FIELD EVALUATION OF SULFICARB THERMAL DESORPTION TUBES DURING SHORT TERM, PASSIVE SAMPLING OF ISOFLURANE IN A UNIVERSITY LABORATORY ANIMAL MEDICINE FACILITY

by

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DEDICATION

This work is dedicated to my daughters, Shannon and Angela. They have given me the drive and desire to be a strong role model as a Mom, Scientist, U.S. Army Officer, Leader, and my most rewarding role as a Grandmother to Bryson, Hayden and Hunter.

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ABSTRACT

Field Evaluation of Sulficarb Thermal Desorption Tubes During Short-Term, Passive Sampling of Isoflurane in a University Laboratory Animal Medicine Facility

CPT Bonnie Parks, 2019

Thesis directed by: Dr. Edward Benchoff, CDR, MSC, USN

Objective: The objective of this study was to investigate the levels of agreement between passive samplers and an active sampling method during short-term isoflurane sampling of animal surgeries in a University Laboratory Animal Medicine (LAM) facility.

Methods: Short-term passive sampling was performed in the USU veterinary operatory using thermal desorption tubes, passive badges and Anasorb tubes. Bland-Altman plots were constructed to determine the level of agreement between sampler types.

Results: Bland-Altman indicates 50% level of agreement between thermal desorption tubes and passive badges, and 35% between thermal desorption tubes and active sampling.

Discussion: Thermal desorption tubes reported a higher concentration than passive badge and active sampler suggesting thermal desorption tubes are more sensitive to the collection of isoflurane during area sampling.

Conclusion: This study provided a baseline for future studies to further investigate the levels of agreement between different devices for isoflurane in a veterinary operatory.

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CHAPTER 1: Introduction

1.1 Statement of Purpose

The objective of this study was to compare agreement between a passive sampling method not widely used in the environmental and occupational health field, an approved passive dosimeter badge sampling method and an active sampling method while conducting short-term (15 minute) sampling of isoflurane during animal surgeries. The passive samplers included stainless steel thermal desorption tubes containing SulfiCarb carbon molecular sieve sorbent (Markes International, Llantrisant, UK) and conventional passive dosimeter badges (ATLabs #574; ChemDisc™ Monitor for Halogenated Anesthetic Gases). The active sampling method followed the requirements of the OSHA Method 103: Enflurane, Halothane, Isoflurane which utilized Anasorb 747 226-81A synthetic carbon sampling tubes (SKC, Inc., Eighty Four, PA).

1.2 Introduction

1.2.1 History of Isoflurane

Isoflurane was developed around 1970 by Dr. Ross Terrell and his associates as a safer-alternative to older halogenated inhalational anesthetic agents, such as halothane and methoxyflurane.

1.2.2 Properties of Isoflurane

Isoflurane (2-chloro-2-(diflouromethoxy)-1,1,1-trifluorethane) is a halogenated methyl ethyl ether with a difluoromethyl group and a fluorinated ethyl group (30). It has a mildly pungent odor (7) and according to Goa, Marshall and Vickers, the odor threshold of approximately 200 parts per million (ppm) can be detected by half of the exposed

population (30) (13). Isoflurane is a stable, clear, colorless liquid (30). Isoflurane has a molecular weight of 184.5 g/mol, a vapor pressure of 238 mm Hg at 20°C, and a boiling point of 48.5°C.

Isoflurane has been shown to pose few adverse health effects as an inhalation anesthesia for both human and animal patients. Isoflurane provides a quick onset and recovery from the effects of anesthesia for the patient thereby potentially reducing the occupational exposure to the healthcare providers (7; 30). Occupational exposure to halogenated anesthetics, including isoflurane, can impact the liver, the central nervous system, the cardiovascular system, the respiratory system, and the kidneys. Furthermore, isoflurane affects the muscular system, the endocrine system, the hematological system, the immune system and reproductive system (29).

1.2.3 Public Health and Military Relevance

With over 490,000 medical and veterinary professionals potentially exposed to harmful levels of isoflurane in the United States per year, it is vital for the environmental health and safety community to explore new sampling methods (44). New sampling or assessment methods can be more reliable, provide greater confidence in the results and lead to work method changes resulting in reducing the health effects experienced by the workers.

The Department of the Army (DA) as outlined in Technical Bulletin 510 (TB MED 510); Guidelines for the Recognition, Evaluation, and Control of Occupational Exposures to Waste Anesthetic Gases currently prescribes passive sampling with dosimeter badges and active sampling utilizing OSHA Method 103 (41). An analytical tool frequently used to compare medical devices or assess the agreement between two

measurement methods is the Bland-Altman. The Bland-Altman is frequently used in deciding if a new device or method can replace an older device or method. A novel approach for sampling WAGs in the operating room is using stainless steel Sulficarb thermal desorption tubes (Markes International, Llantrisant, UK). The Bland-Altman is not frequently used in occupational settings but appropriate in determining agreement between a new method and two older methods (14). Determining the level of agreement between the sampling methods has not been done.

1.2.4 Potential Health Impacts

The potential health impacts of workers to inhalation anesthetics include short-term, acute adverse health impacts as well as long-term, chronic adverse health impacts.

1.2.4.1 Acute Health Effects

The negative health impacts associated with acute exposure to the waste anesthetic gases, such as isoflurane, include nausea, headache, irritability, reduced reaction times, fatigue, loss of consciousness, and loss of coordination (42). Acute occupational exposure to isoflurane has the potential to impair the medical or veterinary staffs' ability to perform their duties competently.

1.2.4.2 Chronic Adverse Health Impacts

Occupational exposures to inhalational anesthetics, to include isoflurane, are also associated with chronic health impacts to include spontaneous abortion, premature birth, low birth weight, DNA damage, liver toxicity, kidney toxicity and neurological damage (24).

1.2.4.3 Common Exposure Controls

The exposures associated with isoflurane use in the veterinary operatory are due to waste anesthetic gases (WAG). WAGs are small quantities of anesthetic gases which leak into the air. The leaks can occur during intubation of the animal, or from the patient's anesthetic breathing circuit which can include the exhaled breath of the patient during and after the procedure (38). WAGs can leak from connectors in the vaporizer or scavenging equipment (38). The control and elimination of WAG gas exposure within the operatory, requires a scavenging system be used. A scavenging system may be an active system or a passive system. The active system which draws WAG through a vacuum exhaust system and evacuates it away from the operating room. The passive system collects the waste anesthetic gases into a local canister attached to the breathing circuit. Saber and Hougaard (2009) compared the levels of isoflurane in the operatory using both active and passive methods. With the active method of scavenging the isoflurane levels were <0.15 ppm on average while the isoflurane levels in the operating room in which passive scavenging was utilized found peak levels >300 ppm (30).

1.2.5 Air Sampling and Analysis

Assessing the veterinary staffs' exposure to isoflurane is currently performed in one of several ways based on the data needs, resources and experience of the industrial hygienist. The current methods for sampling the occupational environment for isoflurane includes the use of passive dosimeter badges and an active sampling method utilizing guidance from the OSHA Method 103 (Enflurane, Halothane, Isoflurane). The active sampling method requires that air be drawn through a sorbent media via an air sampling pump. Passive sampling operates through the maintenance of a concentration gradient between isoflurane in the ambient workplace air and the space inside the sampler. This

gradient enables isoflurane to diffuse into the sampler's sorbent material at a constant rate (6). Technical Bulletin 510 (TB MED 510); Guidelines for the Recognition, Evaluation, and Control of Occupational Exposures to Waste Anesthetic Gases recommends either active or passive sampling for personal and area sampling (41). As the objective of this study was to assess the agreement between the samplers and not to investigate the exposure of veterinary personnel, area sampling was performed.

This research compares, a different method of sampling the occupational environment for isoflurane, Markes stainless steel Sulficarb thermal desorption tubes (Markes International, Llantrisant, UK). Like the passive badges, the sorbent in the thermal desorption tubes collect isoflurane due to a concentration gradient, requires no additional equipment and can be easily clipped on to the lapel at the user level, but unlike the passive badges which can be used only once, thermal desorption tubes can be reused up to 100 times before the sorbent needs replacing. Thermal desorption tubes have advantages over the active sampling method. The active sampling method requires additional equipment such as an air sampling pump calibrated to a known flow rate. These ancillary pieces of equipment rely on an electrical source or charged batteries to operate the pump and the calibrator. The active sampling method requires training. Thermal desorption tubes can be used by the medical or veterinary staff minimal training. One of the greatest advantages of thermal desorption tubes is in the analysis of the samples. Thermal desorption tubes rely on heat to desorb the isoflurane from the sorbent located in the tube after sampling. Heat desorption preserves the total mass of the isoflurane collected in the sample. Passive badges and the active sampling tubes utilize

solvent extraction, which, in general can be up to 1000 times less sensitive than thermal desorption methods (45).

By comparing the thermal desorption tubes with the passive badges and the active sampling method in a field environment, the levels of agreement between the methods may be identified.

HYPOTHESIS AND SPECIFIC AIMS

Hypothesis: There will be no statistically significant difference in the mean isoflurane concentrations measured by the thermal desorption tubes, conventional passive badges, and active sampling method using the reference sampling method, OSHA Method 103 (Enflurane, Halothane, Isoflurane) during short-term (15 minute) sampling of isoflurane anesthetic agent.

Within this hypothesis, there are two specific aims;

Specific Aim #1

Obtain isoflurane measured concentration data using Sulficarb thermal desorption tubes, conventional passive badges and an active sampling method by performing area sampling in the Uniformed Services University Laboratory Animal Medicine (LAM) research facility.

Chapter 2: Literature Review

2.1 At Risk Population

There are over 494,000 medical and veterinary personnel in the United States potentially exposed to inhalation anesthetics annually (44). Surgeons, surgical technicians, nurses, anesthesiologists, nurse anesthetists, veterinarians, veterinary animal technicians, post anesthetic care unit nursing staff and researchers are potentially exposed to the inhalation anesthetics. Exposures can occur from the breathing circuit, or the waste anesthetic gas (WAG) scavenging system. The exhaled breath of the patient or animal, both during and after the procedure is complete can also pose a hazard to the worker (34; 44). Smith and Bolon (2006) suggest exposed workers may expose others to their contaminated exhaled breath. Co-workers and family exposed to this "anesthetic pollution" could experience similar symptoms as those working with anesthetized patients or animals (34). The potential exposures lie with not just the civilian population but the military population as well.

The Department of Defense utilizes the United States Army Veterinary Corps veterinarians and veterinary technicians worldwide as the sole provider of care to Military Working Animals, which includes working dogs, horses, and service members' privately-owned animals. The Army Veterinarian is also involved in research devoted to protecting the Warfighter and supporting the National Military Strategy (35). Military Working Animals are cared for by Army veterinarians and ensure they are in peak physical condition. Currently, isoflurane is one of several inhalation anesthetics utilized by the U.S. Army (41).

2.2 Occupational Exposure Limits

Isoflurane currently has no Occupational Safety and Health Administration (OSHA) Permissible Exposure Limit (PEL) as an Occupational Exposure Limit (OEL). Likewise, the National Institutes of Occupational Safety and Health (NIOSH), American Industrial Hygiene Association (AIHA), or American Conference of Governmental Industrial Hygienists (ACGIH) has not established or developed an OEL for isoflurane. In 1977, NIOSH developed a Recommended Exposure Limit (REL) of 2 ppm (averaged over 60 minutes) for halogenated anesthetics such as halothane (42). However, isoflurane was not yet in use in 1977 so this OEL was not applicable to its use (12; 39). Although the OEL is not applicable to isoflurane, and with lack of any additional guidance, the Department of the Army (DA), within Technical Bulletin 510: Guidelines for the Recognition, Evaluation, and Control of Occupational Exposure to Waste Anesthetic Gases (TB MED 510), has adopted the 1977 NIOSH recommendation of 2 ppm ceiling for personal sampling (41). The NIOSH REL is not enforceable by OSHA. Personal sampling exposure limits do not apply to area sampling. Area sampling may be utilized.

2.3 Toxicokintetics

Isoflurane has a low blood-gas partition coefficient which leads to quick induction and recovery (7; 9; 10; 30). As the anesthetic gas moves into and out of the vascular system, equilibrium will be reached and the concentration of isoflurane in the body will cease to increase (29).

Halothane was implicated in producing more liver metabolites, such as trifluoroacetic acid and inorganic fluoride (20). While isoflurane also produces these

metabolites, it moves more quickly through the body when compared with older inhalation anesthetics, such as halothane. This reduced time in the body reduces the amount of metabolites produced in the liver from 15-20% biotransformation with halothane to 0.2% biotransformation with isoflurane (5). Halothane was shown to be associated with fulminant hepatitis with a potentially lethal outcome. This association has also led to the identification of immunological mechanisms with halothane not seen with isoflurane (20; 29).

Preckel and Bolten (2005) state the earliest volatile anesthetics, such as chloroform, contained chlorine for halogenation (29). The newer inhalation anesthetics replaced the chlorine with fluorine. This change resulted in the patient or animal metabolism being more resistant to the anesthetic (29).

Isoflurane is highly desirable as an anesthetic because only about 0.2% is biotransformed in the surgical patient (37). Saber and Hougaard (2009) go on to say much of the dose is eliminated unchanged through the exhaled breath. The low degree of biotransformation leads to the creation of fewer toxic metabolites, such as trifluoroacetic acid and inorganic fluoride, than previous halogenated anesthetic agents (30).

2.3.1 Absorption and Distribution

The main route of exposure of isoflurane in the occupational setting is through inhalation. The occupation exposure can occur via the exhaled breath of the patient in a medical setting or an animal in a veterinary setting. Exposure has also been associated with leaks from the breathing circuit of the anesthetic equipment into the operating room environment (30). Absorption of isoflurane can also occur via the skin. While this is not a common route of exposure, it typically occurs during spills while filling the anesthetic

vaporizer (38). Post exposure, isoflurane is distributed and reaches equilibrium quickly within the body (30).

2.3.2 Biotransformation and Elimination

Isoflurane has a lower solubility and faster elimination from the body than older anesthetics. Saber and Hougaard (2000) describe the elimination process as having 3 phases, short-phase, inner-phase and long-phase with various half-times. Short phase is the speed of elimination over the alveolar membrane with a half-time of 2 minutes. The inner-phase, is the elimination of isoflurane from the organs and has a half-time of 19 minutes while the long-phase half-time is 233 minutes for the elimination of isoflurane from the muscle and adipose tissue (30). Compared with short-phase elimination of ether at approximately 7 minutes and halothane at 14 minutes, isoflurane shows a decrease in elimination times (4). This faster elimination leads to faster return to normal mental function as well as the reverse of circulatory, neuromotor and respiratory depression (9) (30).

2.4 POTENTIAL HEALTH IMPACTS

2.4.1 Acute Health Effects of Isoflurane

The health effects for acute exposure to isoflurane include mild symptoms from irritation of the skin, mouth and throat to headaches, dizziness, drowsiness, vomiting as well as affect the central nervous system and the cardiovascular system (46). In addition to nasal irritation, Saber and Hougaard (2009) have identified the possibility of increased metabolite sensitivity to isoflurane due to isoflurane exposure (30). According to Hoerauf et al. (1999) there is also the possibility of short-lived DNA strand breaks which can occur during occupational exposure to isoflurane anesthesia. This paired study in

which medical personnel, exposed versus non-exposed to waste anesthetic gases, compared sister chromatid exchanges in lymphocytes to determine if there was an increase in DNA strand breaks. An 8-hour time weighted average concentration of 5.3 ppm for isoflurane and 12.7 ppm nitrous oxide over the course of a week resulted in genetic damage equivalent to smoking 11-20 cigarettes per day. However, it was unclear if the damage was a result of the isoflurane or the nitrous oxide or the combination of the two waste anesthetic gases (16).

2.4.2 Chronic Health Effects of Isoflurane

Damage to DNA has been shown to have not only acute effects in occupationally exposed workers but has chronic health effects as well (1; 28; 30; 33).

In conducting research on the effects of WAG, non-specified waste anesthetic gas, Deng et al (2017) highlighted several studies relating the length of time of exposure to chronic health effects (8). Turkan, Aydin and Sayal (2005) attributed at least three years of occupational exposure to inhalation anesthetics to oxidative stress in the operating room staff . By comparing blood samples, consisting of superoxide dismutase and glutathione peroxidase, of the 30 participants to controls, the antioxidant activity was significantly lower suggested the staff were under oxidative stress (40). Costa et al (2014) considered occupational exposures of 15 medical residents from Anesthesiology, General Surgery, Neurosurgery and Orthopedics, exposed to mainly isoflurane as well as sevoflurane and nitrous oxide. The residents were followed for 22 months with blood sampling conducted at 8, 16 and 22 months. The results of this study concluded there was an increase in DNA lesions and oxidative stress during this time frame with the

highest levels occurring at the 16 month mark (28). Additionally, long term isoflurane exposure can lead to sensitivity and in rare cases, anaphylactic reactions (33).

2.5 Occupational Exposure Control

According to Saber and Hougaard (2009), occupational exposure to the halogenated anesthetic agent, isoflurane, used in the veterinary operatory, may occur during the surgical procedure due to leaks while opening and closing induction chambers, leaks around the animal's face mask/nose cone, leaks in the delivery system, or leaks in any of the connection tubes or connectors, and well as the ventilator itself.

These WAG, can occur when the waste anesthesia scavenging system is not operating correctly. The scavenging system can be either active or passive in nature. The active scavenging system consists of an anesthetic circuit being connected to an evacuation system which removes the anesthesia via pulling the air out of the area and refilling with fresh clean air. The passive system is designed to have a collection canister with an activated charcoal sorbent which traps the isoflurane while the animal is under anesthesia. The air is then exhausted back into the room (11). The U.S. Army Technical Bulletin 510 (TB MED 510) states the scavenging systems are designed to collect gases and vapors that are vented from the breathing circuit. The gases are then directed to a safe area for exhaust or collection (41).

The vaporizer allows the veterinary staff to control the inhalation anesthetic, isoflurane, given to the patient or animal. The machine allows for the mixture of oxygen, isoflurane and ambient air to be delivered to the subject either by mask induction or intubation. Liquid isoflurane is added to the vaporizer which allows vaporization of isoflurane to take place in the machine (30).

TB MED 510 notes there is no Federal standard for WAG exposures, however facilities can be cited for high WAG exposures under the General Duty Clause. The General Duty Clause is defined by OSHA under the Occupational Safety and Health Act, Section 5(a)(1) which requires each employer to furnish to each of its employees a workplace that is free from recognized hazards that are causing or likely to cause death or serious physical harm to its employees and shall comply with occupational safety and health standards promulgated under this act (26). TB Med 510 further defines the need for a combination of engineering and administrative controls such as the use of gas scavenging equipment, general dilution ventilation, proper maintenance of the equipment, application of work practices by anesthetists to minimize the release of anesthetic agents into the room and application of surgical procedures designed to reduce the potential for WAG exposure (41).

2.5.1 Engineering Controls

OSHA has defined a hierarchy of various control measures to protect the worker from occupational hazards. The first control would be the elimination of the hazard followed by substitution with a less hazardous material. In this work environment neither of these control measures are an option as an aesthetic agent is needed for surgical procedures and isoflurane is the replacement for halothane. OSHA then suggests the use of engineering controls. In this study, the use of isoflurane as a substitute for halothane is a control measure frequently used (27).

2.5.1.1 Active Scavenging

An active scavenging system is used to control for WAG via an active airflow system. This system may include a downdraft surgery table, fume hood or a local exhaust

system (11). The proper use of active scavenging systems has been shown to reduce the occupational exposures to isoflurane to well within the occupational exposure limits (30). Mulvenon (2015) states an there is an 86% reduction in isoflurane exposure when an active scavenging system is utilized (23).

2.5.1.2 Passive Scavenging

Passive scavenging systems are used when an active system is not available. The passive scavenging system utilizes a charcoal absorptive material within a canister for removal of WAG from the anesthetized animals breathing circuit before air is released back into the roomt (11). The absorptive canisters require frequent weighing to ensure the collected isoflurane does not exceed the manufacturers predetermined weight. While it is recommended that small canisters be disposed of properly upon the weight reaching 50g more than the initial weight and large canisters may see a weight increase of 200g, Smith and Bolon (2003) showed that cannisters still well within the recommended disposal weight can have isoflurane breakthrough with peak emissions at the port being greater than 100 ppm (15; 36). This also explains why the charcoal absorbent canisters when full or meet the specified weight must be managed as a hazardous material (11). Eliminating occupational exposures to isoflurane while using only a passive scavenging system is most probably impossible and secondary measures such as utilizing an active scavenging system or adding a facemask with a built-in evacuation line to reduce or eliminate exposure may be necessary (36).

2.5.2 Administrative Controls

According to OSHA, administrative controls are used to help change the way people work. This work culture change can be accomplished with organizational,

published standard operating procedures in which everyone is expected to adhere (27). TB Med 510 outlines recommended work practices to reduce occupational exposure by conducting daily inspections of the scavenging equipment to check for leaks throughout, verify all connections and fittings are tight and functioning properly prior to administering isoflurane, and to fill vaporizers in a well ventilated area (41).

2.6 SAMPLING

2.6.1 Active Sampling

Active sampling is conducted following guidance contained in Occupational Safety and Health Administration (OSHA) Enflurane, Halothane, Isoflurane Method 103. The active sampling method requires additional equipment, such as a calibrated air pump and tubing to connect the pump to the media, as well as laboratory support for analysis (25). The OSHA Method 103 uses solvents to remove the isoflurane from the sorbent. Solvent based extraction is problematic for several reasons. The first reason being the loss of analyte due to the inefficiency of this process. During solvent desorption only $1/1000^{th}$ of the available sample can be detected during the analysis process. The second area of concern is the use of hazardous materials, such as toluene as the solvent in the process. And lastly, OSHA Method 103 stipulates the minimum amount of air sampled per minute for short term sampling but relies on the researcher to determine the time limit (2; 25).

2.6.2 Passive Sampling

Passive badges are easy to use, require no specialized training to use, are small and lightweight, and do not require energy to collect isoflurane (6). This study used both traditional passive dosimeter badges, X574 provided by Assay Technologies, as well as

Markes Stainless Steel thermal desorption tubes with Sulficarb sorbent. Assay Technologies, the manufacturer of the passive badges, recommends a minimum of 30 minutes sampling time to allow the sorbent an opportunity to collect enough isoflurane to provide analytical results above the LOQ (2). The passive badges use a modified OSHA 103 Method for analysis, which also uses a liquid solvent to desorb the isoflurane from the media.

2.7 TYPES OF SAMPLING MEDIA

2.7.1 Thermal Desorption

Axial diffusive samplers such as stainless-steel thermal desorption (TD) tubes containing SulfiCarb molecular sieve sorbent (Markes International, Llantrisant, UK) measuring 89 mm x 6.35 mm outer diameter with a 5 mm inner diameter is designed for passive sampling of volatile organic compounds (VOC's) such as isoflurane (22).

Thermal desorption allows for concentration enhancement and a virtually full transference of analytes to the gas chromatography process, whereas methods requiring solvent extraction result in a significant reduction in sensitivity (45). The method requires heat to desorb the isoflurane from the sorbent. This contrasts with standard methods which use solvents to extract collected isoflurane from the sorbent media. Solvent based methods dilute the sample thereby reducing the analytical sensitivity up to factor of a thousand-fold (45).

Benchoff (2017) investigated and validated the performance of stainless-steel thermal desorption tubes containing SulfiCarb sorbent to Carbograph 5TD for short-term (<15 minutes) passive sampling of isoflurane. SulfiCarb thermal desorption was shown to have an excellent desorption efficiency of 99-100%, had good stability when stored at

ambient as well as refrigerated temperatures, had an uptake sampling rate of 4.4 ng ppm^{-1} min^{-1}, retained isoflurane effectively, and was not affected significantly by humidity (3). Woolfenden adds additional advantages found with the use of thermal desorption tubes by stating a pack of 10 prepacked and preconditioned tubes is available at similar cost to one 6-L passivated canister. Thermal desorption tubes are clean and ready for immediate reuse after TD-GC analysis. The only apparatus required for area air sampling is a diffusive cap placed on the end of the tube. Thermal desorption tubes can be reused at least 100 times before the sorbent needs replacing, Thermal desorption provides excellent sensitivity and the tubes are small and convenient for personal and static monitoring. Additional advantages of thermal desorption tubes is their small size, they are compact, easy to use, and durable (45).

SulfiCarb thermal desorption tubes utilize Gas-Chromatography-Mass-Spectrometry to determine the presence of isoflurane in the sample. This process requires multiple pieces of equipment, advanced software and some level of technical knowledge to determine the overall mass of the isoflurane in the sample.

To overcome sensitivity losses due to solvent desorption, thermal desorption may be the most effective approach to short-term sampling needed to provide an accurate level of isoflurane exposure to veterinary personnel.

2.7.2 Anasorb 747 Sampling Tubes

SKC Inc. first introduced the first commercial sorbent tube in 1973 and Anasorb became trademarked in 1990. Since that time Anasorb 747 has been made available for sampling polar organic compounds. In addition to being a synthetic carbon with low ash content, Anasorb 747 is hydrophobic, and has a higher surface area than conventional

coconut charcoal. The tubes are 7 cm x 4 mm (inner diameter) x 6 mm (outer diameter) Anasorb Carbon Molecular Sieve (CMS) glass sampling tubes packed with two separate section of sorbent (150/75 mg). SKC recommends using OSHA Method 103 in conjunction with Anasorb 747 tubes while actively sampling for isoflurane in an occupational setting (32).

The OSHA Method103; Enflurane, Halothane, and Isoflurane is the active sampling method stated in TB MED 510 for isoflurane sampling in a military veterinary setting. A sampling pump calibrated to a minimum of 0.05 L/min (50 mL/min) is used for sample collection (43).

2.7.3 X574 Passive Badges

Assay Technology AT574 Diffusive Air Samplers are a convenient way to passively sample occupational exposures of isoflurane in the veterinary operatory. The laboratory analysis of the passive badges utilize solvent desorption with toluene and are able to recover approximately 93.8% of samples spiked with a known mass of isoflurane (21). The sampling medium is activated carbon. A modified OSHA Method 103 for analysis with solvent desorption utilizing toluene followed by conducting gas chromatography with flame ionization detector (GC/FID) is recommended (2). On visual inspection, it was noted the badges have a larger surface area than either the thermal desorption tubes or the Anasorb 747 active sampling tubes. The passive badges are simple to use. Except for providing the required information on the sampling card and checking the contaminant of choice, “isoflurane”, on the return envelope there is little pre-sampling preparation required.

Overall, thermal desorption has numerous advantages over the passive dosimeter badges and the active sampling method. These include the small size of the tubes, ease of use, multiple uses as a cost savings, improved sensitivity with heat desorption over diluted solvent extraction, and 99-100% recovered sample (3; 22; 45).

Chapter 3: Materials and Methods

3.1 MATERIALS

The following materials were used to conduct analytical analysis and field sampling:

- Stainless Steel thermal desorption tubes with a Sulficarb molecular sieve sorbent (Markes International, Llantrisant, UK)
- AT574 Diffusive Air Sampler passive personal dosimeter badges for Volatile Organic Compounds to include Halogenated Anesthetic Gases (Assay Technology, Boardman, OH)
- SKC Sorbent tubes, Anasorb 747 Catalog # 226-81A (SKC, Inc., Eighty Four, PA)
- Velocicalc, Model # 9565-P, ECN 017175 (TSI Incorporated, Shoreview, MN)
- AirCheck Sampler, Model #224-44XR, Serial 028910, Pump-J (SKC, Inc., Eighty Four, PA)
- Defender™ 510 Calibrator, ECN# 017233, Item# 662501ASCO383 (BIOS International, Butler, NJ)
- Forane, (Isoflurane, USP) (Baxter Healthcare Corporation, Deerfield, IL)
- Gas Chromatograph (GS) coupled to a Mass Selective Detector (MS) (Agilent, Santa Clara, CA)
- J & W PoraPLOT Q Column (Agilent, Santa Clara, CA)
- Markes Unity 2™ (Markes International, Llantrisant, UK)

- Markes ULTRA™ (Markes International, Llantrisant, UK)
- Markes TC-20™ (Markes International, Llantrisant, UK)
- Rae System LP1200 piston pump (Sunnyvale, CA)

3.2 METHODS

3.2.1 Pilot Studies

Initial observations and two pilot studies were conducted in the Laboratory Animal Medicine (LAM) operatory at Uniformed Services University of the Health Sciences (USUHS). These studies consisted of convenience sampling due to researcher's schedules, protocols, and approval by LAM personnel. All initial observations and pilot studies were conducted during normal operations within the LAM.

Several pilot studies were conducted in December 2018 to determine 1) the mass of isoflurane collected in the environment during area sampling at 15, 30, 45 and 60 minutes, 2) what minimum mass of isoflurane could be collected on the thermal desorption tubes at 15 minutes, and 3) determine the best placement of the samplers during the sampling event. The pilot studies also served to validate the analytical methodology needed for the construction of the GC/MS calibration curve and the optimal equipment parameters for analysis.

Initially, the thermal desorption tubes were placed either next to the nose-cone/face mask area or two to three feet away. This placement proved to be problematic as warming blankets and routine animal care interfered with the sampling analysis by interfering or blocking air circulation around the thermal desorption tube. Placing the samplers seven feet from the nose cone/intubation area allowed for good mixing of isoflurane emissions in the air, while ensuring there was no interference with routine

animal care. The sampling location also allowed for convenient changing of the thermal desorption tubes every 15 minutes.

Figure 3.1 illustrates the typical surgical area configuration in which the samplers are on the right (red circle), the vaporizer and passive scavenging equipment (yellow circle) is in the middle and nose cone/intubation area is on the left (pink block). The distance from the nose cone/intubation area to the samplers was seven feet.

Figure 3.1. Common surgery area configuration for sampling. The blue circle shows the vaporizer and passive scavenging system, the red circle shows the location of the sampler set and the orange block shows the nose-cone/induction area.

3.2.2 Area Sampling Device Arrangement

Each sampler set (comprised of one each of the samplers) was stationed near the operating table and was changed out every 15 minutes and labeled. Figure 3.3 illustrates

the configuration of the sampling devices. The devices were approximately 2 inches apart. All samplers remained capped and covered until sampling commenced. When sampling was complete, the SKC Anasorb tubes were shipped overnight to the Navy Comprehensive Industrial Hygiene Laboratory (CIHL) lab, an AIHA-accredited laboratory, in Norfolk, VA. The ATLabs passive badges were shipped to Assay Technologies, an AIHA-accredited laboratory (Boardman, OH) for analysis. Sulficarb TD tubes were analyzed using gas chromatography/mass spectrometry at Uniformed Services University of the Health Sciences (USU). One blank of each sampler type per every 10 samples taken was submitted with the samples to the respective laboratories.

3.2.3 Sampling Location

3.2.3.1 Area Sampling

Area sampling to collect isoflurane reduced the intrusive nature associated with personal sampling in which the individual is required to wear the components of the pump, tubing and sampling media. Area sampling occurred in the same locations as the pilot studies and initial observations and was performed during veterinary procedures utilizing one of each of the sampler types. Observations, pilot studies and sampling events were conducted in three distinct areas of the LAM; the preparation area, the da Vinci surgical room and the traditional surgical suite (Figure 3.2). In all cases observation and sampling occurred 7 feet of the intubation or nose cone/face mask of the anesthetized pig.

3.2.3.2 Preparation Area

The preparation room was the first location in which anesthetized animals were delivered under intravenous anesthesia, placed on mask induction with isoflurane as the inhalation

anesthetic. This was typically followed by intubation prior to moving to one of the two operatory suites. The length of time the anesthetized animals remained in this area prior to being transported to the surgical area varied from minutes to several hours. While the pig was in the preparation area, observations were recorded regarding the procedures the veterinary staff used to prepare the pig for surgery to include the intubation process. During the pilot studies, area sampling was also performed.

Using the thermal desorption tubes, several sampling iterations allowed for collection of isoflurane mass on the tubes, controlled for time or distance. One sampling iteration was performed to test the mass of isoflurane collected at 15, 30, 45, and 60 minutes. Figure 3.2 shows the thermal desorption tubes ready for area sampling and labeled for the indicated time. All tubes were uncapped and collecting isoflurane at the start of the timer. At each time point, the designated tubes were removed from the sampling event. For example, the timer was begun and when it reached 15 minutes, the tubes labeled 15 were removed from the tray, capped and stored for analysis.

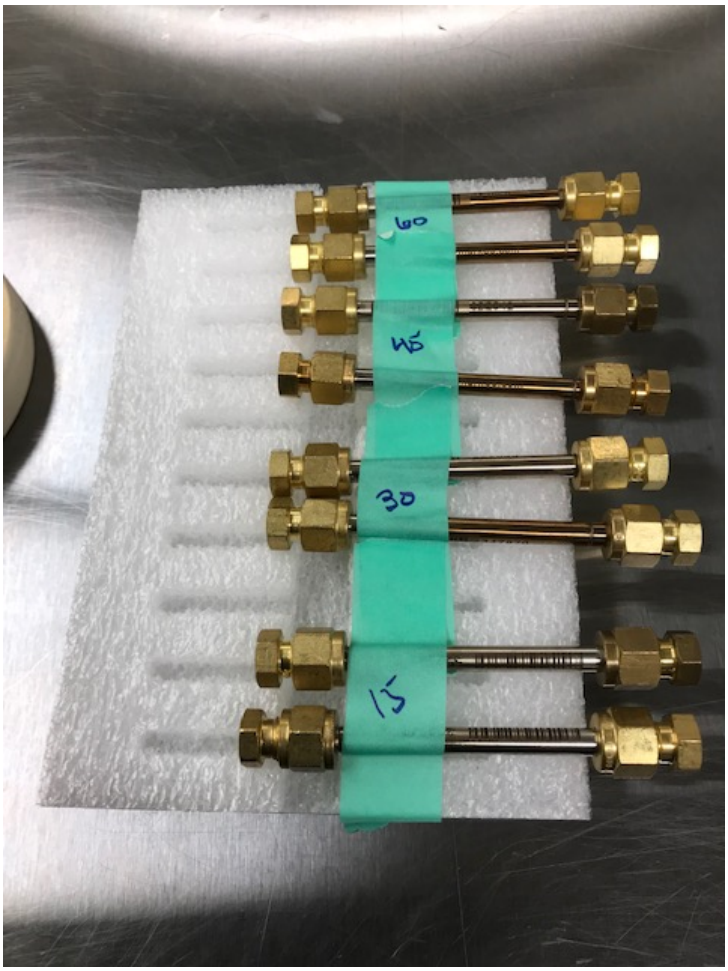

Figure 3.2: Sulficarb TD tubes prepared for timed sampling during pilot study.

A second sampling iteration considered the distance from the nose-cone/induction area of the pig. A tray of thermal desorption tubes were placed on the preparation table next to the pig's head. It was noted this placement interfered with the veterinary staffs care of the pig.

The sampling tray was moved to an empty adjacent table for sampling. This secondary location was seven feet from the nose-cone/induction area of the pig and was not an interference for the veterinary staffs care. During the pilot studies, at this distance the thermal desorption tubes collection isoflurane mass for analysis.

3.2.3.3 da-Vinci Robotic-Assisted Surgical Area

Anesthetized animals were taken from the preparation area to either a small operatory room in which the da-Vinci Robotic-Assisted Surgery system was utilized or the surgical suite. The da-Vinci system allows the surgeons to operate through a few small incisions and features a magnified 3D high-definition vision system and small instruments attached to robotic arms with various tools that bend and rotate far greater than the human hand (18). Anesthetized pigs were delivered to this surgical area after being prepared and intubated in preparation area. The room typically had 2 to 10 personnel working around the equipment. At least one member of the veterinary staff was always in attendance, monitoring the vital signs and the depth of anesthetic induction to the pig. The sampling devices were set up within two feet of the passive scavenging equipment and within seven feet of the intubation area of the anesthetized pig.

3.2.3.4 Surgical Suite Area

The surgical suite was located adjacent to the preparation area. The area can accommodate up to three anesthetized pigs as well as independent passive scavenging equipment for each. All anesthetized pigs were transported to the surgical suite after preparation was complete. Within the surgical suite sampling was conducted in one of two locations (Figure 3.3). The first location was in a corner near the pig and closest to the entrance from the preparation area and the scavenging equipment within the stated distance from the anesthetized pigs nose cone/intubation area. The second location was on an adjacent wall near the foot of the operating table with the scavenging equipment furthest away but the intubation area still within the stated distance.

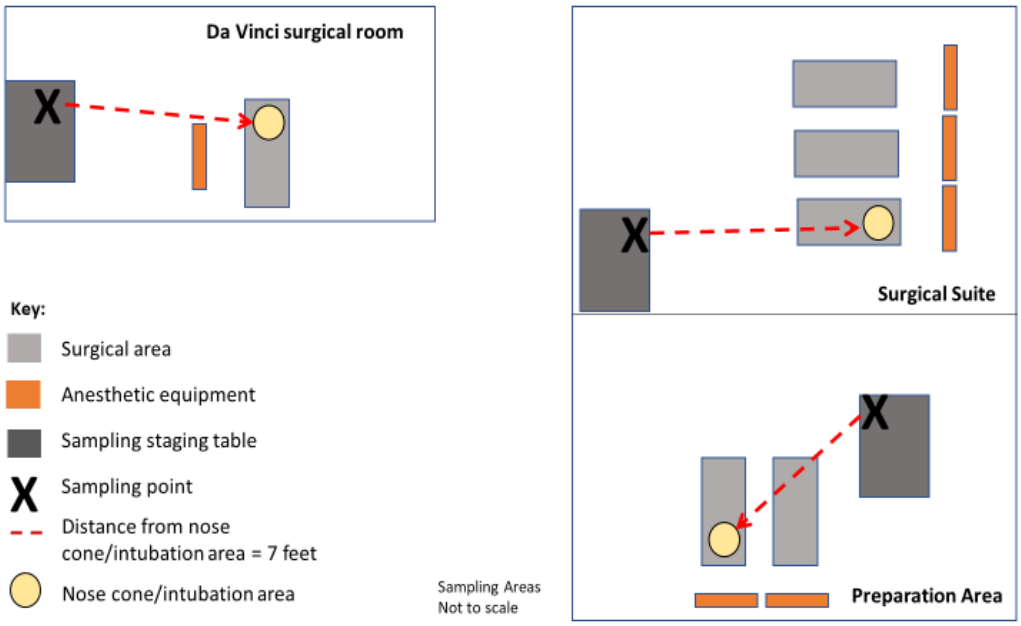

Figure 3.3. Location of sampling including the preparation area, the da Vinci surgical room and the surgical suite.

3.3 Sampling Methods

3.3.1 Active Sampling

Active area sampling was conducted following guidance contained in Occupational Safety and Health Administration (OSHA) Enflurane, Halothane, Isoflurane Method 103. This active method of sampling requires additional equipment, such as a calibrated air pump and tubing to connect the pump to media, as well as laboratory support for analysis (25). Short-term, 15-minute area sampling was done by drawing 0.75 ml/minute of air through a SKC Aircheck pump attached to a SKC Anasorb tube. One sample was collected every 15 minutes. The SKC Anasorb tube was attached to a metal tray between the passive samplers at two inches apart. The tray was located on a vacant operating table to simulate the approximate height of the anesthetized pig and the work area of the veterinary staff members conducting surgery or routine care on the

animal. The open end of the SKC Anasorb tube faced the nose-cone/intubation area of the anesthetized pig. Figure 3.5 shows the SKC Anasorb tube connected to the AirCheck pump in the center. Upon completing of sampling, the SKC Anasorb tubes were packaged and shipped overnight to the Comprehensive Industrial Hygiene Laboratory (CIHL), a lab accredited by the American Industrial Hygiene Association (AIHA) for analysis.

3.3.2 Passive Sampling

The simplest and least intrusive method for sampling for isoflurane involves the use of passive dosimeter badges. Two types of passive samplers were used; Stainless Steel SulifCarb thermal desorption tubes (Markes International, Llantrisant, UK) and AT574 Diffusive Air Sampler passive personal dosimeter badges for Volatile Organic Compounds to include Halogenated Anesthetic Gases (Assay Technologies, Boardman, OH). The passive sampler devices were attached to a metal tray 2 inches on either side of the SKC Anasorb tube with open end/side facing toward the nose-cone/intubation area of the anesthetized pig. The metal tray sat atop a vacant operating table to simulate the approximate height of the anesthetized pig. Figure 3.5 shows the ATLabs Passive badge on the left and the SulfiCarb TD tube on the right. Upon completion of the sampling event, the passive badges were overnight mailed to an accredited AIHA lab while the thermal desorption tubes were carried to the USU analytical laboratory for analysis.

3.4 Sampling Analysis Equipment

3.4.1 Analytical Equipment

In the USU laboratory, a gas chromatograph (7890B, Agilent Technologies, Santa Clara, CA) coupled to a mass selective detector (5977, Agilent Technologies) was used in

association with a Markes Unity 2 thermal desorption unit connected to a Series 2 ULTRA autoloader (Markes International, Llantrisant, UK).

The gas chromatograph contained a J & W Capillary GC PLOT Q Column (30 m length x 250 µm inner diameter x 0.25 µm film thickness). The analytical parameters included the column flow rate was 1.1 mL/min, with the initial column temperature at 40°C and held for 1.0 minute. The temperature was then ramped at 20°C per minute to a maximum temperature of 220°C (held for 6 minutes). The total gas chromatography (GC) analysis time was 17 minutes. Agilent Enhanced Chem Station software (Ver. F.01.03.2357) was used to manually integrate the peak areas. Ultra-high purity helium (99.999%) was used as the carrier gas (Roberts Oxygen, Rockville, MD).

3.4.1.1 Markes Unity 2 Parameters

The thermal desorption system cold trap was programmed to −15°C during desorption of the Sulficarb TD tubes. The focusing trap was then flash heated to a maximum temperature of 300°C and held for 3 minutes. This hold time ensured full desorption of the isoflurane collected on the cold trap from the Sulficarb TD tube. The desorption temperature was 300°C with a 5-minute desorption time. The total split flow of 32.9:1.

3.4.2 Chemicals

Forane (Isoflurane (99.99%) (Baxter International Deerfield, IL) was used to generate the calibration curve in the USU laboratory. The Agilent carrier gas was ultra-high purity helium (99.999%) and was purchased from Roberts Oxygen (Rockville, MD). High purity nitrogen (99.99%) was used as for operating the valves on the Markes Unity II and was purchased from Roberts Oxygen (Rockville, MD).

3.4.3 Thermal Desorption Tube Selection

Stainless steel (89 mm x 6.35 mm outer diameter (5 mm inner diameter) SulfiCarb thermal desorption tubes were used in this study. SulfiCarb TD tubes were purchased from Markes International. Prior to use, each tube was pre-conditioned using a TC-20 sorbent tube conditioner (Markes International, Llantrisant, UK). The manufacturers' recommendations were used in the conditioning process. Passive sampling was performed with a diffusion cap (Markes International, Llantrisant, UK) installed on the collection end of the tube and a brass storage cap installed on the opposite end of the tube. After sampling, and prior to analysis each tube was capped with DiffLok caps (Markes International, Llantrisant, UK) and loaded into the Markes Series 2 ULTRA autoloader.

3.5 Sample Analysis

3.5.1 Active Sampling Analysis (OSHA Method 103)

The OSHA Method 103: used for this study to conduct active sampling has been validated with an accuracy of 25%. The OSHA Method 103 uses solvents (Toluene) to remove the isoflurane from the sorbent.

3.5.2 Passive Dosimetry Badge Sampling Analysis

Assay Technologies (2018) has validated the analytical method used for the passive badges to be ≤ 25%. The National Institute of Occupational Safety and Health (NIOSH) lists ≤ 25% as the acceptable range for air samplers (19). The sampling time was 15 minutes.

3.5.3 Thermal Desorption Calibration Curve

In order to quantitatively measure the isoflurane masses collected on the thermal desorption tubes during the sampling event, a calibration curve was constructed.

The masses needed to construct the calibration curve were derived from the uptake rate of 4.4 ng ppm^{-1} min^{-1} from the research conducted by Benchoff (2017). The temperature and pressure were measured utilizing the VelociCalc (TSI Incorporated, Shoreview, MN).

The range of measurements desired for the calibration curve was from 25 ng to 7688 ng corresponding to 0.4 to 125 ppm.

The initial step in constructing the calibration curve was to generate a stock Tedlar (SKC, Inc., Eighty Four, PA) bag (5L).

Equation (1) was used to determine the liquid volume of isoflurane needed to make the stock bag.

$$C = \frac{(MV)(106)\rho VL}{VM} \qquad (1)$$

Where:

C = concentration (ppm) of isoflurane

MV = molar volume (24.09 L/mol at 21.9°C and 756.3 mmHg)

ρ = density of isoflurane (1.496 g)

VL = volume (μL) needed for 400 ppm stock bag

V = volume of diluent bag (5 L)

M = molecular weight of isoflurane (184.5 g/mol)

The stock bag was created to have a theoretical concentration of 400 parts per million (ppm) using Equation (1). Using 10.2 µL of liquid isoflurane the actual concentration of the stock bag was 400 ppm.

For this research, the stock bag was the source for each dilution. Equation (2) was used to determine the gas volume of isoflurane needed to make the dilution bags.

$$C_1V_1 = C_2V_2 \qquad (2)$$

Where:

V_1 = injected volume (µl, ml)

C_1 = concentration (ppm) of isoflurane

V_2 = total volume of stock bag (5.0 L)

C_2 = desired concentration in ppm

The stock bag was used to create individual 5-liter Tedlar bags for the parallel dilution. The diluted bags were 25 ng, 100 ng, 200 ng, 300 ng, 400 ng, 500ng, 600 ng, 700 ng, and 800 ng. Triplicate samples (100 ml) were drawn from each sample bag using the Rae System LP1200 piston pump (Sunnyvale, CA).

The thermal desorption tubes were analyzed using the Agilent 7890B/5977B GC/MS. The peak areas were compared against the triplicate mass averages for resulting in the calibration curve shown in Figure 3.4.

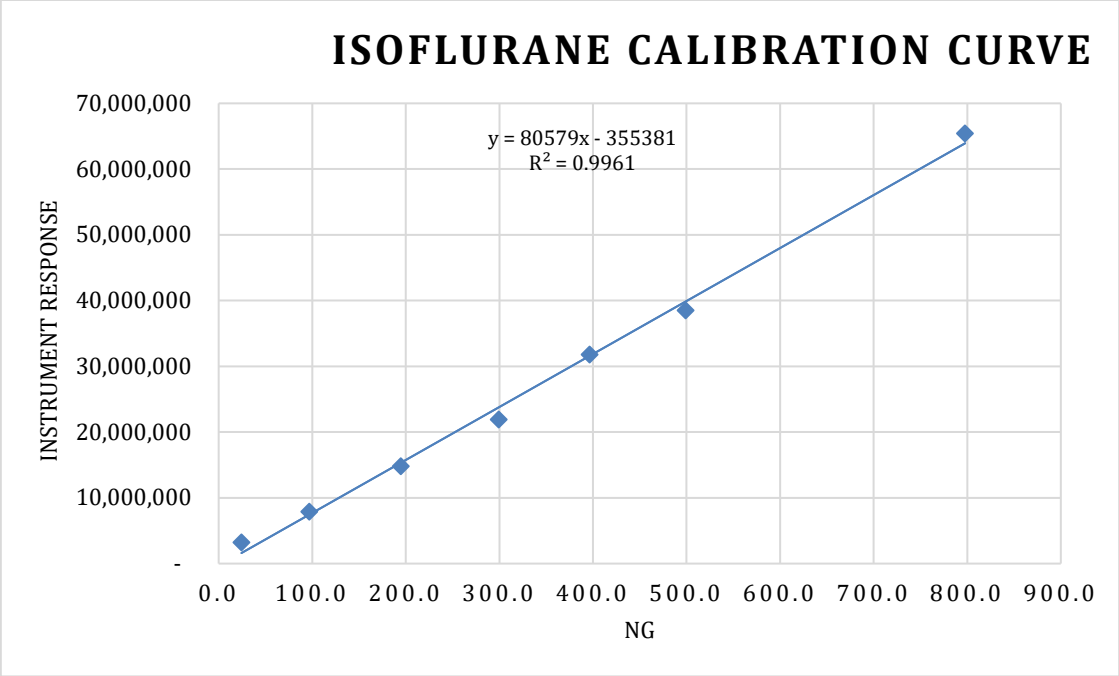

Figure 3.4. Isoflurane Calibration Curve with an uptake rate of 4.4 ng/ppm/min

3.5.4 Calculating Mass and Concentration for Thermal Desorption Tubes

The mass collected by each thermal desorption tube was placed in the calibration equation shown in Figure 3.4.

3.5.5 Pre-Sampling Activities

- Prior to each sampling event, pre-calibration procedures on the AirCheck Sampler, Model #224-44XR, Serial 028910, (SKC, Inc., Eighty Four, PA) were conducted. The pump was calibrated to approximately 75 mL/min. All thermal desorption tubes were conditioned according to the product literature shipped with the tubes
- The sample number, SKC Anasorb tube number, ATLabs Passive badge number and SulfiCarb TD tube were all recorded.
- The start time and stop time for each sample set were recorded.
- Any anomalies, such as refilling the anesthesia vaporizer during the sampling period were noted.

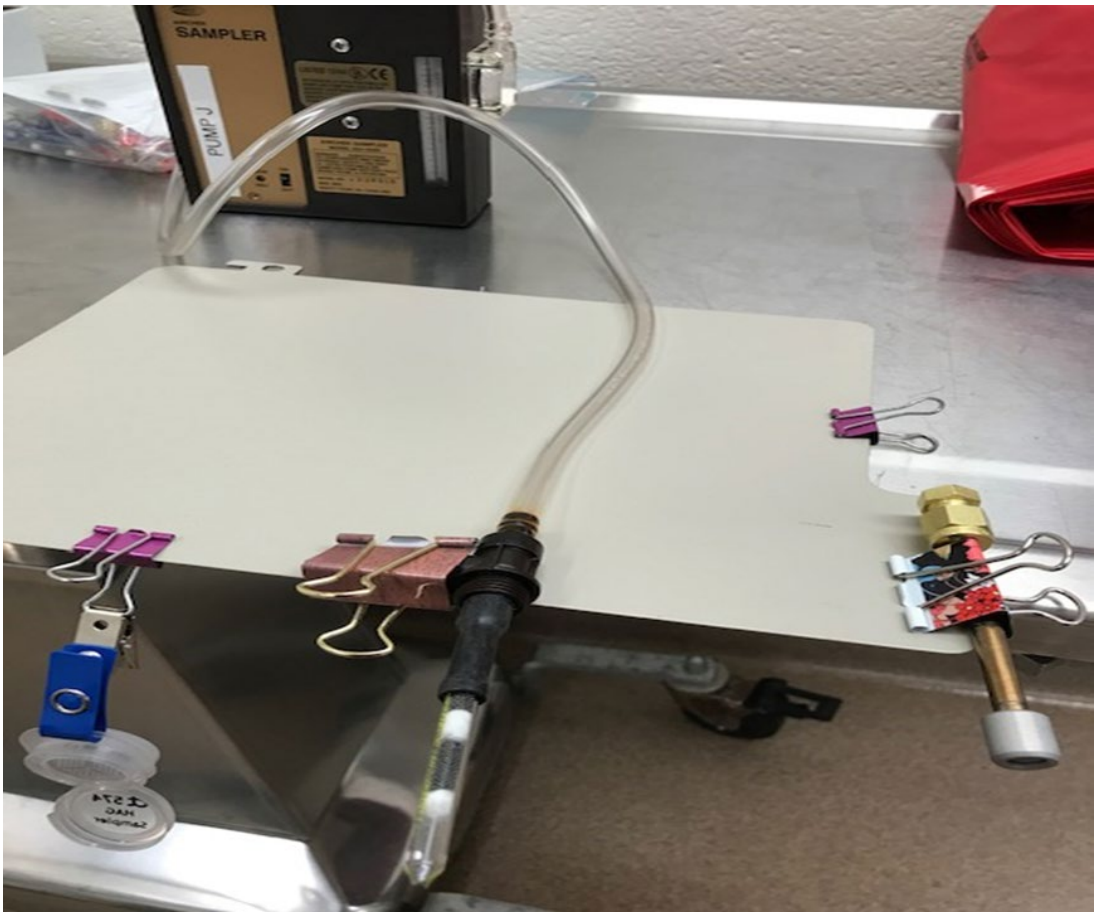

Figure 3.5. Assay Technology AT574 passive dosimeter badge, SKC 575-002 Anasorb 747 and Markes stainless steel SulfiCarb thermal desorption tube (from left to right)

3.6 Statistical Analysis

3.6.1 Statistical Analysis

The minimum sample size required to identify a statistically significant difference in the sample means was calculated using the University of California San Francisco Clinical and Translational Science Institute's (2018) sample size calculator (17). A total of n = 20 samples was calculated as the minimum sample size needed to ensure statistical significance at a 0.05 level of significance, 80% power and an estimated sample standard deviation of 0.80.(25) A total of 23 Sulficarb TD tubes, ATLabs Passive badges and 23

SKC Anasorb tubes, not including blanks, were targeted for sampling and analysis. A total of 25 samples of each sampling device was taken.

3.6.2 Statistics

3.6.2.1 Bland-Altman

The Bland-Altman statistical analysis is based on the quantification of the agreement between two quantitative measurements or methods by studying the mean difference and constructing limits of agreement (14). The Bland-Altman analysis was used to determine the level of agreement between the isoflurane concentrations measured between the thermal desorption tubes versus the passive badges and the thermal desorption tubes versus the active sampling tubes. There were no *a-priori* limits set prior to sampling.

3.6.2.2 Censored Data

Approximately 50% of the passive badges and the active sampling tube results were returned from the respective laboratories as levels being below the laboratories limit of quantification. Of the three sampling events, day 1 provided the results used for development of the Bland-Altman plots.

Chapter 4: Results

4.1 Descriptive statistics

A total of 23 sample sets, each comprised of an Assay technologies AT574 Passive Dosimeter badge, a SKC 575-002 Anasorb 747 tube, and a Markes stainless steel thermal desorption tube containing Sulficarb molecular sieve sorbent, were collected over three days. Two field blanks of each sampler type were also collected and shipped to the appropriate laboratory for analysis.

The sampling period for each sample set was 15 minutes. Samples were collected in either the LAM preparation area, the da-Vinci robot surgical room, or the traditional surgical suite. During one sampling event, 11 samples collected in the da-Vinci robot surgical room reported enough mass collected to determine the level of agreement between the sampling methods.

The analyzed samples collected on days two and three resulted in measurements which fell below the limit of quantification (CIHL, 0.9 ppm, Assay Technologies, 1.5 ppm and were recorded as Non-Detects (ND) from the laboratories.

On day three, the Markes Stainless Thermal Desorption tubes detected isoflurane at 0.1 ppm but this value fell outside the range of Agilent's calibration curve. Confidence in the accuracy would be higher if the value fell within the calibration curve. The lowest value falling within the calibration curve was for day three at 0.8 ppm.

4.1.1 Normality

A requirement for using Bland-Altman analysis to determine the level of agreement between devices or methods is that the assumption of normality is met (14).

The assumption of normality for this data set was determined by visually inspecting a histogram of the standard deviation between the thermal desorption tubes and the passive badges, and the thermal desorption tubes and the active sampling method. Each histogram followed the guidelines from Samuels and Marshall (2019) in which there was a peak in the middle, the tails were approximately equal on each side and resembled the normal (Gaussian) distribution (31). Figure 4.1 illustrates normality between the thermal desorption tubes and the active sample utilizing SPSS (IBM, Armonk, NY).

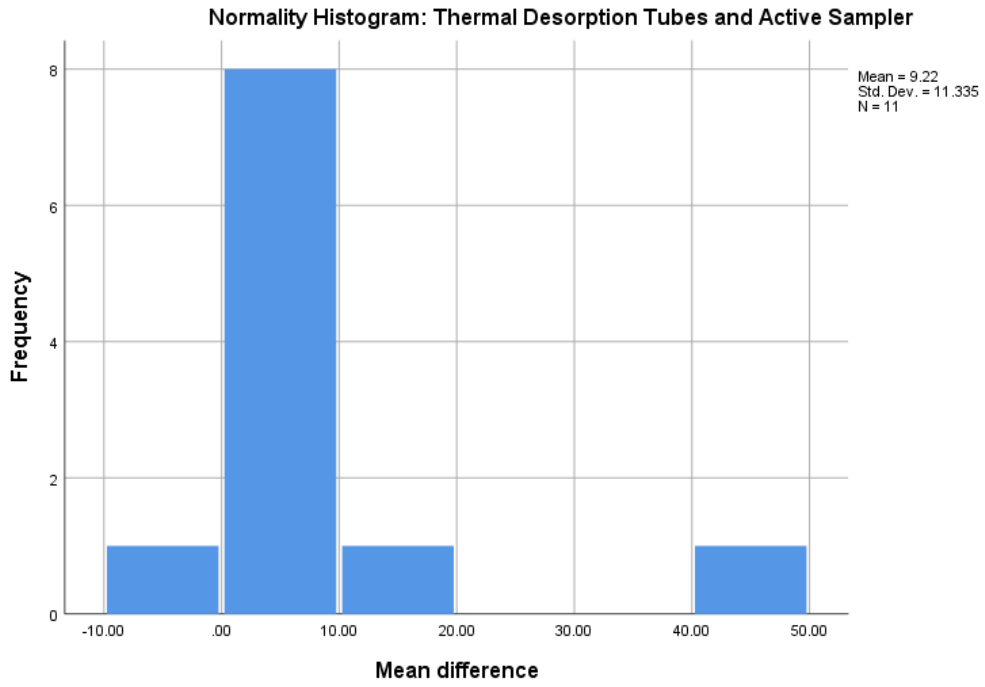

Figure 4.1: Histogram illustrating normality of thermal desorption tubes and active samplers.

4.1.2 Bland-Altman Level of Agreement (LOA)

The Bland-Altman plots were constructed to visually compare the agreement between the thermal desorption tubes with the active sampling tubes, and the thermal desorption tubes with the passive badges. There were no *a-priori* limits of agreement

selected to be “acceptable” as the objective of this study was to report the observed level of agreement between the sampling methods. It was observed the level of agreement between the thermal desorption tubes compared to the active sampling tubes was 35% and is shown in figure 4.3. The level of agreement between the thermal desorption tubes compared to the passive badges was 50% and is shown in figure 4.5.

The upper and lower levels of agreement represent the area in which 95% of data points should fall and were calculated by using Equation (4)

$$Bias \pm 1.96 * s \qquad (4)$$

The bias represents the mean between the samplers. The closer to zero or the line equality the bias falls, the less variability there is between the samplers mean values. A positive value represents the first sampling method measuring more concentration than the second sampling method. Consequently, a negative value represents the second sampling method reading was higher than the first method.

4.1.1.1 LOA Thermal Desorption Tubes and Anasorb 747

Figure 4.2 is a Bland-Altman plot of thermal desorption tube concentration minus active sampling tubes concentration results. The limits of agreement in which 95% of the data points should fall are 34.7 as the upper limit and -9.7 as the lower limit are represented by the dashed lines. Ten of eleven data points fall within the 95% distribution range. The line of equality is the horizontal line at zero. The bias, representing the gap between zero differences in reading and the actual differences in the readings is 12.7. The positive value for the bias indicates, on average, the thermal desorption tubes reported a higher concentration of isoflurane than the active sampling tubes. For this data ten of

eleven points are above the line of equality indicating the thermal desorption tubes collected a higher mass of isoflurane.

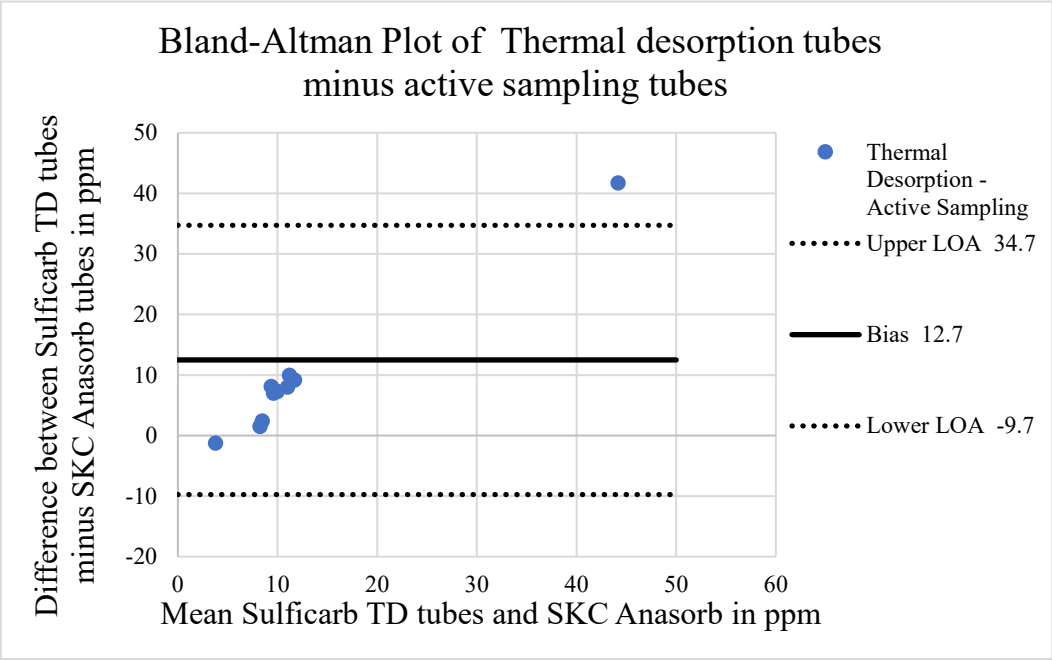

Figure 4.2. Bland-Altman Plot showing Sulficarb TD tube concentration minus SKC Anasorb tube concentration with bias at 12.7, upper LOA 34.7 and lower LOA -9.7

Figure 4.3 highlights a calculated 35% agreement between the thermal desorption tubes and the active sampling tubes and is shown in the heavy dashed lines. The level of agreement percentage was determined by multiplying the upper level of agreement, 34.7, and the lower level agreement, -9.7 by 0.35 resulting in 12.15 and -3.41, respectively. The 35% agreement does not include the data point in the upper right of the plot.

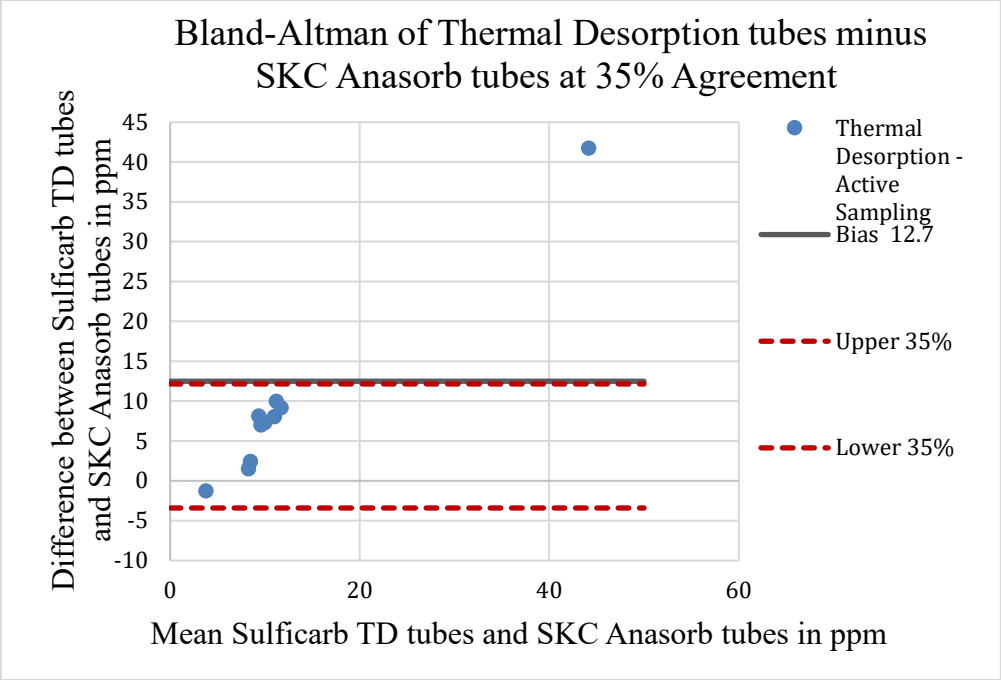

Figure 4.3. Bland-Altman Plot showing 35% agreement between Sulficarb TD tube concentration minus SKC Anasorb tube Active sampler.

4.1.1.2 LOA Thermal Desorption Tubes and Passive Badges

Figure 4.4 is a Bland-Altman plot of thermal desorption tube concentration minus the passive badge concentration results. The limits of agreement in which 95% of the data points are shown as the horizontal dashed lines at 47.1 for the upper limit of agreement and -12.4 for the lower level of agreement. Ten of eleven data points fall within the 95% distribution range. The line of equality is the horizontal line at zero. The bias is 17.4. The positive value for the bias indicates, on average, the thermal desorption tubes reported a higher concentration of isoflurane than the active sampling tubes. For this data eight of eleven points are above the line of equality.

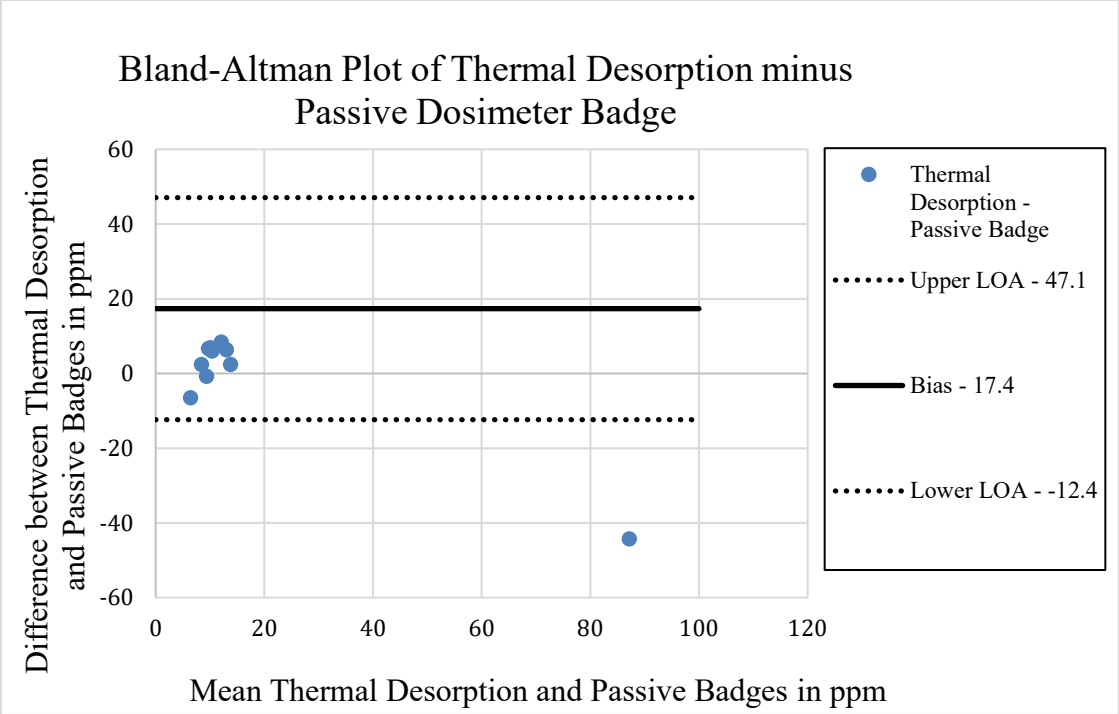

Figure 4.4. Bland-Altman Plot showing Thermal Desorption Tube concentration minus Passive Dosimeter Badge concentration with bias at 17.4 ppm, upper LOA 47.1 ppm and lower LOA -12.4 ppm

Figure 4.5 highlights a calculated 50% agreement between the thermal desorption tubes and the active sampling tubes and is shown in the heavy dashed lines. The level of agreement percentage was determined by multiplying the upper level of agreement, 47.1, and the lower level agreement, -12.4 by 0.50 resulting in 24.0 and -6.2, respectively. The 50% agreement does not include data point at the higher concentration falling outside the 95% range.

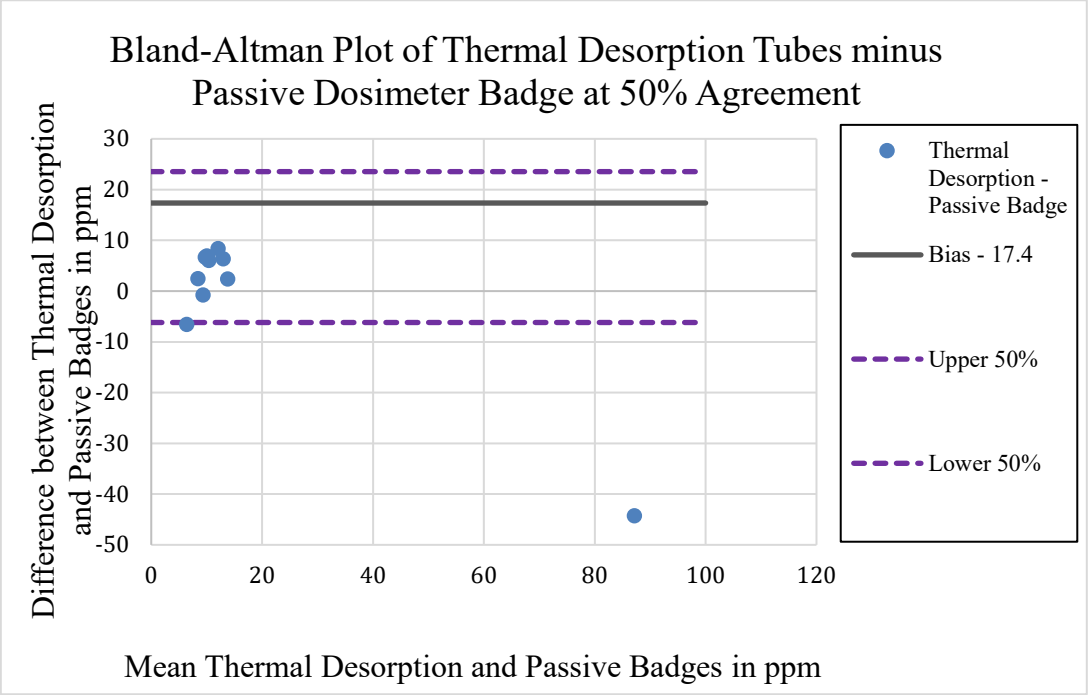

Figure 4.5. Bland-Altman Plot showing 50% agreement between Thermal Desorption Tube concentration minus Passive Dosimeter Badge concentration

Chapter 5: Discussion

5.1 Short term sampling

This study was designed to determine if any agreement exists between three devices sampling for isoflurane during 15-minute, short-term sampling period in a laboratory animal medicine facility. Technical Bulletin 510 (TB MED 510); Guidelines for the Recognition, Evaluation, and Control of Occupational Exposures to Waste Anesthetic Gases 3.3c(1-3) recommends multiple ways to sample for volatile anesthetic gases such as isoflurane. The sampling methods recommended include using a direct reading type instrument such as a Miran® infrared analyzer (not used in this study), adsorption tubes connected to an air pump (SKC Anasorb tubes) and passive badges (ATLabs Passive badges) (41). This study included an additional approach to passive sampling not mentioned in TB MED 510, SulfiCarb thermal desorption tubes.

5.2 Levels of agreement

The Bland-Altman analysis explores the differences (mean and standard deviation) by representing every difference between the two paired methods against the average of the measurements. These variables are then plotted against each other to plot the level of agreement. Traditionally, the level of agreement is determined and stated prior to conducting sampling (14). In this study, no *a-priori* limits of agreement were set prior to the study as the objective was to determine any level of agreement.

5.3 Sample Size

The original sampling size included 25 sample sets, two of which were field blanks. Each sample set consisted of one each of a passive badge, the active sampler tube

and thermal desorption tube. Of the 23 samples collected, 11 were used for analysis and determination of the level of agreement between the methods. Twelve sample sets with laboratory analysis <LOQ were excluded. If the excluded data had been included, the assumptions of normality would have been rejected and determining any level of agreement would not have been possible. The resulting sample size is small.

5.3.1 Thermal Desorption Tubes and Passive badges

The sample size of 10, determined the level of agreement between the thermal desorption tubes and passive badges to be 50%. One data point was excluded from determining the level of agreement because it fell outside the 95% (2 standard deviation) range.

With the eleven samples analyzed, the thermal desorption tubes reported higher concentrations than the passive badges in 73% of the samples and is reflected in the number of sample points above the horizontal zero line as seen in figure 4.5.

5.3.2 Thermal Desorption Tubes and Active Sampling

The sample size of 10 found a 35% level of agreement between the thermal desorption tubes and the active sampling tubes. The same sample point was excluded because it fell outside the 95% (2 standard deviations) range.

Again, with the 11 samples analyzed, the thermal desorption tubes reported higher concentrations than the active sampling tubes 91% of the time and is reflected in the number of sample points above the horizontal zero line as seen in figure 4.3.

5.4 STUDY LIMITATIONS

5.4.1 Sampling

All sampling was convenience sampling due to the limited number of researchers conducting surgical procedures on the pigs. One sampling location, the da-Vinci robotic surgical room, was a small space holding multiple large pieces of equipment and cramped conditions in which to work. Comparatively, the preparation area and the surgical suite were similar in size, work area and overall conditions. The differences in the sampling areas may have contributed to the number of samples exceeding the 2ppm ceiling OEL (da-Vinci surgical room) and led to the number of censored data points (preparation area and surgical suite).

5.4.2 Thermal Desorption Tubes and Passive badges

5.4.2.1 Day 1 Sampling in the da-Vinci Surgical Room

The samples taken in the da-Vinci operating room were collected at the farthest point from the door and approximately three feet from the vaporizer and scavenging equipment and seven feet from the intubation area of the animal. Two likely explanations for the higher concentration of reported isoflurane exists.

The higher reported concentration in the thermal desorption tubes may be the result of differences in the cross-sectional area of the devices. The increased cross-sectional area of the passive badges increases the likelihood turbulent air will negatively affect the uptake rate. The samplers were placed two inches on either side of the active sampling method, and it is possible the movement of the air toward the active sampling tube could have created turbulence.

The location of the samplers in proximity of the vaporizer/scavenging equipment suggest the equipment itself could be the source of occupational exposures. This was the only sampling conducted with the vaporizer/scavenging equipment between the

intubation area of the animal and the samplers. There could have been leaks in the tubes, hoses, connectors, the vaporizer or the scavenging equipment or the collection canister. The vaporizer and scavenging equipment were not tested for leaks prior to the sampling.

5.4.2.2 Days 2 and 3 Sampling in the Preparation Area and Surgical Suite

On days two and three the passive badge samples collected in the preparation area and surgical suite were reported to be below the limit of quantification by the analyzing laboratory. The laboratory analyses of the thermal desorption tubes reported quantifiable concentration of isoflurane. Several samples were outside the range of the calibration curve and were considered censored data points.

The samplers were placed on a table with ingress/egress doors on either side of the table. Staff, doctors and observers entered and/or exited the room by these doors. The location of the samplers and the movement by personnel may have provided an opportunity for air turbulence which may have negatively affected the isoflurane uptake rate of the passive badges. Compared with the passive badges, the small cross-sectional area of the thermal desorption tubes makes them more robust against turbulence and could explain the low levels of quantifiable isoflurane concentration.

5.4.3 Thermal Desorption Tubes and Active Sampling

5.4.3.1 Day 1 Sampling in the da-Vinci Surgical Room

The samples taken in the da-Vinci operating room were collected at the farthest point from the door and approximately three feet from the vaporizer and scavenging equipment and seven feet from the intubation area of the animal.

The higher reported differences in the thermal desorption tubes may be the result of using a low, 75ml/minute flow rate to sample with the active sampling method. The

OSHA Method 103 recommends no less than 50 ml/minute when active sampling (25). The AirCheck Sampler Pump (SKC, Inc., Eighty Four, PA) was pre and post calibrated with the Defender™ 510 Calibrator (BIOS International, Butler, NJ) within ± 5%, suggesting equipment issues were not of concern.

Additionally, the samplers were placed approximately three feet from the vaporizer and scavenging equipment and seven feet from the intubation area of the animal. This was the only sampling period in which this configuration occurred. The equipment was not leak tested prior to the sampling event. The possibility isoflurane was able to leak from the connections, hoses, vaporizer or collection canister into the room should not be ruled out.

5.4.3.2 Days 2 and 3 Sampling in the Preparation Area and Surgical Suite

On days two and three the active samples collected in the preparation area and the surgical suite were reported to be below the limit of quantification by the analyzing laboratory. The laboratory analyses of the thermal desorption tubes reported quantifiable concentration of isoflurane. Several thermal desorption tube samples were outside the range of the calibration curve and were considered censored data points.

The samples were collected from a table seven feet from the intubation area of the anesthetized animal. The table was located between two doors in which personnel used frequently to enter and exit the areas. The thermal desorption tubes are robust against air turbulence and the active samplers would not be affected as the pump was actively pulling air into the tube and across the sorbent. The resulting censored data suggests the flow rate on the pump may been too low or the sampling period was too short to collect enough quantifiable mass on the active sampling tubes.

5.4.4 Sampling Analysis

At concentration below 13 ppm the passive badges reported the same or slightly higher concentrations (>13 ppm) when compared with the active sampling tubes. The average percent difference was 6.9%. At higher concentrations the difference was considerably large (32.4%). For the thermal desorption tubes compared with passive badges, the average percent difference at the lower concentrations was 7.9%, while at -12.7% for the higher concentrations. In comparing the thermal desorption tubes with the active sampling tubes, the average percent difference was 14.1% at the concentrations <13 ppm and at 23.6% >13ppm. The differences are due to one sampling point in which all three methods reported large concentrations of isoflurane. The laboratory analyses reported 23 ppm (CIHL), 65 ppm (USU) and 109 ppm (Assay Technologies). This may be indicative of variances in the devices themselves, in the laboratory analysis or in the reporting procedures.

5.4.5 Censored Data

More than 50% of the 23 samples taken were determined by the AIHA accredited laboratories conducting the analysis to be below the LOQ. The LOQ given by Assay Technologies was 1.5 ppm for the ATLabs Passive badges, while the CIHL laboratory provided an LOQ of 0.9 ppm for the SKC Anasorb tubes.

All samples collected in the preparation area and surgical suite were considered to be censored data as the results fell below the LOQ. As the objective of this study was to compare the agreement between the methods, the censored data was excluded from the Bland-Altman analysis as it would have heavily skewed the data and confounded the

results relating to the levels of agreement. For this same reason, the censored data was excluded from the statistical analysis.

5.4.6 Sampling Time

The thermal desorption tubes used during short-term 15-minute sampling were able to collect a quantifiable mass of isoflurane in every sample whereas the passive badges and active sampling tubes did not collect enough mass for the laboratory to detect. This may be an indication the short-term 15-minute sampling period may not be adequate to identify quantifiable mass when using the prescribed methods within TB MED 510 to collect isoflurane.

5.5 Future Studies

It is recommended to set limits of agreement *a-priori* in order to define the maximum acceptable differences between two methods of sampling (14). While this study, the first of its kind, did not follow this recommendation, it has identified the starting point in determining limits of agreement for future studies.

Sampling in several locations during the three days of sampling may have introduced variables which were not controlled for. One such instance was the difference in the distance between the vaporizer/scavenging equipment and the samplers in the da-Vinci room. It is probable the high reported concentrations were the result of leaks in the equipment.

Sampling in the preparation area and surgical suite resulted in all the samples taken in those areas to report concentration <LOQ suggesting the general ventilation as well as the scavenging system may be working appropriately to remove isoflurane from the areas. The samples were taken at more than seven feet from the vaporizer/scavenging

equipment and may have been too far away to collect isoflurane. It is recommended sampling being consistent in the distance from the equipment instead of the distance from the intubation area.

Future studies should include considering conducting personal sampling instead of area sampling. Area sampling was convenience sampling but only highlights isoflurane is in the room and not what the workers are being exposed to.

There was wide variation in reported results for sample 1, 23 ppm for active sampler, 109 ppm for the passive badges and 65 ppm for the thermal desorption tubes. It is unclear if the variation is due to the reporting methods or differences in the sampling devices. It is recommended the samplers be evaluated in a closed, controlled system such as a dynamic flow chamber to determine the cause of this variation.

Chapter 6: Conclusions

6.1 Conclusions

The objective of this study was to compare the agreement between three sampling devices. Thermal desorption tubes were compared with two suggested methods of sampling for isoflurane as identified by the Department of the Army in TB MED 510. Overall, the thermal desorption tubes may be a viable substitute for either passive sampling or active sampling for isoflurane. The thermal desorption tubes were able to detect levels of isoflurane as low as 0.1 ppm, well below the OEL of 2 ppm for a 60-minute ceiling. The laboratory analysis was able to confidently report quantified levels > 0.50 ppm while the laboratory for the passive badges and the active sampling analysis reported as low as 1.5 ppm and 0.9 ppm, respectively (LOQ). Further research in controlled atmospheric conditions may provide additional insight into the limitations of the sampling devices and resolve the variability between them.

Of the 11 data points used, 10 were similar across the devices (<13 ppm reported by all devices). The data point with the highest reported concentration was the first sample collected in the da-Vinci robot operatory. The variability in this data point (23 ppm to 109 ppm) should be investigated. It is unclear if this variability was due to the samplers themselves or some other unknown cause. Further research generating short-term spiked samples under controlled atmospheric conditions may illuminate the cause of the variability.

This study set out to collect 23 samples in order to meet the minimum sample size as outlined by the UCSF Clinical and Translational Science Institute's sample size calculator (17). The required number of samples were collected, however, the number of

samples used to perform the Bland-Altman analysis to determine agreement was reduced due to >50% of the samples reported results falling below the LOQ. By excluding the data <LOQ, the Bland-Altman analysis was able to determine there was 35% and 50% level of agreement among the devices. In contrast, the reduced sample size (n=11) may have impacted the power of this study. Repeating this study with a larger sample size may produce a stronger level of agreement among the devices. Additionally, this study could be repeated with consideration for sampling only in the da-Vinci robotic surgery room.

The short-term 15-minute sampling period was sufficient to collect quantifiable mass of isoflurane on the thermal desorption tubes but may have impacted the reporting for the passive badges and the active sampling. A longer sampling period would be recommended when considering isoflurane sampling in the surgical or preparation areas of the LAM.

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