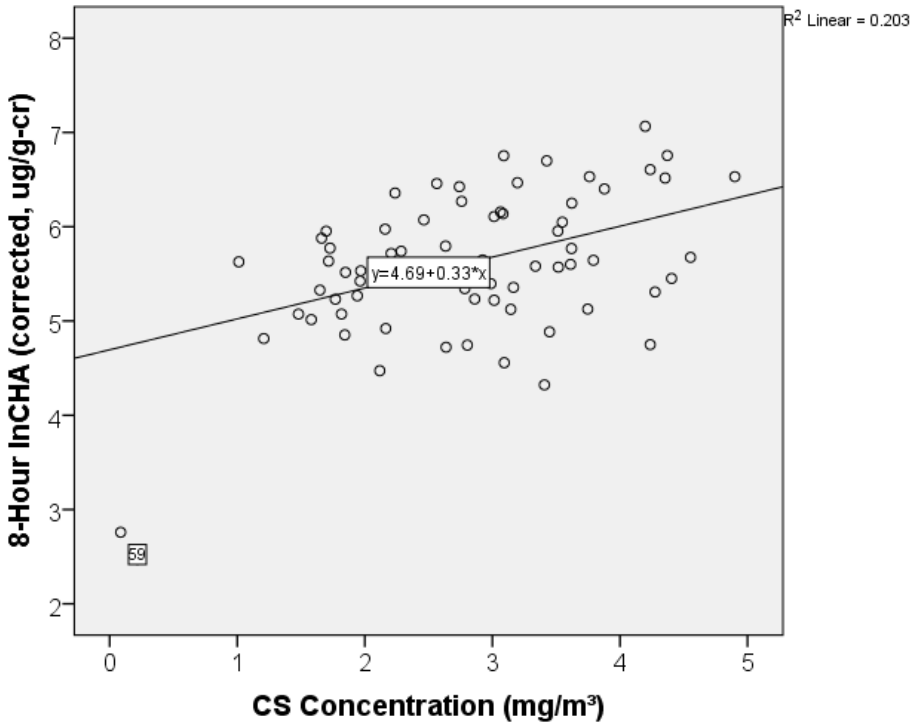

Figure 16. Relationship between CS exposure (mg/m³) and CHA levels corrected for creatinine (µg/g-cr) at the eight-hour sample interval
Note: Case no. 59 had a chamber stay-time of only 23 s due to apparent mask leak

24-hour Time Interval

Of the 87 subjects who provided at least one post-exposure urine specimen, 76 subjects provided a sample at approximately 24 hours post-exposure. However, the distribution was highly skewed, with 22 subjects providing specimens after the 30-hour mark. Based on the distribution, a new sample time interval was created for samples after the 24-hour mark and was labeled the 30-hour time interval. A total of 30 samples were moved to the 30-hour sample time interval while 46 samples remained in the 24-hour interval. Of the 46 samples, four samples were removed from the 24-hour time interval

for having extremely high CHA levels, as identified by the outlier testing procedure. The extremely high CHA can be attributed to a combination of long chamber stay-time, high CS exposure concentration, short time sampled, and high BMI. The 24-hr sample times ranged from 20.4 – 23.9 hrs ($\bar{x}$=21.9 hrs). Laboratory analysis of CHA metabolite levels in 24-hr specimens ranged from 1.350 – 131.0 ng/mL ($\bar{x}$=40.91 ng/mL). Correcting CHA levels for creatinine at this time interval resulted in a range of 4.00 – 53.1 µg/g-cr ($\bar{x}$=19.3 µg/g-cr). The Shapiro-Wilk test for normality indicated that CHA (corrected) data was not normally distributed ($p<0.01$). Based on these findings, natural log transformation of CHA (corrected) levels was necessary for statistical analysis. Natural log transformation resulted in an lnCHA (corrected) range of 1.378 – 4.116 µg/g-cr ($\bar{x}$=2.814 µg/g-cr). The Shapiro-Wilk test of normality revealed a normal data distribution ($p=0.609$) and allowed for parametric analysis.

Pearson correlation coefficient revealed lnCHA (corrected) was significantly correlated CS concentration, $r = 0.442$ ($P<0.05$). The scatter plot of 24-hr lnCHA (corrected) versus CS concentration is shown in Figure 17. Simple linear regression showed the following relationship:

$$\ln(\text{CHA 24-hr, µg/g-cr}) = 1.957 + 0.305\ (\text{CS conc., mg/m}^3)$$

Multiple regression analysis showed that age, gender, or BMI did not significantly affect the levels of CHA (corrected) among test subjects at this time interval.

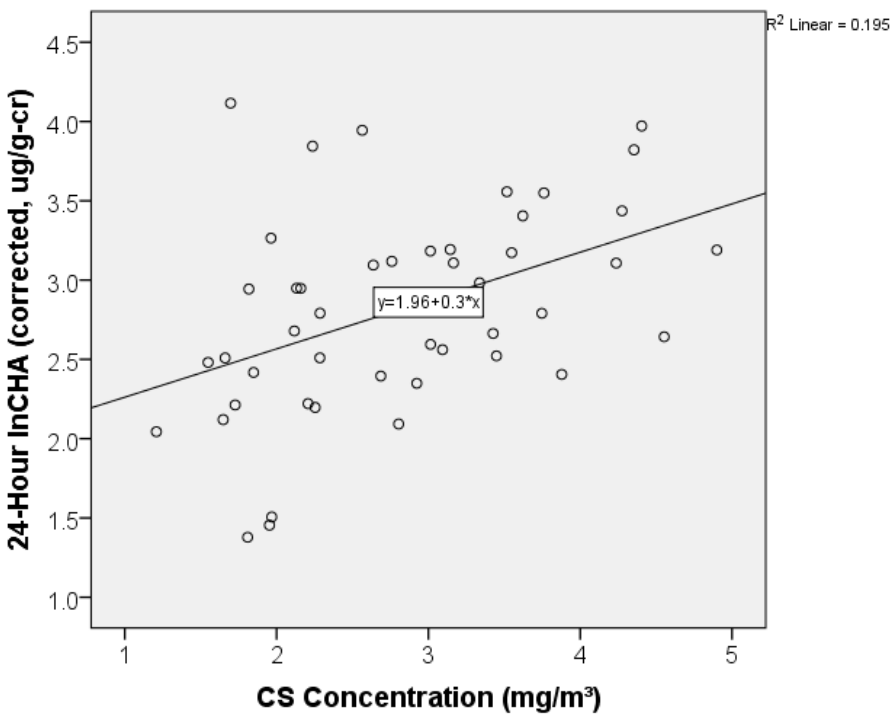

Figure 17. Relationship between CS exposure (mg/m³) and CHA levels corrected for creatinine (µg/g-cr) at the 24-hour sample interval

30-hour Time Interval

Of the 87 subjects who provided at least one post-exposure urine specimen, 30 subjects provided a sample approximately 30 hours post-exposure. Utilizing the outlier testing procedure for 30-hour sample times, no samples were removed from the study due to sample time alone. Two samples from this time interval were removed for high CHA (corrected) values based on the outlier testing procedure. After removing outliers, the 30-hour interval sample times ranged from 28.1 – 34.4 hrs ($\bar{x}$=31.5 hrs). The Shapiro-Wilk test indicated that 30-hour sample times were normally distributed (p=0.256) and allowed for parametric analysis. Laboratory analysis of CHA metabolite levels in 30-hour specimens ranged from <LOD – 65.00 ng/mL. CHA levels below the LOD were accounted for by dividing the LOD (1 ng/mL) by the square root of two, resulting in a

CHA range of 0.71 – 65.00 ng/mL ($\bar{x}$=27.10 ng/mL) (35). Correcting CHA levels for creatinine at this time interval resulted in a range of 1.99 – 28.4 µg/g-cr ($\bar{x}$=10.63 µg/g-cr). The Shapiro-Wilk test for normality indicated that CHA (corrected) data was not normally distributed ($p<.05$). Based on these findings, natural log transformation of CHA (corrected) levels was necessary for statistical analysis. Natural log transformation resulted in an lnCHA (corrected) range of 0.69 – 3.35 µg/g-cr ($\bar{x}$=2.185 µg/g-cr). The Shapiro-Wilk test of normality revealed a normal data distribution ($p=0.266$) and allowed for parametric analysis.

Pearson correlation coefficient revealed that lnCHA (corrected) was significantly correlated with CS concentration, $r = 0.626$ ($p<0.01$). The scatter plot of 30-hour lnCHA (corrected) versus CS concentration is shown in Figure 18. Simple linear regression revealed the following relationship:

$$\ln(\text{CHA, µg/g-cr}) = 1.084 + 0.437\ (\text{CS conc., mg/m}^3)$$

Multiple regression analysis showed that age, gender, or BMI did not significantly affect the levels of CHA (corrected) among test subjects at this time interval.

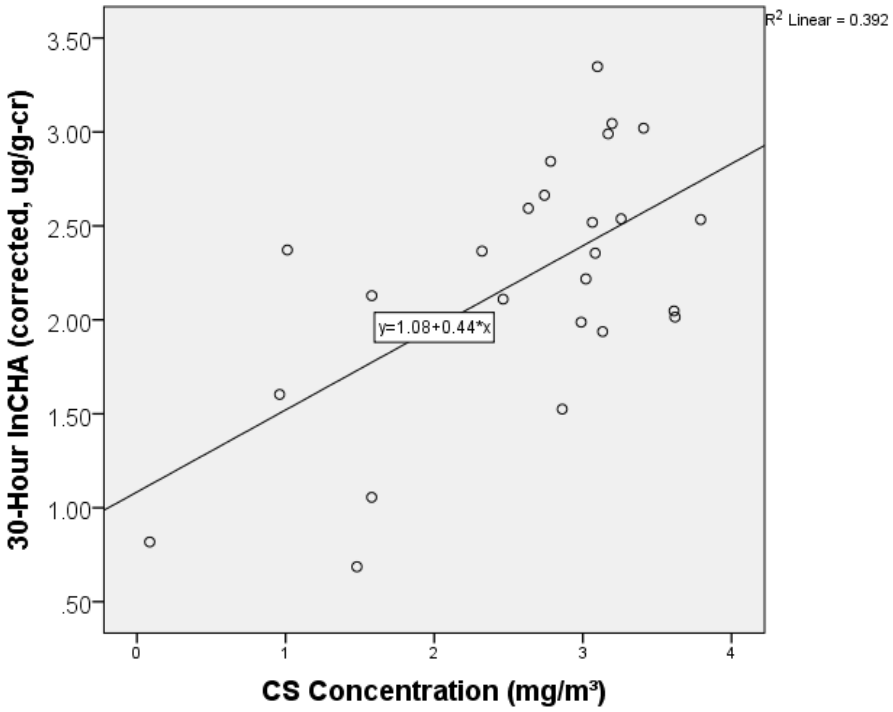

Figure 18. Relationship between CS exposure (mg/m³) and CHA levels corrected for creatinine (µg/g-cr) at the 30-hour sample interval

Table 3. Summary of CS Exposures and CHA Levels in US Army Trainees

Group	Parameter	CS Exposure (mg/m^3)	CHA (ng/mL)			
Unexposed (Tenn. Blood Service)	n	--	108			
	n > LOD	--	23			
	Range	--	1.36 - 32.5			
Pre-Exposure (Army Trainees in this study)	n	--	72			
	Mean ± SD	--	1.20 ± 1.34			
	Median (range)	--	0.71 (<LOD - 8.27)			
	n > LOD	--	12			
	Mean ± SD >LOD	--	3.56 ± 2.59			
	Median > LOD	--	2.46 (1.02 - 8.27)			
	Time Interval		2-hour	8-hour	24-hour	30-hour
Post-Exposure (Army Trainees in this study)	Mean ± SD	2.72 ± 0.963	509.3 ± 486	709.9 ± 705	43.77 ± 37.40	18.68 ± 17.12
	Median (range)	2.77 (0.086 - 4.90)	330 (63.4 – 2180)	432 (17.20 - 3800)	31 (1.350 – 175.0)	19 (<LOD – 65.00)
	n	87	68	69	47	28

Time as a Continuous Variable

The following analysis accounted for time as a continuous variable over the 34-hour sampling period. No samples were removed from the study based on sample time deviation from the mean. The 87 test subjects who provided at least one post-exposure urine specimen provided a total of 212 post-exposure samples. Four samples were removed using the outlier labeling procedure for excessively high CHA levels. The high CHA levels outside the normal distribution are likely a result of a combination of one of the following factors: high chamber stay-time, high CS concentration, short sample time, and high BMI. Sample times ranged from 1.18 – 34.4 hours. Five of the 87 (5.7%) test subject's final sample returned CHA levels <LOD, suggesting that these individuals metabolized virtually all CS during the sample period.

CHA levels for all samples ranged from 0.71 – 3800 ng/mL ($\bar{x}$=406.62 ng/mL). CHA corrected for creatinine levels for all samples ranged from 1.986 – 1170.2 µg/g-cr ($\bar{x}$=241.92 µg/g-cr). The Shapiro-Wilk test for normality revealed the CHA (corrected) data was not normally distributed ($p<0.01$). Based on these findings CHA (corrected) levels required natural log transformation for statistical analysis. Natural log transformation of CHA (corrected) data revealed an lnCHA (corrected) range of 0.6861 – 7.064 µg/g-cr ($\bar{x}$=4.620 µg/g-cr). The Shapiro-Wilk test of normality revealed a non-normal distribution ($p<0.01$) and required non-parametric analysis.

Utilizing Spearman's rho non-parametric correlations, lnCHA (corrected) was strongly correlated (negatively) with time sampled, $r = -0.750$ ($p<0.01$). LnCHA (corrected), was weakly correlated with CS concentration, $r = 0.243$ ($p<0.01$). CS concentration was not correlated with time sampled, as expected. Multiple regression was

applied to account for multi-variable analysis. Regression analysis resulted in a significantly strong association between lnCHA (corrected), CS concentration and time of urine sample, R = 0.910 (R^2 = 0.829, $p<0.01$). The relationship was determined to be:

$$\ln(\text{CHA, } \mu g/g\text{-cr}) = 5.519 + 0.279\ (\text{CS conc., } mg/m^3) - 0.002\ (\text{time sampled})$$

Multiple regression analysis showed that age, gender, or BMI did not significantly affect the levels of CHA (corrected) among test subjects when time was considered as a continuous variable.

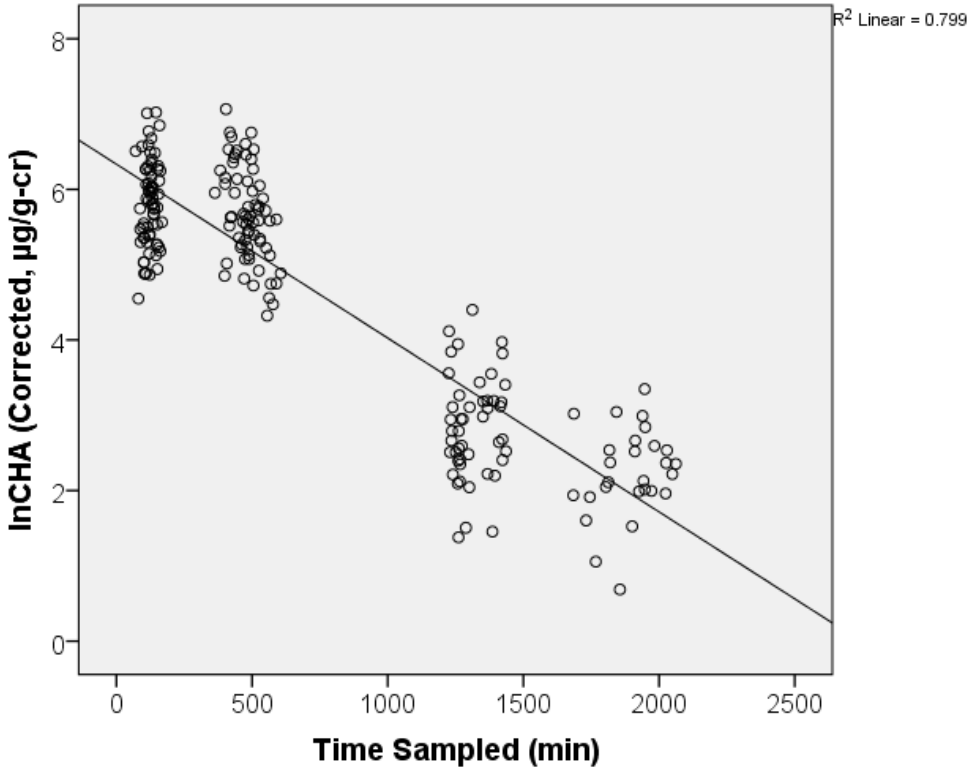

Figure 19. lnCHA corrected for creatinine versus post-exposure time sampled

The following analysis excluded all subjects who had pre-existing CHA levels to determine the association without pre-exposure as a potential confounder. Removing the

12 subjects from the study removed 33 total specimens from this analysis. Utilizing Spearman's rho non-parametric correlations, lnCHA (corrected) was strongly correlated with time sampled, $r = -0.741$ ($p<0.01$) and was weakly correlated with CS concentration, $r = 0.290$ ($p<0.01$). Multiple regression was applied to account for multi-variable analysis. Regression analysis of this model resulted in a significantly strong association, $R = 0.912$ ($R^2 = 0.831$, $p<0.01$). The relationship was determined to be:

$$\ln(\text{CHA, } \mu\text{g/g-cr}) = 5.289 + 0.366\ (\text{CS conc., mg/m}^3) - 0.002\ (\text{time sampled})$$

Consequently, the removal of individuals from this study who had a baseline CHA level >LOD (1 ng/mL) increased the strength of association between lnCHA (corrected), CS concentration, and time sampled by only 0.2%.

Chapter 5: Discussion

This research tested the following hypotheses:

1) Personal CS exposures exceed ACGIH TLV-C[skin] during MCT post ALARACT 051/2013 implementation
2) Personal CS exposures exceed NIOSH IDLH during MCT post ALARACT 051/2013 implementation
3) A statistically significant relationship exists between exposure to CS and concentration of CHA metabolite excreted in urine after US Army MCT exercises

Research objectives of this study included determining CS exposure concentration, determining CHA metabolite levels in urine of test subjects pre and post-exposure, and using inferential statistics to assess the strength of association between CS concentration and post-exposure CHA metabolite level. All specific aims were carried out and research objectives were accomplished.

General Study Completion and Exposure Assessment

Of the 91 subjects who volunteered for this study and signed a consent form, four subjects self-dropped from this study after the MCT exercise and prior to providing urine specimens. Of the four subjects who dropped, two subjects voluntarily stated that they would not provide samples due to the onset of menstruation. The other two subjects did not return to provide urine for reasons unknown. Of the 87 subjects who provided at least one post-exposure urine sample, 86 wore personal monitoring during the MCT event. One test subject was overlooked by researchers during event staging and completed the event without being issued a personal monitoring sampling train. This subject, however,

remained in the study and provided post-exposure urine specimens. CS exposure for this subject was estimated using the mean of his/her exposure group, SEG 26, which was not significantly different from the mean of the day's SEGs.

During the MCT, four of the 87 test subjects who provided post-exposure urine specimens left the chamber before the 200 s ($\bar{x}$=341 s) mark due to apparent mask seal leaks. Three of the four subjects' data was removed from the study based on CHA levels significant deviation from the mean based on the outlier testing procedure. One subject had low CS concentration and low CHA levels associated with his/her low chamber stay-time but was included in the normal distribution and was retained in the dataset. Subject out-of-mask times ranged from 4 – 19 s ($\bar{x}$=8 s; 95% CI [8, 9]). Statistical analysis revealed that subject out-of-mask times were not statistically different from one another (p=0.53) nor was lnCHA correlated with out-of-mask times at any time interval or using time as a continuous variable. This data suggests that variation in urinary CHA output was not associated with variation in time out-of-mask during this evolution or that the actual dose the subjects received was not related to uptake through inhalation.

Seventeen test subjects had pre-post pump calibration greater than the industrial hygiene industry acceptable ±5% (32). Of the 17 subjects with greater than acceptable pump calibration difference, 11 subjects had pre-post calibration difference less than 1% greater than acceptable, two subjects had less than 2% greater than acceptable, two were less than 3% greater, and one was less than 5% greater. For flow rate pre-calibration and post-use check reporting, US Army TG 141 guidance states that if the difference between pre-calibration flow rate and the post-use check is equal to or less than 5%, report the average of the two (14). The manual also states that if the difference is greater than 5%,

to use the lower flow rate to ensure overestimate of airborne concentration in the sampling environment (14). This study, however, was not performed to determine conservative exposure profiles for reducing hazard severity in occupational settings. This study sought obtain most accurate exposure levels possible for the purpose of association evaluation between exposure and metabolite. Therefore, pre and post-use sampling pump flow rates were averaged for all samples except one. The results of CS concentration were not significantly different from their respective SEG and the averages of pre and post-flowrates were used in the total air volume calculation. For the one exception, the subject had a pre-post pump calibration difference of >200% and spent over seven minutes in the chamber, however, this participant's exposure measured only 0.004 mg/m^3. These results indicate this subject had an apparent critical pump malfunction, which in other settings, would require pump repair or replacement and retesting (25). With CHA urine level data in the normal distribution range however, and the infeasibility of repeating the MCT event, this subject's CS exposure was categorized using the mean for SEG 11 and his/her data remained in this study.

SPATIAL VARIATION INSIDE MCT CHAMBER

Due to the assembly of the test subjects in the chamber and the local procedures used by MCT instructors, participants were arranged in a circle-like formation around the point of CS generation located in the middle of the room. Because of this, test subjects were located approximately equidistant from the exposure source while performing drills and during the mask removal portion of the exercise. The BOLC MCT chamber layout drawing is included in Figure 20. In a chamber with little air movement, the CS "plume" was observed to distribute throughout the chamber in a relatively even manner as the

instructor wafted to the vapor each side. Also, as two test subjects removed his/her masks and evacuated the chamber, the next two participants in line to remove their masks moved into the position vacated by the exiting trainees. OSHA allows area samples to be taken in a fixed location and results may represent the potential risk from airborne contaminants or physical agents to workers in that area (34). However, for evaluation of the association between exposure and metabolite in this study, personal monitoring allowed a more subject-specific exposure profile to be used in statistical analysis. From these observations and OSHA sampling guidance, spatial variation inside the chamber played a small role, if any, in CS exposure concentration and was considered negligible during this study.

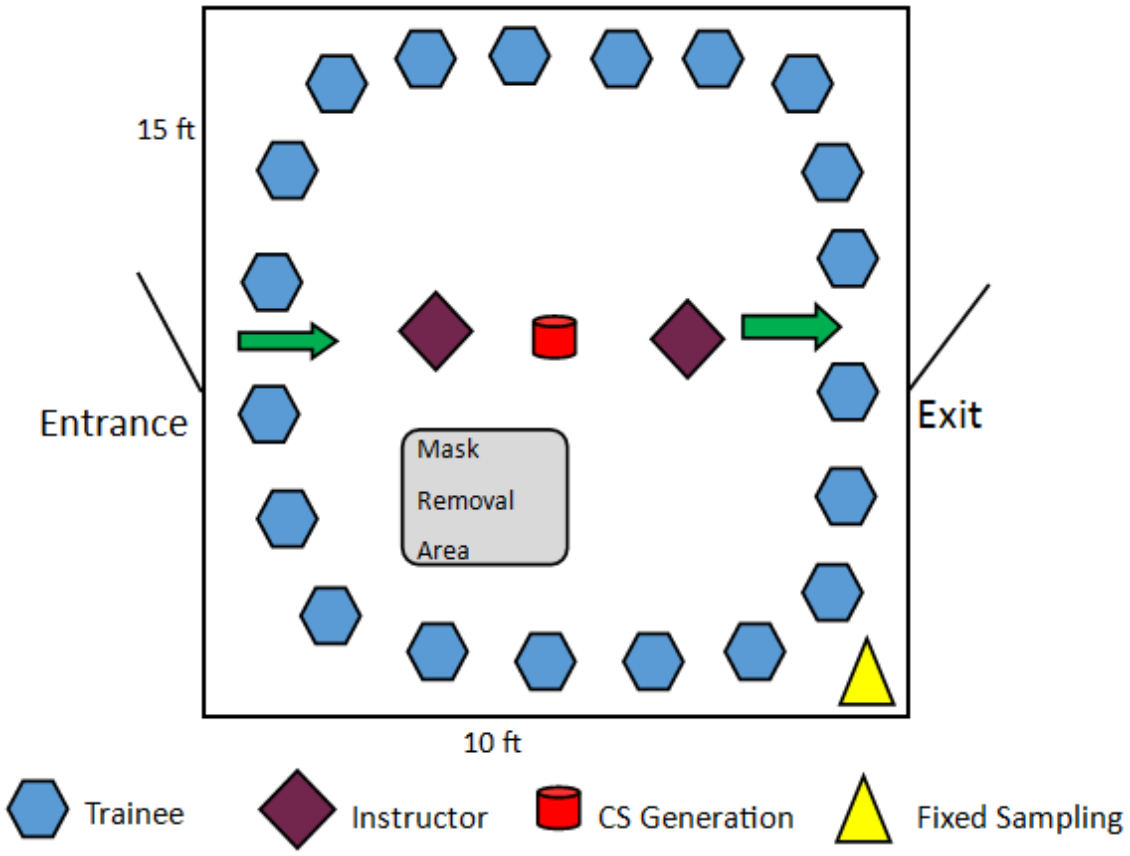

Figure 20. BOLC MCT Chamber Layout.

EXPOSURE HYPOTHESES

Results of this study showed that CS exposure concentrations from all test subjects enrolled in the study (n=91) ranged from 0.086 – 4.900 mg/m³ ($\bar{x}$=2.741 mg/m³). 89 of the 91 participants (98%) received an exposure concentration greater than the ACGIH TLV-C[skin] (0.39 mg/m³). The two subjects who were exposed to less than the TLV-C[skin] had chamber stay-times of less than 70 s due to apparent mask seal leaks. Based on these results, researchers fail to reject hypothesis number one as personal exposures exceeded ACGIH TLV-C[skin]. Furthermore, 25 of 91 participants (27%) received an exposure concentration greater than NIOSH IDLH (2.0 mg/m³). Based on these results, researchers fail to reject hypothesis number two as CS concentration levels and stay-times resulted in exposure above the IDLH limit. These findings suggest that there was insufficient implementation of ALARACT 051/2013 to reduce CS concentration during MCT events at BOLC (29).

ASSOCIATION HYPOTHESIS

CHA vs. CS Concentration

After review of urine and CS exposure data, two main analyses of association between lnCHA and CS exposure were explored. The following analysis placed urine sample times into one of four time intervals. After removal of outliers, subjects provided urine samples close enough to the 2, 8, 24, and 30-hour post-exposure sample time means that differences in time were not significant. Categorizing sample times into one of the four time intervals did not account for the specific time the urine sample was provided, therefore, lnCHA (corrected) levels were not significantly associated with time within the individual sample time intervals.

There were weak to moderate correlations of CS exposure to urine metabolite levels with significance at all time intervals of in this method of analysis. Summary of regressions of CHA on exposure among subjects exposed to CS in this study are provided in Table 4. A weak correlation for this method was expected as these relationships were developed without adjusting for the exact time the urine specimen was drawn which was a highly dependent variable.

Table 4. Summary of time interval regressions of CHA and CS concentration

Time Interval	n	CS Exposure Range (mg/m^3)	CHA (corr) Range (µg/g-cr)	Exposure-CHA relationship*	r
2-hour	68	0.086 - 4.900	94.6 – 1121.6	ln(y) = 5.182 + 0.219 (x)	0.361
8-hour	69		15.80 – 1170.20	ln(y) = 4.695 + 0.328 (x)	0.360
24-hour	46		4.00 – 53.10	ln(y) = 1.957 + 0.305 (x)	0.442
30-hour	30		1.99 – 28.40	ln(y) = 1.084 + 0.437 (x)	0.626

*Note that relationships are expressed as (natural) logarithms of y (CHA concentration (corrected), ng/mL), and x (CS concentration, mg/m^3)

CHA vs. CS Concentration & Time Sampled

The following analysis explored the association between CHA levels, CS exposure, and sample time by performing multiple regression to include the specific time (in minutes from end of MCT exercise) that the urine specimen was provided. This analysis was predicted to have a higher strength of association results when accounting for time, particularly with the short half-life of the CHA metabolite (82 – 95% excreted within 96 hours of exposure) (8).

The bivariate correlation analysis resulted in a very strong, significant correlation, ($r = 0.910$, $r^2 = 0.827$, $p<0.05$). A summary of this regression equation is provided in Table 5.

Table 5. Association of CHA, CS exposure, and time of sample among test subjects

Time Interval (min)	n	CS Exposure Range (mg/m^3)	CHA (corr) Range (ng/mL)	Exposure-CHA relationship*	R
71 - 2062	208	0.086 - 4.900	1.99 - 1170.20	ln (y) = 5.519 + 0.279(x) - 0.002(z)	0.910

*Note that relationships are expressed as (natural) logarithms of y (CHA concentration (corrected), ng/mL), x (CS conc., mg/m^3), and z (time sampled, min)

PREVIOUS STUDIES

A comparison of this study and the results of the previous Hout et al. MCT sampling study revealed significant differences in chamber concentration during the MCT events. In the Hout study of the Army basic training MCT and associated ARIs, CS concentrations reached 53.3 mg/m^3 (21). The highest concentration seen in the Hout study was approximately eight times higher than the highest concentration reached in this study, 4.90 mg/m^3. Also, chamber stay times in the Hout study ranged from 5.0 – 15.0 minutes while the mean stay time in this study was 5 min 37 s (21). Based on this data, it is apparent that exposure levels during BOLC MCT at Fort Sam Houston were much less than that in Army Basic Training (before implementation in ALARACT 051/2013) at Fort Jackson. Utilizing exposure monitoring methods developed in the Hout study and comparison of long-area, short-area, and personal monitoring samples in this study, it was determined that CS exposure characterization was completed satisfactorily in this study. Based on exposure monitoring results from this study, it was revealed that

implementation of ALARACT 051/2013 into the BOLC MCT resulted in lower mean exposures than recorded in the Hout et al. study, however, implementation was not successful overall as 64 of the 87 test subjects (74%) were exposed to CS concentration levels above the IDLH limit of 2.0 mg/m³ (36). While evaluation of the implementation of ALARACT 051/2013 or thermal dispersion techniques was not a goal of this study, the higher range of exposure was helpful to evaluate a potential biomarker using CHA metabolite for retrospective quantification of CS exposure in this cohort.

A comparison of this study to the Riches et al. study of the development of an analytical method for urinary metabolites revealed a significant difference in two-hour CHA levels measured between the two studies. In the Riches study, two-hour post-exposure CHA (non-corrected) ranged from 3 – 135 ng/mL ($\bar{x}$=29.2 ng/mL) and creatinine concentration mean was 256 mg/dL (37). Creatine concentration in the Riches study was considerably higher than the reference values for randomly collected urine of 155 mg/dL and 172 mg/dL (388 non-Hispanic white US subjects and US Navy recruits, respectively) (15; 39). However, quantitative measurements for CHA in the Riches study were not creatinine corrected prior to analysis. In this study, two-hour post-exposure CHA (non-corrected) ranged from 63.4 – 2180 ng/mL ($\bar{x}$=509.33 ng/mL) and the mean creatinine for two-hour samples was 143 mg/dL, a value much closer to the mean creatinine reference values. It is important to note that all exposures measured in this study were lower than the lowest estimate of exposure in the Riches study. Based on these results and the lack of CS sampling in the Riches study, evidence suggests that the CS concentration estimate between 5 and 15 mg/m³ based on volume of chamber (55m³) in the Riches study may have been considerably over-estimated, LC/MS laboratory

analysis for post-exposure CHA levels insufficiently captured metabolite levels in urine specimens, and/or lack of creatinine correction for quantitative measurement of 2-CHA may have negatively impacted their method development. The Riches study did conclude that a sensitive method for analysis of CHA could not be developed.

PROJECT IMPLICATIONS AND LIMITATIONS

The recent deployment of RCAs during periods of civil unrest in the US and around the world has motivated the development of analytical methods of quantification of CHA detection and analysis of a potential biomarker for exposure to CS. Previous studies have attempted to create a sensitive method for analysis of CHA but were unsuccessful, likely in part due to concentrated urinary creatinine levels (37). While creatinine levels in this study were slightly outside of the ACGIH recommended range for analysis (30 – 300mg/dL), the mean concentration of creatinine in this study was 143 mg/ dL, not considerably different from the reference values for randomly collected urine of 155 mg/ dL (388 non-Hispanic US subjects) and 172 mg/ dL (US Navy recruits) (15; 39).

Pre-exposure CHA Baseline Levels

According to the CDC, background levels of CHA that are not attributed to exposure to CS are caused by either biotransformation of prescription medication or exposure to 2-chlorobenzoic acid, which is also a precursor in the metabolic pathway of CS to CHA (8). Chlorobenzoic acid is an anthropogenic compound not known to occur in nature. It is used in the manufacturing of glues, paints, dyes, fungicide and other agricultural chemicals (43). It has also been found to be a by-product of some municipal wastewater chlorination processes (7). Pharmaceuticals containing chlorobenzoic acid

include ticlopidine (a platelet drug), isoprophenamine (a bronchodilator), bupropion (an anti-depressant also used for smoking cessation), chlormezanon (a muscle relaxant), 4-chlorobenzotrichloride (pharmaceutical intermediate), and lofepramine (an antidepressant) (8). The possibility of pre-exposure to these products were not screened in this study. Future research could design a study that determines potential pre-exposure from occupation or medical treatment sources.

It is currently unknown how CS exposure affects CHA metabolism for individuals who had pre-existing CHA levels from previous exposures of pharmaceuticals or using products containing 2-chlorobenzoic acid. This study performed statistical analysis with the pre-exposure CHA baseline included and excluded. Results indicate that exclusion of subjects with pre-exposure CHA increased strength of association by only 0.2%. Therefore, final correlations and associations did not exclude participants with a CHA baseline from suspected pre-exposure for three reasons: 1) there was statistically no difference between subjects with pre-exposure CHA baseline and those without 2) the percentage of subjects in this study with baseline exposure was within four percent of the percentage of the non-exposed random convenience sample group provided by the Tennessee Blood Service and 3) practical use of the retrospective regression equation would include the suspected portion of the population having pre-exposure CHA baseline. Based upon this, it appears this association applies to individuals whether or not they had pre-exposure to 2-chlorobenzoic acid.

Of the 12 subjects in this study who had baseline CHA, five subjects provided pre-exposure urine samples prior to any trainees in this BOLC iteration being exposed to CS in the MCT chamber. This reduced the suspicion that elevated baseline CHA levels in

this population could be due to secondary or indirect exposure from uniforms of classmates who completed the MCT exercise earlier in the day. In addition, one BOLC student reported sensing CS in the mats of the gymnasium tent of the FOB at some point after MCT exercises began during the sampling week. The potential indirect exposure effect on this study's participants is unknown but this report reveals that the possibility exists that, while unlikely, baseline urine CHA levels for day two and three MCT exercise participants were attributed to secondary pre-MCT exposure.

Inhalation vs. Dermal Exposure

There are several other limitations and implications associated with this observational study. This study evaluated participants of an MCT exercise that spent their majority of time in the chamber while exposed to CS wearing a M40 chemical protection mask and filter. The range of out-of-mask times were 4 – 19 s ($\bar{x}$ = 8 s). Based on this evidence, an average of greater than 97% of an individual's time in the chamber was spent being exposed via the dermal route only. Consequently, an average of only 3% of an individual's time in the chamber was spent being exposed through both dermal and inhalation routes. This study did not attempt to distinguish the portion of the absorbed dose attributed to CS uptake through the skin as opposed to the CS uptake was through respiration. OSHA P&CAM 304 and Army TG 141 prescribes sampling pump flow rates to be set at 1.5 L/min for CS sampling (14). However, evidence shows that individual respiration rates may have varied during the out-of-mask portion of the exercises. In the post-exposure survey, 6% of the subject population admitted to taking no breaths during the out-of-mask portion. 41% reported only taking one breath and 48% report taking more than one breath. Regardless of whether an individual did or did not take breaths

during the out-of-mask portion of the exercise, elevated CHA levels found in all urine samples in 2, 8, and 24-hour samples indicate CS uptake and metabolism in all subjects. This study did not attempt to account for self-reported breathing rates in statistical analysis these reports are subjective, and shallow and erratic breathing in a CS rich atmosphere may vary widely from person to person. This study relies upon the OSHA and US Army industrial sampling guidance for a sampling rate of 1.5 L/min, which is based upon the chemical, sample media, and analysis platform.

Post-MCT Residual Exposure

Another limitation of this study and likely confounder is the potential for continued exposure after completing the MCT chamber exercise from residual CS emanating from the participant's body. Trainees completed the event wearing the general issue ACU without additional MOPP gear, except for the M40 mask and filter. Wearing only the ACU left the subjects' hands, wrists, neck, and head exposed to the CS rich atmosphere. In addition, the ACU blouse and trousers are made of a 50/50 cotton/nylon blend, which may allow permeation and absorption of CS into uniform and underclothing. This is supported by participants that reported the ability to sense CS emanating from their own clothing as well as from their fellow trainee's clothing for a period after the event. This absorption into clothing, skin, and hair create a secondary exposure source that likely results in a relatively small exposure for a period of time after completion of the chamber event. In previous research, evidence suggests that secondary exposure to CS could be a notable occupational hazard for health care workers (emergency department personnel, and anesthetists) (16). It is unknown whether the subjects showered or changed clothing within the 34 hours after exposure during urine

specimen collection. This study did not account for CS exposure from residual "off-gassing" from uniform, hair, skin or a receiving a secondary exposure from a fellow MCT trainee. Future research may include a study that quantifies residual exposure giving future CHA metabolite studies the ability to account for post-chamber residual exposure.

Creatinine Concentrations

Even though mean creatinine levels in this study were closer to the reference values, a limitation and possible confounder in this study was the range of measured creatinine levels used in statistical analysis, 17.40 – 507.80 mg/dL. According to ACGIH guidelines, urine specimens that are highly dilute or highly concentrated are generally not suitable for biological monitoring and suggests utilizing urine specimens with creatinine concentrations only within the range of 30 – 300 mg/dL (2; 13). In order to avoid repeat testing due to improper creatinine concentration, it has been suggested that the collection site perform a specific gravity screen of urine prior to shipment and to accept samples only within the range of 1.015 – 1.030 (23). The infeasibility to efficiently perform on-site measurements of specific gravity in this austere environment in urine specimens prior to freezing and shipment resulted in numerous samples being outside the recommended creatinine range. This variation would affect creatinine corrections, and could have possibly lowered the precision of CHA (corrected) levels used in regression analysis.

The presence of CHA background levels found in 17-21% of the combined population of this study and the Tennessee Blood Service randomly selected unexposed convenience sample group reflects implications in the use of CHA metabolite levels as a potential biomarker for CS exposure. Obtaining medical history with prescription drug

use or occupational exposure records would be necessary to account for possible confounders or false positives in individuals during investigations of alleged chemical agent use during international warfare. However, further research could possibly determine a CHA value that distinguishes occupational exposure to 2-chlorbenzoic acid or CHA from medications from that of exposure to CS riot control agent.

Chapter 6: Conclusion

This is the first study to use personal monitoring to sample CS concentration and the CDC's DLS Method Code: 2437, CS Metabolite: 2-chlorohippuric acid (CHA), to quantify urinary metabolite levels for the purpose of finding the association of exposure to CS Riot Control Agent and the primary metabolite, CHA. The application of the CDC's method for sensitive analysis of CHA measured in urine by HPLC/MS, which was used in statistical analysis for association testing is reported here, and resulted in a limit of detection of 1.0 ng/mL for CHA. The method was applied to samples from an all Army volunteer population during the BOLC MCT (n=87) with urine specimens taken approximately of 2, 8, 24 and 30 hours post-exposure. The detection of elevated CHA levels above LOD for all subjects at the 2-hour and 8-hour time intervals and in 80 of 85 subjects at the 24-hour and 30-hour time intervals reveals the potential for this assay to be used successfully to monitor for CS exposure.

A significant correlation was found in all time intervals and using urine collection time as a continuous variable. A strong and significant association in the analysis of CHA over time and CS concentration ($R = 0.910$, $R^2 = 0.827$, $p<0.01$) allowed for the development of a regression equation that could potentially be used for retrospective analysis of exposure to CS with a urine specimen drawn within 34 hours of exposure quantified levels of CHA and creatinine. Accounting for an exposed individual's potential baseline CHA levels from specific pharmaceuticals or exposure to certain glues, paints, or dyes containing 2-benzoic acid (a precursor in the biotransformation of CS to CHA), these regression equations have the potential to identify an individual's exposure to CS.

FUTURE RESEARCH

Future research is recommended to better understand the proportion of absorbed dose of a CS exposed individual that is attributed to the inhalation versus the dermal routes of exposure. This could perhaps be conducted by CHA metabolite analysis from chamber operators who only wear M40 masks and no chemical protective garments. MCT events limit trainee inhalation times to less than 15 s. In practical application scenarios, it is unlikely for exposed individuals to wear chemical protection respirators during the time of exposure. In addition, the CS concentration range measured in this study was low and narrow compared to previous studies, 0.086 – 4.900 mg/m^3. A similar study conducted during an MCT event where CS concentrations significantly exceed the maximum concentrations measured in this study could broaden the range predictability of a retrospective regression equation to higher levels. Future research that carried out urine specimen draws over 34 hours, possibly to 96 hours, may help researchers better understand the metabolism of CS in exposed populations and how elimination is affected by various personal characteristics. Studies may also be conducted to quantify potential exposure from residual "off-gassing" from clothing, hair, and skin to an individual who had spent time in a CS rich atmosphere with no personal decontamination.

DISCLAIMER

The views expressed in this article are those of the authors and do not reflect the official policies or positions of the Uniformed Services University of the Health Sciences, Department of the Navy, Department of the Army, Department of Defense, or the US Government.

Appendix

A. Results from personal monitoring for all study subjects

B. IRB Authorization Letter

C. Information Sheet and Consent Form

D. Pre-exposure questionnaire

APPENDIX A: RESULTS FROM PERSONAL MONITORING FOR ALL STUDY SUBJECTS.

Study ID	Day	SEG	Time (s)	CS (mg/m³)	Study ID	Day	SEG	Time (s)	CS (mg/m³)	Study ID	Day	SEG	Time (s)	CS (mg/m³)
1	2	26	408	2.633	32	1	17	330	1.48	62	2	26	419	2.686
2	2	26	390	2.564	33	2	21	337	3.792	63	3	33	262	4.275
3	3	36	337	4.198	34	1	12	349	1.769	64	2	21	316	2.923
4	2	25	347	2.861	35	2	21	353	3.408	65	2	21	337	3.093
5	2	27	433	3.426	36	SELF REMOVED FROM STUDY				66	2	23	315	1.941
6	1	13	339	2.463	37	1	13	318	1.968	67	3	34	359	3.144
7	2	27	412	3.170	38	2	27	432	2.988	68	1	17	330	1.58
8	2	26	400	3.165	39	3	32	418	3.677	69	3	33	244	3.878
9	2	24	411	2.285	40	1	13	330	2.159	70	3	33	237	4.403
10	2	24	398	3.064	41	1	11	390	0.96	71	3	36	328	3.622
11	2	27	435	3.517	42	1	14	313	2.164	72	3	35	388	4.354
12	2	24	411	2.741	43	1	11	441	1.012	73	3	36	78	2.637
13	1	14	325	2.237	44	1	14	326	1.716	74	3	32	417	3.761
14	2	22	313	3.133	45	SELF REMOVED FROM STUDY				75	3	34	332	3.337
15	2	23	326	1.963	46	2	25	371	3.513	76	3	37	315	4.405
16	1	17	302	1.579	47	1	15	323	1.848	77	SELF REMOVED FROM STUDY			
17	1	11	400	1.208	48	1	15	324	1.697	78	1	17	324	1.953
18	2	21	208	3.613	49	1	17	279	1.843	79	1	16	329	1.809
19	3	34	345	3.089	50	1	12	379	1.648	80	2	23	346	2.04
20	3	33	275	4.553	51	2	24	397	3.098	81	2	26	398	2.286
21	3	35	361	4.237	52	1	17	330	1.727	82	3	32	419	4.237
22	3	31	356	2.254	53	1	11	435	1.06	83	3	35	389	4.371
23	3	31	262	2.117	54	1	13	346	2.181	84	2	22	294	3.257
24	3	38	312	3.013	55	2	25	369	3.62	85	2	22	353	2.804
25	3	38	311	2.759	56	2	24	387	3.02	86	2	25	354	3.196
26	2	22	339	3.014	57	1	12	381	1.549	87	2	25	361	3.747
27	1	17	287	2.321	58	2	26	420	2.783	88	2	22	326	3.083
28	3	32	406	3.448	59	2	26	23	0.086	89	3	37	317	4.9
29	2	23	338	1.661	60	SELF REMOVED FROM STUDY				90	3	31	369	2.207
30	3	35	40	1.953	61	2	23	347	1.817	91	3	34	360	3.548
31	1	13	329	2.132										

APPENDIX B: IRB AUTHORIZATION LETTER

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
4301 JONES BRIDGE ROAD
BETHESDA, MARYLAND 20814-4712
http://www.usuhs.mil

July 02, 2015

MEMORANDUM FOR LTJG MACCON BUCHANAN, MS, USN, PREVENTIVE MEDICINE AND BIOSTATISTICS

SUBJECT: USU IRB #1 (FWA 00001628; DoD Assurance P60001) Approval of Protocol TO-87-3516 for Human Subjects Participation

Congratulations! The Initial Review for your No More Than Minimal Risk human subjects research protocol TO-87-3516 entitled "Evaluation of Urinary Biomarker Assay of Exposure to O-Chlorobenzylidene Malononitrile during U.S. Army Mask Confidence Training Exercises " was reviewed and approved for execution on July 02, 2015 by Dr. Edmund Howe, M.D., J.D., Chair IRB #1 under the provision of 32 CFR 219.110(b)(1)Suppl.F(3). This approval will be reported to the USU IRB #1 scheduled to meet on July 16, 2015.

The purpose of this observational study is to determine the concentration of 2-chlorohippuric acid in urine after participant exposure to CS (tear gas) vapor and particulates during the mandatory Mask Confidence Training (MCT) exercise conducted during Basic Office Leadership Course (BOLC). Volunteers will provide urine samples (10-20mls) prior to their regularly scheduled MCT exercise and again at approximately 2, 8 and 24 hours post MCT and wear a personal air sampling device (for determination of individual CS exposure). Up to 120 individuals are authorized to participate in this study. This study is conducted in concert with protocol TO-87-3564, entitled "Respiratory Health Effects of CS Riot Control Agent Exposure in Army Medical Department Basic Officer Leadership Course Trainees."

You are reminded that local Command approval must be obtained prior to implementation of research activities.

Authorization to conduct protocol TO-87-3516 will automatically terminate on July 01, 2016. If you plan to continue data collection or analysis beyond this date, IRB approval for continuation is required. Please submit a USU Form 3204 A/B, application for continuing approval 60 days prior to your termination date. You will receive a reminder from IRBNet.

You are required to submit amendments to this protocol, changes to the informed consent document (if applicable), adverse event reports, and other information pertinent to human research for this project in IRBNet. No changes to this protocol may be implemented prior to IRB approval. If you have questions regarding this IRB action or questions of a more general nature concerning human participation in research, please contact Micah Stretch at 301-295-9534 or micah.stretch@usuhs.edu.

Edmund G. Howe, M.D., J.D.
Chair, IRB #1

Concur/ Nonconcur

JUL - 2 2015

Arthur L. Kellerman, MD, MPH
Professor and Dean, School of Medicine

Date

This document has been signed electronically.

"Electronic Signature Notice: In accordance with the "Government Paperwork Elimination Act" (GPEA) (Pub.L. 105-277; codified at 44 USC 3504); Federal and DOD applicable instructions, directives and regulations, documents have been electronically signed and authorized by all who have been required to do so. These signatures have the same effect as their paper-based counterparts. Verification is retained within our protected electronic records and audit trails."

Learning to Care for Those in Harm's Way

APPENDIX C: INFORMATION SHEET AND CONSENT FORM

UNIFORMED SERVICES UNIVERSITY

BETHESDA, MARYLAND

This consent form is valid only if it contains the "USUHS IRB Approved" stamp. Do not sign this form or participate in this research if the IRB stamp is not present or if it has expired.

Consent for Voluntary Participation in a Research Study

1. **INTRODUCTION OF THE STUDY**

You are being asked to participate in concurrent research studies entitled, "Evaluation of Urinary Biomarker Assay for Exposure to O-chlorobenzylidene malononitrile (CS Riot Control Agent) during U.S Army Mask Confidence Training (MCT) Exercises" and "Self-Reported Respiratory Outcomes of CS Riot Control Agent Exposure in AMEDD Basic Officer Leadership Course Trainees" coordinated by the Uniformed Services University of the Health Sciences (USUHS), Bethesda, Maryland. Your participation is voluntary. Refusal to participate will not result in any punishment or loss of benefits to which you are otherwise permitted. Please read the information below, and ask questions about anything you do not understand, before deciding whether to take part in these studies.

2. **PURPOSE**

a. **"Evaluation of Urinary Biomarker Assay for Exposure to o-chlorobenzylidene malononitrile (CS Riot Control Agent) during U.S Army Mask Confidence Training (MCT) Exercises" (Study A)**

The purpose of this study is to investigate the relationship between exposure to CS riot control agent and the concentration of the metabolite 2-chlorohippuric acid (CHA) found in urine after exposure. A better understanding of the relationship between exposure and metabolite concentration would assist medical and law enforcement professionals to more effectively perform their duties in CS exposure cases.

b. **"Self-Reported Respiratory Outcomes of CS Riot Control Agent Exposure in AMEDD Basic Officer Leadership Course Trainees" (Study B)**

The objective of this research study is to document any relationship of CS exposure to specific acute respiratory symptoms in U.S. Army Officers enrolled in the Army Medical Department (AMEDD) Basic Officer Leadership Course (BOLC). The study will examine any potential association between CS exposure during MCT and new-onset acute respiratory outcomes post-exposure at two points in time (24 hours and 14 days). Previous studies have described an association between CS exposure (during MCT) and increased rate of acute respiratory illness and so results from this study could inform policy regarding BOLC training to reduce respiratory morbidity and lost duty days.

3. **PROCEDURES**

Depending on which part of this research you agree to participate in, you will be asked to do different things.

If you agree to participate in the biomarker study (study A), you will be asked to provide urine samples (approximately 10-15ml or 2-3.5 teaspoons) the day of your regularly scheduled BOLC training in the morning before your MCT exercise and again at approximately 2, 8 and 24 hours after you complete the MCT exercise. Within 24 hours of collects, samples will be sent to the CDC for analysis. In addition, you will be fitted with a personal air-sampling pump during the MTC exercise. The pump will allow us to know how much CS vapor and particulates you have been exposed to and is a standard occupational exposure sampling method.

If you agree to participate in the respiratory outcome study (study B), you will be asked to complete a brief questionnaire prior to your MCT exercise and again at approximately 24 hours and 14 days after completing the MCT exercise. You will be asked to complete these questionnaires at the same time urine samples are collected for study A. The questionnaires ask about your basic demographic information, general respiratory health and symptoms you may have experienced after completing the MCT exercise. You may skip any questions that make you feel uncomfortable. To understand the relationship (if any) between your self-reported respiratory health/ symptoms and CS exposure, we will need to use the data collected from your personal air-sampling pump used in study A. **As a result, to participate in study B you must also participate in study A.**

Standard operating procedures, personal protective equipment, training tasks, and time in the CS chamber will not be altered in any way by either study.

4. **POSSIBLE BENEFITS FROM BEING IN THIS STUDY**

These projects are being conducted for research purposes only and are not intended to directly benefit you.

5. COMPENSATION

There is no financial compensation for your participation in this research.

6. POSSIBLE RISKS OR DISCOMFORTS FROM BEING IN THIS STUDY

There are no known expected risks or discomfort from being in either or both studies. You may skip any survey questions that make you feel uncomfortable.

7. RIGHT TO WITHDRAW

You may decide to stop taking part in either or both studies at any time. Your relations with both BOLC and USU faculty and staff will not be changed in any way if you decide to end your participation in the study.

8. RECOURSE IN THE EVENT OF INJURY

If at any time you believe you have suffered an adverse event as a result of participating in this research project, you should contact the Director of Human Research Protections Programs at the Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814-4799 at (301) 295-9534. This office can review the matter with you, provide information about your rights as a subject, and may be able to identify resources available to you. If you believe the government or one of the government's employees (such as a military doctor) has injured you, a claim for damages (money) against the federal government (including the military) may be filed under the Federal Torts Claims Act. Information about judicial avenues of compensation is available from the University's General Counsel at (301) 295-3028.

9. PRIVACY AND CONFIDENTIALITY

All information you provide as part of this study will be confidential and will be protected to the fullest extent provided by law. Your surveys and urine samples will contain no personally identifiable information. Only the key linking your name to a unique study identifier and the consent form will contain your name. Both documents will be physically secured and held separate from the completed surveys and urine samples. The CDC will receive coded samples and will destroy any remaining samples once the analysis has been completed. Once the final round of surveys is complete and data are linked in the analysis data set, the masker key file will be destroyed. All records related to this study will be accessible to those persons directly involved in conducting this study and members of the USUHS Institutional Review Board (IRB), which provides oversight for protection of human research volunteers. In addition, other federal agencies that help protect people who are involved in research studies may need to see the information you give us. Other than those groups, records from this study will be kept confidential to the fullest extent of the law. Scientific reports that come out of this study will not use your name or identify you in any way, and all reporting of results from this study will be in the aggregate. As a military service member, please be advised that under Federal Law, your confidentiality cannot be strictly guaranteed.

USUHS IRB APPROVED
02 JULY 2015
Expires: 01 JULY 2016

10. CONTACT FOR QUESTIONS OR PROBLEMS

If you have questions about this study, please contact either, LTJG Maccon A Buchanan (maccon.buchanan@usuhs.edu) or CPT Matthew Holuta (matthew.holuta@usuhs.edu) . If you have questions about your rights as a research subject, you should call the Director of Human Research Protections Program at USUHS at (301) 295-9534. The director is your representative and has no connection to the researcher conducting this study.

****IF YOU HAVE ANY QUESTIONS PLEASE FEEL FREE TO ASK THEM****

SIGNATURE OF RESEARCH PARTICIPANT OR LEGAL RESPRESENTATIVE

You have read (or someone has read to you) the information in this consent form. You have been given a chance to ask questions and all of your questions have been answered to your satisfaction.

BY SIGNING THIS CONSENT FORM, YOU FREELY AGREE TO TAKE PART IN THE RESEARCH IT DESCRIBES.

I consent to participate in the study (A): "Evaluation of Urinary Biomarker Assay for Exposure to O-chlorobenzylidene malononitrile (CS Riot Control Agent) during U.S Army Mask Confidence Training (MCT) Exercises"

Signature	Print Name	Date

I consent to participate in the study (B): "Self-Reported Respiratory Outcomes of CS Riot Control Agent Exposure in AMEDD Basic Officer Leadership Course Trainees"

Signature	**Print Name**	**Date**

SIGNATURE OF INVESTIGATOR

You have explained the research to the participant, or his/her legal representative, and answered all of his/her questions. You believe that the volunteer subject understands the information described in this document and freely consents to participate.

Investigator's Signature	Date (must be the same as the participant's)

Investigator's Printed Name

APPENDIX D: PRE-EXPOSURE QUESTIONNAIRE

Pre-Mask Confidence Training Questionnaire

1. **Use the scale below to rate the <u>average severity</u> of your symptoms over <u>the last three (3) days</u>:**

(please mark one circle for each symptom)

	Mild Symptoms	Moderate Symptoms	Severe Symptoms	Do not have this symptom
Sneezing	○	○	○	○
Runny Nose	○	○	○	○
Stuffy Nose	○	○	○	○
Sore Throat	○	○	○	○
Cough	○	○	○	○
Headache	○	○	○	○
Feeling Tired	○	○	○	○
Chills	○	○	○	○

2. Do you think that you have a <u>cold</u> or **may be getting a** cold? *(choose one)*

   □ Yes
   □ No
   □ Don't know

3. **In the past <u>three (3) days</u>, have you taken medication for any of the following reasons?**

<u>Yes</u>	<u>No</u>	
□	□	A cold
□	□	Cough
□	□	Fever
□	□	Runny nose

4. **In the past <u>three (3) days</u>, have you gone to sick call for any of the following reasons?**

<u>Yes</u>	<u>No</u>	
□	□	A cold
□	□	Cough
□	□	Fever
□	□	Runny nose

5. Has a doctor or other health professional ever told you that you have respiratory allergies?
(choose one)

- □ Yes
- □ No
- □ Don't know

6. According to the scale below, please rate the <u>average severity</u> of your respiratory allergies over the past <u>three (3) days.</u> *(choose one)*

- □ I don't have respiratory allergies

Very Mild	Mild	Moderate	Severe	Very Severe	No Symptoms
○	○	○	○	○	○

7. Have you used any medications to ease your respiratory allergies in the past <u>three (3) days</u>?
(choose one)

- □ I don't have respiratory allergies
- □ Yes
- □ No

8. Has a doctor or other health professional ever told you **that you have asthma?** *(choose one)*

- □ Yes
- □ No
- □ Don't know

9. According to the scale below, please rate the average severity of your asthma symptoms in the past <u>three (3) days</u>. *(choose one)*

- □ I don't have asthma

Very Mild	Mild	Moderate	Severe	Very Severe	No Symptoms
○	○	○	○	○	○

10. Have you used any medication(s) or an inhaler to control your asthma symptoms in the past <u>three (3) days</u>? *(choose one)*

- □ I don't have asthma
- □ Yes
- □ No

11. **In the past year, how many days did you miss work or school due to respiratory illness?** *(choose one)*

    - □ No days missed
    - □ 1 or 2 days missed
    - □ 3 or more days missed

12. **In the past week, has your roommate had any of the following: a cold, cough, fever, or runny nose?** *(choose one)*

    - □ Yes
    - □ No
    - □ Don't know
    - □ No Roommate

13. **In the past week, has anybody in your platoon had any of the following: a cold, cough, fever, or runny nose?** *(choose one)*

    - □ Yes
    - □ No
    - □ Don't know

14. **Since arriving at Camp Bullis, has anybody staying in your tent had any of the following: a cold, cough, fever, or runny nose?** *(choose one)*

    - □ Yes
    - □ No
    - □ Don't know

15. **On average, about how many hours of sleep per night did you get in the past two weeks?** *(choose one)*
    [Seep is defined from the time you laid down until the time you got out of bed, minus any time intentionally spent awake (example: watching TV)]

    - □ Less than 7 hours of sleep
    - □ 7 hours of sleep or more

16. **Have you smoked at least 100 cigarettes in your entire life?** *(choose one)*

    - □ Yes
    - □ No
    - □ Don't know

17. **Do you now smoke cigarettes every day, some days or not at all?** *(choose one)*

    - □ Every day
    - □ Some Days
    - □ Not at all

18. Have you <u>ever</u> been exposed to CS gas during a military mask confidence training exercise?
(choose one)

- □ Yes, more than one time
- □ Yes, one time
- □ No, I have never completed Mask Confidence Training

19. Age **_____**

20. Gender

- □ Male
- □ Female

21. Height: _____ feet _____ inches

22. Weight: _____ pounds

23. What was your most recent Army Physical Fitness Test (APFT) Score?
(choose one)

- □ Less than 180
- □ 180 - 269
- □ 270 or greater
- □ I have not taken an APFT

24. What is your branch of service in the Army Medical Department (AMEDD)?

- □ Dental Corps (DC)
- □ Medical Corps (MC)
- □ Medical Service Corps (MS)
- □ Medical Specialist Corps (SP)
- □ Nurse Corps (AN)
- □ Veterinary Corps (VC)
- □ Other_____

25. To which Company are you assigned while completing BOLC? *(choose one)*
(This refers to your BOLC Company (not A Co. 187th Med BN).

- □ A Company
- □ B Company
- □ C Company
- □ D Company
- □ Other_____

26. To which Platoon are you assigned while completing the BOLC? *(choose one)*

- □ 1st Platoon
- □ 2nd Platoon
- □ 3rd Platoon
- □ 4th Platoon
- □ Other_____

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