

Development and evaluation of active and passive sampling methods to improve
characterization of worker dose and exposure to isoflurane anesthetic

by

Edward A. Benchoff

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Name of Student: Edward Benchoff

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Place: Room 3176L AFRRI/OEHS Conference Room

DECISION OF EXAMINATION COMMITTEE MEMBERS:

BRUEGGEMEYER.MARY.T.1103870310 Digitally signed by
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Date: 2017.07.31 13:22:40 -04'00'

PASS

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Mary T. Brueggemeyer, MD, MPH, Col, MC, USAF
DEPARTMENT OF PREVENTIVE MEDICINE & BIostatISTICS
Committee Chairperson

JUAREZ.THEODORE.JOSEPH.1170475786 Digitally signed by
JUAREZ.THEODORE.JOSEPH.1170475786
DN: c=US, o=U.S. Government, ou=DoD, ou=PKI,
ou=USAF, ou=USAF, ou=USAF,
cn=JUAREZ.THEODORE.JOSEPH.1170475786
Date: 2017.07.27 10:56:40 -07'00'

Theodore T. Juarez, PhD, CIH
DEPARTMENT OF PREVENTIVE MEDICINE & BIostatISTICS
Dissertation Advisor

PHILIP SMITH Digitally signed by
PHILIP SMITH
DN: c=US, o=U.S. Government, ou=Department of Labor,
ou=Occupational Safety and Health Administration, cn=PHILIP
SMITH, o=9.2342.19200300.100.1.1=16001003043855
Date: 2017.07.31 06:30:52 -06'00'

Philip A. Smith, PhD, CIH
DEPARTMENT OF PREVENTIVE MEDICINE & BIostatISTICS
Committee Member

STUBNER.ALEX.HARRY.1014173257 Digitally signed by
STUBNER.ALEX.HARRY.1014173257
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ou=USA, cn=STUBNER.ALEX.HARRY.1014173257
Reason: I am approving this document
Date: 2017.07.26 16:48:16 -04'00'

Alex Stubner, PhD, LTC, USA
DEPARTMENT OF PREVENTIVE MEDICINE & BIostatISTICS
Committee Member

ROY.MICHAEL.JOSEPH.1008084293 Digitally signed by
ROY.MICHAEL.JOSEPH.1008084293
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ou=OSD, cn=ROY.MICHAEL.JOSEPH.1008084293
Reason: I am the author of this document
Date: 2017.07.31 09:03:57 -04'00'

Michael Roy, MD, MPH
DEPARTMENT OF MEDICINE
Committee Member

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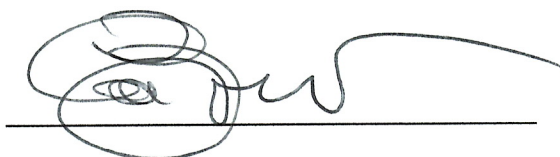
I dedicate this dissertation to my beautiful wife Mirasol Española. Without your love, support, listening skills, and understanding, I would have never been able to complete this dissertation or deal with all the pain that came with it. I thank you and love you always.

“Just Keep Swimming.”

- Mirasol Española (and Dory the fish)

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Edward A. Benchoff
Preventive Medicine and Biostatistics
Uniformed Services University
November 3, 2017

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ABSTRACT

Title of Dissertation: “Development and evaluation of active and passive sampling methods to improve characterization of worker dose and exposure to isoflurane anesthetic”

Name of Candidate: Edward A. Benchoff
Doctor of Philosophy Degree
November 3, 2017

Thesis directed by: Theodore J. Juarez, PhD, Department of Preventive Medicine and Biostatistics & U. S. Naval Medical Center San Diego, San Diego, CA

Isoflurane is an occupational hazard with over 250,000 workers exposed in the United States alone. Conventional exposure assessment challenges include cumbersome and expensive air pumps, or passive samplers that can require lengthy sampling times. Third party analysis can delay results, and solvent desorption can decrease analytical sensitivity. Additionally, air sampling estimates absorbed dose based solely on the inhalational route of exposure. This research proposed to address these challenges by 1) assessing the ability of the HAPSITE[®] ER portable gas chromatograph/mass spectrometer to quantify isoflurane in humidified air as a surrogate for biological monitoring of exhaled breath, and 2) validating two types of thermal desorption tubes for passive sampling of isoflurane.

The INFICON HAPSITE[®] Extended Range gas chromatography-mass spectrometer hand-held probe and thermal desorption sampling system were assessed for their ability to quantify isoflurane in surrogate exhaled breath with an accuracy of $\pm 25\%$.

The HAPSITE® hand-held probe offered the most convenient sampling and greatest overall accuracy, displaying an average error of 8% across the tested concentration range when calibrated with high humidity standards.

Performance of Sulficarb, Carbograph 5TD, and Carbotrap 349 thermal desorption tubes with the HAPSITE® ER was further investigated by assessing desorption flow rate, desorption efficiency, breakthrough, and ability to achieve the target desorption temperature. Desorption temperature varied significantly by tube casing type (glass vs. steel) and sorbent type, and no tube achieved the target temperature of 315°C. This resulted in poor desorption efficiency of Sulficarb (69 – 71%). Carbotrap 349 and Carbograph 5TD achieved good desorption efficiency (98 – 100%).

Sulficarb and Carbograph 5TD were partially validated for 15-minute passive sampling of isoflurane. Sulficarb and Carbograph 5TD achieved average sampling rates of approximately 4.4 and 4.2 ng ppm⁻¹ min⁻¹, respectively, and humidity did not significantly affect the sampling rate of either type of tube. Both tubes achieved good desorption efficiency (99-100%), but Sulficarb displayed more efficient retention of isoflurane and greater stability in storage.

Thermal desorption, passive sampling, biological monitoring, and field-portable instrumentation techniques may offer industrial hygienists and other environmental health scientists methods to enhance the characterization of worker exposure to isoflurane in a variety of work environments.

TABLE OF CONTENTS

LIST OF TABLES	xiii
LIST OF FIGURES	xiv
CHAPTER 1: Introduction	1
1.1 Description and Background of Isoflurane Anesthetic	1
1.2 Potential for Exposure to Isoflurane	1
1.2 Potential Health Impacts	2
1.3 Air Sampling and Analysis	3
1.4 Portable Gas Chromatograph / Mass Spectrometer Instrumentation	4
1.5 Analysis of Exhaled Breath	5
1.6 Public Health / Military Health Relevance	6
1.7 Hypothesis and Specific Aims	7
1.7.1 Hypothesis	7
1.7.2 Specific Aims	7
CHAPTER 2: Literature Review	9
2.1 Introduction	9
2.2 Isoflurane as an Inhalational Anesthetic	9
2.3 Occupational Exposure Limits	10
2.4 Health Effects	11
2.5 Exposures and Controls	13
2.5 Toxicokinetics	21
2.5.1 Absorption and Distribution	21
2.5.3 Biotransformation and Elimination	22
2.6 Biological Monitoring	22
2.7 Thermal Desorption Techniques	26
2.7.1 Passive Sampling of Isoflurane	28
2.7.2 Thermal Desorption Tube Selection	30
2.8 Inficon HAPSITE® ER Portable GC/MS	32
2.9 Conclusion	37
CHAPTER 3: Analysis of Isoflurane in Humidified Air Using the INFICON HAPSITE® ER Portable Gas Chromatograph/Mass Spectrometer	40
Abstract	40
3.1 Introduction	41
3.2 Materials and Methods	43
3.2.1 Analytical Equipment	43
3.2.2 Analytical Method Parameters	44
3.2.3 Thermal Desorption Tube Selection	45
3.2.4 Preparation of Isoflurane Standards	46
3.2.5 Sample Introduction	47

3.2.5.1 Sample Introduction via Probe Inlet	47
3.2.5.2 Sample Introduction via Thermal Desorption	47
3.2.6 HAPSITE® Calibration and Analysis	47
3.2.6.1 Generalizability of HAPSITE® Calibration Using the Probe Inlet	48
3.2.7 Temporal Humidity Effects on Instrument Response	48
3.2.8 Performance of the Internal Concentrator	49
3.2.8.1 Probe Inlet Method	49
3.2.8.2 Thermal Desorption Method	50
3.2.8.3 Concentrator Temperature	50
3.2.9 Statistical Methods	51
3.3 Results	51
3.3.1 Precisions of the Internal Standard Peak Areas	51
3.3.2 Measurement Accuracy, Precision, and Linearity	51
3.3.2.1 Low Humidity Calibration	51
3.3.2.2 Effects of Humidity on Analytical Accuracy, Precision, and Instrument Response	52
3.3.2.3 Expected Performance of Calibration with High Humidity Standards	53
3.3.2.4 Low Humidity Probe Calibration for High Humidity Sampling	53
3.3.3 Concentrator Performance	53
3.4 Discussion	54
3.4.1 Instrument Response to Internal Standards	54
3.4.2 Instrument Response: Probe vs. Thermal Desorption	55
3.4.2.1 Low Desorption Temperature	55
3.4.2.2 Concentrator Breakthrough	56
3.4.2.3 Reduced Instrument Response Due to High Humidity	57
3.4.2.4 Sorbent Strength	58
3.4.3 Performance of the Probe and Thermal Desorption Tubes	59
3.4.4 Study Limitations	60
3.5 Conclusions and Recommendations	61
3.6 Acknowledgements	62
4.1 Introduction	75
4.2 Materials and Methods	77
4.2.1 Analytical Equipment	77
4.2.1.1 Analysis Using the HAPSITE®ER GC/MS	77
4.2.1.2 Analysis Using the Agilent 7890B / 5977B GC/MS Instrument	78
4.2.1.3 Markes Unity 2 Parameters	79
4.2.2 Thermal Desorption Tube Selection	79
4.2.3 Preparation of Isoflurane Atmospheres	80
4.2.4 HAPSITE® Instrument Calibration and Analysis	81
4.2.5 Sample Loading onto Thermal Desorption Tubes	81
4.2.6 Determination of Desorption Flow Rate and Volume	81
4.2.7 Desorption Efficiency	82
4.2.8 Thermal Desorption Tube Breakthrough	83
4.2.9 Thermal Desorption Tube Temperature Measurement	84
4.2.10 Statistical Methods	84
4.3 Results	85

5.3.3 Method Validation Tests	115
5.3.3.1 Sampling Rate and Sampling Capacity	115
5.3.3.1.1 Sampling Rate Over a 15-minute Sampling Period	115
5.3.3.1.2 Sampling Rate and Sampling Capacity Over a 120-minute Sampling Period	116
5.3.4 Sorbent Breakthrough.....	116
5.3.5 Retention of Isoflurane - Reverse Diffusion	117
5.3.6 Desorption Efficiency	117
5.3.7 Storage Stability	117
5.4 Discussion.....	117
5.4.1 Dynamic Flow Chamber Validation.....	117
5.4.2 Sampling Rate and Sampling Capacity	118
5.4.3 Sorbent Breakthrough.....	120
5.4.4 Retention of Isoflurane - Reverse Diffusion	120
5.4.5 Storage Stability	121
5.5 Study Limitations	122
5.6 Conclusions and Recommendations	122
Chapter 6: Conclusion and Future Studies	139
6.1 Analysis of Isoflurane in Humidified Air Using the INFICON HAPSITE® ER Portable Gas Chromatograph/Mass Spectrometer.....	139
6.2 Evaluation of Carbotrap 349, Carbograph 5TD, and Sulficarb Thermal Desorption Tubes for Sampling Isoflurane in Humidified Air Using the INFICON HAPSITE® ER Portable Gas Chromatograph/Mass Spectrometer.....	140
6.3 Chapter 5: Validation of Sulficarb and Carbograph 5TD Thermal Desorption Tubes for Passive Sampling of Isoflurane.....	141
6.4 Summary	143
REFERENCES	144

LIST OF TABLES

Table 2.1. Occupational Exposure Limits (OEL) for Isoflurane	39
Table 3.1. Thermal desorption tube sorbent characteristics	66
Table 3.2. Temporal effects on MS peak area for isoflurane.	67
Table 3.3. Instrument Response to Internal Standards.	67
Table 3.4. Accuracy, precision and difference in sensitivity between low ¹ and high ² humidity measurements.	68
Table 3.5. Expected performance of High Humidity measurements.	69
Table 3.6. Isoflurane average % breakthrough of the internal concentrator during thermal desorption (10-minute desorption).	73
Table 3.7. Qualitative results of the ¹ internal concentrator breakthrough by selected challenge agents.....	74
Table 4.1. Thermal desorption tube sorbent characteristics	96
Table 4.2. Desorption efficiencies of Carbograph 5TD, Sulficarb, and Carbotrap 349 (low concentration isoflurane standards).	97
Table 4.3. Temperature profile data for tested thermal desorption tubes.....	98
Table 4.4. Desorption tube average steady-state desorption temperature.	101
Table 4.5. Statistical analysis of average steady-state desorption temperature.....	101
Table 4.6. Tukey Honest Significant Difference Multiple Comparisons post hoc test. ...	102
Table 5.1. Calibration curve data for Agilent 7890B / 5977B.	125
Table 5.2. Chamber Validation: Average Agreement of Thermal Desorption Tubes (Passive Sampling) and Anasorb 727 Tubes (OSHA 103)	127
Table 5.3. Thermal Desorption Tube Sampling Rates During 15-minute Sampling (20% and 80% Relative Humidity)	129
Table 5.4. Experimental Design of the Passive Sampling Tests.	130
Table 5.5. Estimated Marginal Means for Sulficarb and Carbograph 5TD Sampling Rates.	131
Table 5.6. Two Way ANOVA Results for Sulficarb and Carbograph 5TD Sampling Rates.	132
a. $R^2 = 0.129$ (Adjusted $R^2 = 0.090$).	132
Table 5.7. Reverse Diffusion of Isoflurane from Sulficarb.....	135
Table 5.8. Reverse Diffusion of Isoflurane from Carbograph 5TD.	135
Table 5.9. Desorption Efficiency of Sulficarb and Carbograph 5TD.....	136
Table 5.10. Storage Stability Test Results.....	138

LIST OF FIGURES

Figure 3.1. The HAPSITE® ER GC/MS Instrument.	64
Figure 3.2. Internal concentrator.	64
Figure 3.3. Hand-held direct air sampling probe.....	65
Figure 3.4. The HAPSITE thermal desorption sampling system (TDSS).....	65
Figure 3.5. Instrument response observed during analysis of low humidity standards.....	70
Figure 3.6. Instrument Response: Low Humidity vs. High Humidity.	71
Figure 3.7. Agreement between sample introduction methods.	72
Figure 4.1. Temperature measurement in the HAPSITE® ER's thermal desorption sampling system (TDSS).	99
Figure 4.2. Desorption temperature profiles of tested thermal desorption tubes.	100
Figure 5.1. Agilent 7890B / 5977B Gas Chromatograph / Mass Spectrometer.	124
Figure 5.2. Markes Unity 2 and Series 2 ULTRA Thermal Desorption Autosampler....	124
Figure 5.3. Seven and Ten Point calibration curves for Agilent 7890B / 5977B.....	125
Figure 5.4. Dynamic flow chamber and Miller-Nelson temperature, humidity, and flow control system.....	126
Figure 5.5. Thermal desorption tube mounting system.	126
Figure 5.6 Chamber Validation: Agreement of Thermal Desorption Tubes (Passive Sampling) and OSHA Method 103 (Active Sampling).	128
Figure 5.7. Sulficarb and Carbograph 5TD Sampling Rates and Sampler Capacity During 120-minute Sampling.	133
Figure 5.8. Sorbent Breakthrough.	134
Figure 5.9. Stability of Isoflurane on Sulficarb and Carbograph 5TD Sorbents	137

CHAPTER 1: Introduction

1.1 DESCRIPTION AND BACKGROUND OF ISOFLURANE ANESTHETIC

One of the most common inhalational anesthetics in both medicine and veterinary science is 2-chloro-2-(difluoromethoxy)-1, 1, 1-trifluoroethane, more commonly known as isoflurane. It is a colorless, halogenated ether with a pungent odor commonly administered to maintain anesthesia in humans and animals during invasive surgical procedures (92). Isoflurane, along with sevoflurane, has supplanted many older halogenated anesthetics due to desirable characteristics such as low solubility in blood, relatively high tissue solubility, low rate of metabolism, and low cardio-suppressant effect (25; 31). Isoflurane is often administered in combination with other anesthetics such as nitrous oxide, though it can also be administered alone (92).

1.2 POTENTIAL FOR EXPOSURE TO ISOFLURANE

Undesirable exposure to anesthetic gases and vapors (for simplicity referred to simply as “gases”) is not uncommon in medical, dental, and veterinary environments (81). Potentially exposed occupations include surgeons, doctors, dentists and dental technicians, veterinarians, veterinary technicians, anesthesiologists, nurse anesthetists, operating room and recovery room nurses, operating room technicians, and other hospital personnel (74; 92). Waste anesthetic gases (WAGs), the small amounts of anesthetic gases that inadvertently leak into work areas and that can lead to personnel exposures, may be generated in a variety of ways: 1) through the improper use of, or leaks in, anesthesia delivery systems, 2) from spills that occur during filling of anesthesia vaporizers, 3) during maintenance of those systems, 4) during intubation or extubation of the anesthetized patient, 5) as a result of poorly functioning gas scavenging systems, and

6) from the expired breath of anesthetized patients (74; 92). In 1991, the U.S. Occupational Safety and Health Administration (OSHA) estimated that there were over 250,000 people who may be unnecessarily exposed to harmful levels of WAGs (79). More recently, the U.S. Department of Labor (2016) estimated the number of workers in occupational fields that generally have potential risk of exposure to WAGs. Estimates include over 100,000 workers in the medical research field, over 67,000 anesthesiologists and nurse anesthetists, 140,000 surgeons, surgical technologists, and surgical assistants, and over 250,000 veterinarians, veterinary technologists, and veterinary assistants. These numbers may underestimate the number of potentially exposed workers as they do not include Post Anesthesia Care Unit (PACU) nurses and others (111).

1.2 POTENTIAL HEALTH IMPACTS

Isoflurane has not been definitively proven to negatively impact the health of chronically exposed persons (17). However, most studies that have specifically studied isoflurane have investigated the high acute exposures needed to anesthetize a patient. Studies that have investigated the health effects resulting from chronic, low concentration exposures that are typical of occupational environments are scarce (92). In general, epidemiological studies have associated exposures to waste anesthetic gases and vapors (WAGS) with negative health outcomes such as miscarriages, spontaneous abortions, congenital defects, liver and kidney toxicity, and neurobehavioral changes (21; 22; 31). Several studies also associated occupational exposure to anesthetic gases with decreased cognitive and motor function (15; 16), which could affect medical, dental, and veterinary personnel and could conceivably impact patient safety. Since miscarriages and spontaneous abortions have been associated with exposure to anesthetic gases,

occupations with high numbers of potentially exposed females, such as nursing and veterinary sciences, may be at unique risk. For example, in 2010, females comprised almost 78% of all veterinary school graduates and the proportion continues to rise (5).

1.3 AIR SAMPLING AND ANALYSIS

Monitoring of exposures during the full work shift or during task(s) where potential exposure exists have been performed using conventional sorbent-based active and passive sampling methods as well as by infrared spectrometry (49; 56; 80; 92). Infrared spectrometry can offer continuous monitoring and measurement of peak exposures, which are advantages that conventional air sampling methods lack. However, infrared systems can be expensive, very sensitive to humidity and background interference and can be problematic when used to perform integrated monitoring on workers whose jobs duties require them to be mobile (83; 96). Active and passive methods that use conventional media have been shown to offer good accuracy and precision, however, they can have several disadvantages. For example, active sampling requires the wear of cumbersome pumps to draw ambient air through the sorbent. Also, analysis of traditional sorbent media is typically beyond the capability of onsite health and safety personnel and thus requires shipping of the media to a third-party laboratory for analysis. Interruptions in shipping or analysis at the lab can delay results by days, weeks, or, in extreme cases, even months. These types of delays are common in the military environment, particularly when samples are shipped from a Navy vessel or from deployed settings. These delays can negatively affect the sampling media and reduce confidence in, or even invalidate, results. Traditional sampling media also requires solvents, such as carbon disulfide, to chemically desorb the analyte of interest from the

media. This creates a potential health and fire hazard and makes reuse of the sampling media impossible. Desorption using liquid solvents also has several inherent drawbacks. Liquid solvents are inconvenient to carry into the field. Impurities in the desorption solvent, or even the desorption solvent itself, may mask the agents of interest. Also, solvent desorption dilutes the sample and can reduce analytical sensitivity by a factor of up to a thousand (58; 118).

Thermal desorption is a method that precludes the use of solvents and instead relies on heat to desorb the analyte of interest from the sorbent media. Thermal desorption methods were originally developed to address the limitations associated with traditional liquid chemical desorption and are well-suited for use with field-portable instrumentation (118). Unlike methods that rely on liquid desorption, thermal desorption enables virtually all of the collected analyte to be made available for analysis. This produces a strong advantage in analytical sensitivity that can be up to 1000 times greater than traditional solvent-based methods. Thermal desorption can be a powerful tool that offers industrial hygiene and environmental health scientists additional capabilities needed for rapid, sound, science-based decision making.

1.4 PORTABLE GAS CHROMATOGRAPH / MASS SPECTROMETER INSTRUMENTATION

Use of field-portable GC instrumentation offers investigating personnel a powerful tool enabling on-site, near real-time assessment of volatile organic compounds (VOCs) (102). Additionally, field-portable GC instrumentation can be small and light enough to be easily transported by one person (person-portable) and allow for convenient relocation to specific areas where VOC analysis is needed (102). When combined with an appropriate detector, such as a mass spectrometer (GC/MS), field-portable

instrumentation can identify (and in some cases quantify) unknown or unexpected VOCs contained within complex mixtures in the workplace and outdoor environment to solve exposure problems (100). Field-portable instruments are already commonly used in military, firefighter, and police applications and additional uses in those fields are expected as GC/MS capabilities continue to evolve (100). Some manufacturers of field-portable GC/MS systems integrate into their instruments the capability to directly analyze thermal desorption tube and solid phase microextraction (SPME) media (42; 108), which gives them even greater capabilities.

1.5 ANALYSIS OF EXHALED BREATH

Biological data pertaining to workers' chemical uptake can be very useful in exposure assessment. When correlated with personal exposure data, exhaled breath analysis can be useful in helping to estimate the total dose to the agent of interest. As a result, the American Conference of Governmental Industrial Hygienists (ACGIH) encourages occupational health professionals to accumulate and report biological monitoring data together with exposure and health data whenever possible (3).

Exhaled breath can offer non-invasive biological data and has been used to evaluate exposure to various solvents, fuels, and other VOCs (32; 53; 86; 87). Sampling and analysis of exhaled breath has even formed the foundation for the National Institutes of Occupational Safety and Health (NIOSH) Method 3704, Perchloroethylene (Portable GC) in Exhaled Breath and Air (73). Exhaled breath has also been used to assess exposure to isoflurane and other anesthetic gases and vapors in hospital operating rooms and post-anesthesia care unit environments (23; 31; 32; 48; 89). Analysis of exhaled breath is desirable as it provides an indirect (and less invasive) estimate of the VOC

concentration in the worker's blood and provides an index of an individual's chemical uptake. It has been used to identify increases in exposure over time or to identify personnel within a group who have high exposures relative to the other members of the group (118).

1.6 PUBLIC HEALTH / MILITARY HEALTH RELEVANCE

While researchers have utilized various methods to assess isoflurane exposure, no studies were found in the literature that combined passive sampling, thermal desorption, exhaled breath analysis, and field-portable GC/MS instrumentation. Combining these techniques can be advantageous, especially in a deployed military context, as they are light, convenient, non-invasive, highly sensitive, and no power is required for collection onto the sampling media. Analysis requires no toxic, flammable solvents and can be performed in a field environment. It would also enable rapid presumptive (field) results obtained in a matter of minutes or hours rather than the days, weeks, or even months that can result when shipping samples from a military field environment to a qualified laboratory for analysis. Delay in analysis due to long shipping times is a particularly important point since high temperatures possible in austere military environments can quickly degrade samples, making results unreliable. This study may provide results that can help provide enhanced characterization of isoflurane exposure and dose to military and civilian medical and veterinary personnel. Additionally, these assessment methods could also be leveraged to characterize other public and military-relevant exposures such as solvents, jet fuels, and others.

1.7 HYPOTHESIS AND SPECIFIC AIMS

The research presented in this study investigated the use of portable GC/MS instrumentation and thermal desorption tube sampling to help improve characterization of worker exposure to isoflurane. Chapter 2 provides a review of the pertinent literature, while Chapter 3 provides a proof-of-concept by investigating the suitability of the Inficon Hazardous Pollutants on Site Extended Range (HAPSITE® ER) to quantify isoflurane in surrogate exhaled breath. Chapter 4 further examines the performance of the HAPSITE® ER's thermal desorption sampling system and the three thermal desorption tubes used in Chapter 3, and assesses suitability for their use on that instrument. Finally, Chapter 5 partially validates Sulficarb and Carbograph 5TD thermal desorption tubes for short-term (15 minute) passive sampling of isoflurane using a Markes UNITY 2 thermal desorption pre-concentrator, Markes Series 2 ULTRA autoloader, and a bench-top Agilent 7980B gas chromatograph with a 5977B mass selective detector.

1.7.1 Hypothesis

The INFICON HAPSITE® Extended Range (ER) portable GC/MS and thermal desorption methods, used with active and short-term passive sampling techniques, can successfully quantify isoflurane anesthetic in surrogate exhaled breath and ambient air with an accuracy of $\pm 25\%$.

1.7.2 Specific Aims

(1) Determine whether the HAPSITE® ER is suitable for quantification of isoflurane in highly humidified surrogate exhaled breath with the accuracy of $\pm 25\%$. Both sample introduction methods (whole-air sampling probe and thermal desorption sampling system) will be investigated.

(2) Further evaluate the performance of Sulficarb, Carbograph 5TD, and Carbotrap 349 thermal desorption tubes to determine their suitability for sampling of isoflurane when used in combination with the HAPSITE[®] ER.

(3) Validate Sulficarb and Carbotrap 5TD thermal desorption tubes for short-term passive sampling of isoflurane using a dynamic flow sampling chamber, with analysis conducted using a Markes UNITY 2, Series 2 ULTRA autoloader, and bench-top Agilent GC/MS. At a minimum, parameters to be validated include sampling rates, humidity effects, sample stability, reverse diffusion, sampler capacity, accuracy and precision.

CHAPTER 2: Literature Review

2.1 INTRODUCTION

The first halogenated inhalational anesthetic, chloroform, was initially used in 1847 (81). Since that time, a variety of halogenated anesthetic agents (compounds with chlorine, bromine, fluorine, or a combination of the three as part of their structure) have been used, including trichloroethylene, halothane, fluoroxene, and methoxyfluorene (81). These anesthetics had varying degrees of toxicity, less than ideal physical and chemical properties, and variable levels of effectiveness, so the search for safer, less toxic, and more effective alternatives continued (107). Research efforts eventually led to the development of the fluranes, including isoflurane, desflurane, and sevoflurane. These three halogenated ethers are currently the most widely used FDA-approved halogenated inhalational anesthetics in medicine (115). Isoflurane and sevoflurane are the most common halogenated inhalation anesthetics used in veterinary medicine (95).

2.2 ISOFLURANE AS AN INHALATIONAL ANESTHETIC

According to Eger (1981, 2004), isoflurane has several important properties that make it a desirable choice as an inhalational anesthetic. First, is its potency, displayed by a low Minimum Alveolar Concentration (MAC) of 1.15%. The MAC is defined as the concentration of a vapor that prevents movement in 50% of exposed subjects after a stimulus (pain) is applied. A low MAC is desirable as it enables delivery of the anesthetic with a high concentration of oxygen. When delivered with a mixture of 70%/30% nitrous oxide/oxygen, the isoflurane MAC decreases to 0.50%. Secondly, isoflurane has relatively high solubility in tissues, which is shown by a fat:gas partition

coefficient of 45. Solubility in fat is an important parameter for an inhalational anesthetic, since it must be able to cross the blood/brain barrier in order to exert an effect on the central nervous system. Thirdly, isoflurane has a lower blood:gas partition coefficient (1.4) than older inhalational anesthetics such as enflurane (blood:gas = 1.68), halothane (blood:gas = 2.3), and methoxyflurane (blood:gas = 12). The only inhalational anesthetics that have lower blood:gas coefficients are nitrous oxide (blood:gas = 0.45), sevoflurane (blood:gas = 0.65), and desflurane (blood:gas = 0.45). Isoflurane's low blood:gas coefficient allows rapid build up in the body to anesthesia-inducing levels, offers a greater degree of control over the patient's anesthetic state, and results in more rapid recovery to consciousness. Additionally, isoflurane is resistant to physical and metabolic degradation, which minimizes the formation of damaging byproducts (such as carbon monoxide) after exposure to moist carbon dioxide scrubbers in rebreathing systems or toxic metabolites arising from biotransformation. Lastly, isoflurane is inexpensive in comparison to alternatives such as sevoflurane and desflurane (25; 26).

2.3 OCCUPATIONAL EXPOSURE LIMITS

Occupational Exposure Limit (OEL) values for isoflurane have been implemented by domestic and foreign organizations and vary from 2 – 60 ppm (8 hour time-weighted average), and from 2 – 80 ppm for short-term exposures (2; 18; 43; 75; 92; 110; 112). A list of regulatory, authoritative, and voluntary isoflurane OELs is given in Table 2.1. None of the three primary U.S. organizations that promulgate OELs, OSHA, ACGIH, and NIOSH, have established OELs specific to isoflurane. NIOSH (1977) did develop a Recommended Exposure Limit (REL) of 2 parts per million (ppm) for the halogenated anesthetics gases (HAGs) chloroform, trichloroethylene, halothane, methoxyflurane,

fluroxene, and enflurane (72). According to Field (2015) and the Centers for Disease Control (CDC) (2006), the 2 ppm REL was not based on toxicological or health effects data as NIOSH was unable to determine a safe level of exposure from the toxicological and exposure information available at the time. Instead the REL was based on the lowest concentration in air that contemporary sampling and analytical methods could detect. However, isoflurane was not in wide use when the 1977 REL was published, and the CDC later specifically excluded isoflurane from that recommendation (30; 69). As a result, the applicability of the 2 ppm REL to isoflurane as a “safe” level of exposure is questionable (10; 96). In light of the continuing uncertainty surrounding what exposure level to isoflurane and other halogenated anesthetics is protective of workers, OSHA simply recommends that occupational exposures to these compounds be kept “as low as possible” (81). The U.S. Army and U.S. Navy use (at the time of this writing) the 1977 NIOSH REL of 2 ppm (Ceiling) averaged over 60 minutes (110; 112).

2.4 HEALTH EFFECTS

In general, epidemiological studies have associated chronic exposures to waste anesthetic gases and vapors (WAGs) with negative health outcomes such as miscarriages, spontaneous abortions, congenital defects, liver and kidney toxicity, and neurobehavioral changes (21; 22; 31).

As reported by the American Society of Anesthesiologists Taskforce on Trace Anesthetic Gases (1999), Vaisman published in 1967 the seminal study investigating health effects associated with exposure to inhalational anesthetic gases on male and female anesthesiologists in the Soviet Union. The primary inhalational anesthetics used at the time of the study were diethyl ether, halothane, and nitrous oxide. Exposure was

associated with health effects such as headaches, fatigue, disturbed sleep patterns, loss of appetite, general malaise, and irritability. Exposure was also associated with increased risk of adverse reproductive effects as 18 of 31 pregnancies in the female subjects ended in spontaneous abortion (4; 17). A meta-analysis of studies investigating the increased risk of spontaneous abortion in exposed women estimated a relative risk of spontaneous abortion of 1.9 (12).

A later study by Bruce (1974 & 1975) found a significant associations between exposure to anesthetic gases and decreased cognitive and motor functions when performing a divided-attention audiovisual task ($p < 0.001$), tachistoscopic (eye recognition) tasks ($p < 0.025$), reciting memory passages ($p < 0.05$), and digit span ($p < 0.05$) (15; 16).

Some adverse health effects caused by inhalational anesthetics result from toxic metabolites produced during biotransformation. Pihlainen & Ojanpera (1998) reported that toxic metabolites were the likely cause for several cases of hepatitis in patients who were exposed to high, anesthesia-inducing concentrations of isoflurane. Byhahn (2001) reported a similar finding for antigenic metabolites created through biotransformation of another halogenated inhalational anesthetic (halothane). These metabolites caused an autoimmune response in hepatocytes, which resulted in damage or destruction of those cells (17). While fluoride ion is known to be nephrotoxic, no cases of kidney toxicity have been reported after exposure to isoflurane. Isoflurane has not been found to be carcinogenic (85).

Most studies investigating the health effects associated with waste anesthetic gas exposure have focused on inhalational anesthetics in general and not isoflurane

specifically. Additionally, many studies that have assessed isoflurane have concentrated on health effects resulting from exposure to high concentrations needed to induce anesthesia. As a result, there is a lack of epidemiological data that can definitively determine the association between chronic health effects and occupational isoflurane exposure.

Acute exposure to isoflurane has been associated with central nervous system effects such as headaches, nausea, fatigue, drowsiness, and irritability (78). These effects could conceivably impact patient safety. Hoerauf et al. (1999) found a significant increase ($p < 0.05$) in the frequency of sister chromatid exchanges in the peripheral lymphocytes of operating room personnel exposed to low concentrations of isoflurane (mean = 0.05 ppm) and nitrous oxide (mean = 11.8 ppm). The researches concluded that the genetic damage caused by these exposures were comparable to smoking 11-20 cigarettes per day (40).

As reported by Saber & Hougaard (2009), chronic occupational exposure (greater than 5 years) of nurses to isoflurane, sevoflurane, desflurane, and nitrous oxide was found to be associated with impaired balance control, though the study did not make clear which compound(s) contributed to the effect. This is not uncommon, as co-administration of isoflurane with other anesthetics (such as nitrous oxide) has made causal association between isoflurane and specific health effects challenging in many studies (92).

2.5 EXPOSURES AND CONTROLS

According to NIOSH (2007), potentially exposed occupations include surgeons, doctors, dentists and dental technicians, veterinarians, veterinary technicians,

anesthesiologists, nurse anesthetists, operating room and PACU nurses, operating room technicians, and other hospital personnel. Waste anesthetic gases (WAGs), the small amounts of anesthetic gases that inadvertently leak into work areas and lead to personnel exposures, can be generated in a variety of ways: 1) through the improper use of, or leaks in, anesthesia delivery systems, 2) during maintenance of those systems, 3) during intubation/extubation of the anesthetized patient, 4) as a result of poorly functioning gas scavenging systems, and 5) from the expired breath of anesthetized patients.

Recommended engineering, administrative controls, and work practice controls to reduce exposures include dilution ventilation, use of active and/or passive waste anesthetic scavenging systems, use of key fill vaporizers that minimize the risk of spills during refilling, use of inflatable cuffs on endotracheal tubes to minimize leakage into the room during anesthesia delivery, use of scavenging masks on patients to collect and exhaust exhaled breath, training of workers on prevention of and control of exposures, development of an exposure monitoring program, development of medical surveillance, use of closed system or low fresh flow of anesthesia, and delay flow of anesthetic until the patient is masked or intubated (11; 65; 74). Facility-specific policies that require the use of these controls should be written.

Boiano & Steege (2016) collected self-reported survey responses from 1,783 nurse anesthetists, 1,104 physician anesthesiologists, and 100 anesthesiologist assistants (total n = 2,987) working in outpatient surgical centers and hospitals. The survey collected data on the prevalence, use, and effectiveness of engineering, administrative, and personal equipment used by the respondents to control exposures to waste anesthetic gases. The most highly used inhalational anesthetics used during the week prior to

submittal of the survey were sevoflurane (62%), desflurane (27%), isoflurane (5%), and nitrous oxide (4%). The majority (95%) of respondents reported that they co-administered nitrous oxide with the halogenated anesthetic. Approximately 97% of respondents indicated that scavenging systems were used “every time” gaseous anesthesia was administered. Most of the respondents who did not use scavenging systems indicated that those systems were either not available or were not functional. Approximately 28% of the responding physician anesthesiologists, 13% of the nurse anesthetists, and 9% of anesthesiology assistants indicated that they had never received proper training on the safe handling of anesthetic gases. 81% of those respondents who did receive training indicated that the training had been received over 12 months prior. More than half (54%) of the respondents reported that standard procedures regarding the proper use of anesthetic gases were not available from their employers, and 27% did not know whether standard procedures were available. On average, approximately 14% of respondents began flow of anesthesia before the delivery mask had been applied to the patient. However, the proportion increased (35 – 45%) when anesthesia was administered to pediatric patients. Filling of vaporizers was not always performed using the key fill system. Use of the key fill system during each or most vaporizer fillings was reported by 86% of the respondents, while 17% reported using “open-air” techniques with a funnel. Of these 17%, 7% reported performing the funnel fill in a ventilated area. Approximately 5% of respondents reported that a large spill of liquid anesthetic had occurred within the previous 12 months. Most spills were reported as being cleaned by the person who caused the spill (20% trained, 37% untrained), while 32% of respondents indicated no knowledge of who cleaned the spill. Approximately 8% of spills were

cleaned up by a designated spill response team. No information on personal protective equipment used during spill cleanup was reported. Several respondents (% not reported) indicated that spills evaporated before clean up occurred. Almost all respondents indicated that patients were extubated in the surgical suite prior to transfer to the post anesthesia care unit. Approximately 97% of respondents reported spending time (78% < 1 hour, 19% > 1 hour) with recovering patients in the post anesthesia recovery unit and 42% did not know whether the recovery unit was sufficiently ventilated. Approximately 35% reported that continuous or periodic air monitoring had been performed in the operating room area to detect anesthetic gas leaks, while 15% reported that personal air monitoring had been performed on themselves or a co-worker (11).

Exposures between medical and veterinary personnel are likely to differ due variety of factors. These can include variances in the use and effectiveness of dilution ventilation, scavenging systems, procedures for induction, maintenance, and recovery from anesthesia, patient size, and difficulties in leak control during anesthesia delivery (68). For example, the U.S. Army Guidelines for the Recognition, Evaluation, and Control of Occupational Exposure to Waste Anesthetic Gases (TB MED 510, 2004) describes general design criteria contained in Military Handbook 1191 for military locations that utilize inhalational anesthetics. Minimum ventilation requirements for operating rooms that utilize recirculating ventilation systems are 25 air changes per hour, with a minimum of 5 air changes per hour of outside air. Operating rooms that utilize 100% fresh air (no recirculation of room air) require a minimum of 15 air changes per hour. Post anesthesia recovery areas require a minimum of 6 air changes per hour, with a minimum of 2 air changes per hour of fresh (outside) air. Veterinary surgery areas

require a minimum of 15 air changes per hour (minimum of 5 of fresh air). Veterinary preparation and recovery areas require a minimum of 6 air changes per hour (minimum of 2 air changes per hour of fresh air) (110). Ventilation recommendations contained in the American National Standards Institute / American Society of Heating, Refrigeration, and Air-Conditioning Engineers and American Society for Healthcare Engineering (ANSI/ASHRAE/ASHE) Standard 170-2013 (Ventilation of Healthcare Facilities) is a minimum of 20 air changes per hour with at least 4 air changes per hour of fresh air for occupied operating rooms and 6 air changes per hour with at least 2 air changes per hour of fresh air for patient recovery areas (7).

A search of industrial hygiene air sampling data contained within the OSHA Integrated Management Information System (IMIS) showed that compliance sampling for isoflurane had been performed a total of 215 times from 1984 to 2015. Approximately 50% of the samples were taken in medical environments (hospitals), while 18% and 32% of the samples were taken in industrial and veterinary settings, respectively. Isoflurane was non-detectable in 99% of the hospital samples (103 of 108). For the five samples in which isoflurane was detectable the concentrations reported ranged from 0.66 – 17 ppm. No information on engineering or administrative controls was given. Isoflurane was non-detectable in approximately 56% (23 of 41) of the industrial samples. The reported concentrations in the remaining samples ranged from 0.69 – 45.3 ppm ($GM = 2.2 \text{ ppm} \pm 1.1$). Isoflurane was not detected in approximately 36% (25 of 69) of veterinary samples. The remaining samples ranged from 0.092 – 32 ppm ($GM = 2.1 \text{ ppm} \pm 1.7$). No information was reported on what proportion of the samples may have had insufficient sample volume or analytical sensitivity.

Numerous studies of isoflurane exposure experienced by workers in Danish and Swedish hospitals and veterinary clinics from 1986 – 2003 were reported by Saber & Hougaard (2009). In one of those studies, Wiesner (2000) et al. performed personal air sampling on operating room personnel using photoacoustic infrared methods. The median exposures at three different hospitals with no scavenging systems ranged from 1.7 – 25.9 ppm. Follow-up studies conducted after scavenging system installation resulted in median exposures of 0.3 – 1.4 ppm. Korczynski (1999) performed personal air sampling (single-beam infrared spectrometry) on veterinarians and veterinary assistants. The median exposure for veterinarians was 5.3 ppm (± 2.7) and for veterinary assistants was 4.7 ppm (± 2.5). Area sampling performed in the same veterinary clinics showed a median concentration of 4.6 ppm (± 2.2) (92). Hoerauf et al. (1998) investigated isoflurane exposure of veterinary workers using open-mask anesthesia induction. The results of their study showed 8-hour time weighed average exposures of 5.3 ppm (± 8.1), with peak exposures of up to 300 ppm (39).

Virtually all professional organizations involved with anesthesia recommend the use of anesthetic scavenging systems to control exposures (64). Scavenging systems can be active or passive in nature. Active systems act as local exhaust systems and rely on a pump or fan to create negative pressure that draws in the overflow exhaled by a patient before it can escape into the workplace (92). Passive systems rely on a chemical filters, generally canisters filled with a sorbent such as activated charcoal, to adsorb the waste anesthetic gases from the air exhaled by the patient. Canisters have a finite capacity, which is monitored by weighing the canister prior to each use. If the weight of the canister increases beyond a value predetermined by the manufacturer, then the filter must

be changed or breakthrough and subsequent leakage of the waste anesthetic gases into the surrounding area can occur (68; 99). It has been shown that unacceptable exposures can occur even when effective scavenging systems are used. A study by Smith & Bolon (2003) showed that up to 42% of tested passive scavenging system canisters that were still within their maximum useful life emitted concentrations of isoflurane greater than 100 ppm back into the room air (99). Mulvenon (2015) found that isoflurane exposure to medical researchers was significantly associated with scavenging system type ($p = 0.02$) and that researchers in areas with passive scavenging systems experienced exposures that were almost 4 times higher than exposures in areas with active systems. Mulvenon also found no significant difference in exposures between areas where there was no scavenging and in areas that utilized passive scavenging (68). McGlothlin, Moenning and Cole (2014) conducted a pilot study evaluating a scavenging mask developed for wear by post-operative patients recovering from sevoflurane and nitrous oxide anesthesia in post-anesthesia care units. Ten patients recovering in the post-anesthesia care unit wore scavenging Iso-Gard masks (cases) and nine patients wore non-scavenging nasal cannulas (controls). Exposure of post anesthesia care unit nurses to sevoflurane and nitrous oxide were measured to determine the effectiveness of the Iso-Gard mask in reducing exposures at various distances from the patients' faces. The average nitrous oxide exposure to nurses tending the control group was 69.10 ppm (± 62.77), while the average exposure to nurses tending the case group was 23.99 (± 28.57). Both exposures were at an average of 6 inches from the patients' faces. This indicated a significantly different ($p < 0.05$) exposure for control nurses that was 2.9 times greater than for the control nurses. At three feet from the patients' faces, the control group nurses

experienced an average nitrous oxide exposure of 11.91 ppm ($\pm$ 5.61), while case group nurses experienced an average exposure of 7.40 ppm ($\pm$ 4.61). Again, the control nurses' average exposure was significantly different ($p < 0.05$) and about 1.6x greater than the case nurse exposure. Similar results were observed in exposures to sevoflurane. Control group nurses at an average of 6 inches from the patients' faces experienced average sevoflurane exposures of 5.04 ppm ($\pm$ 5.82), which was significantly different ($p < 0.05$) than the case nurse average exposure of 1.89 ($\pm$ 2.01). At an average of three feet from the patients' faces, control nurses received an average exposure of 0.92 ppm ($\pm$ 0.33), which was significantly different ($p < 0.05$) than case nurses exposures of 0.69 ppm ($\pm$ 0.30). The authors concluded that post-anesthesia care unit nurse exposure to nitrous oxide and sevoflurane exposure emitted in the patients' exhaled breath can exceed the NIOSH RELs of 25 ppm and 2 ppm, respectively even when dilution ventilation exchange rates meet the required standards. Post anesthesia care units generally do not utilize scavenging units to capture exhaled waste anesthetic gases, and the Iso-Gard scavenging mask was shown to provide an effective means to reduce exposures to levels below the respective inhalational anesthetic OELs (65).

Leaks from anesthesia delivery systems can be pervasive. Marx, Froba, Bader, Villwock, and Georgieff (1996) assessed 115 anesthesia ventilators in 60 anesthetic units and found leakage rates that ranged from 28 – 5,794 mL/min. Leakage rates from 20 new systems ranged from 0-321 mL/min. The majority of the leakage resulted from damaged breathing bags units and leaky delivery system connectors and fittings (63). When combined with poorly operating scavenging systems and/or insufficient ventilation, high exposures could occur. Exposure can also occur as a result of diffusion of the anesthetic

through anesthesia delivery system materials. Marx et al. (1998) showed that anesthetic gases can leak through porous and non-porous polymers of parts that comprise anesthesia delivery and scavenging systems, including reservoir bags, tubing, and endotracheal tubes. Testing was performed on silicone, latex, and polyvinyl chloride (PVC) versions of these products. The silicone version of each of the tested products displayed the highest isoflurane permeation rates. The high permeation rate (1.13 mL/hour) through the silicone ventilation tube resulted in an isoflurane concentration of 1,260 ppm in the test chamber after one hour (chamber volume not reported). The lowest permeation isoflurane permeation rate (1.5×10^{-3} mL/hour) was observed with the latex endotracheal tubes, which resulted in the test chamber concentration (volume not reported) of 1.18 ppm after one hour. Other tested anesthetics (enflurane, and nitrous oxide) also displayed their highest observed permeation rates when tested on products made of silicone, and the lowest permeation rates through products made of latex (63). The results of this study highlight the importance of utilizing anesthesia delivery and scavenging system materials that minimize diffusion of the anesthetic. Leakage through poorly chosen or damaged materials could potentially create areas of relatively high isoflurane concentration even in when dilution ventilation and scavenging systems are in place.

2.5 TOXICOKINETICS

2.5.1 Absorption and Distribution

According to OSHA (2000) and NIOSH (2007), the primary occupational route of exposure is via the lung through inhalation. Absorption through the skin can also occur during clean up of spills and during refilling of anesthesia vaporizers (74; 81). The route of exposure for medical and veterinary patients is solely through the lungs. After

exposure, isoflurane rapidly equilibrates between the alveolar air and the blood and is distributed throughout the body (92).

2.5.3 Biotransformation and Elimination

Approximately 0.2 – 1% of the total isoflurane dose undergoes biotransformation, while < 95% is excreted unchanged in the exhaled breath and approximately 0.5% is excreted through the skin. (92). According to research by Kharesh, Hankins, and Cox (1999), biotransformation occurs by the hepatic cytochrome P450 2E1 enzyme. The primary metabolic products resulting from the biotransformation of isoflurane are F⁻ (fluoride ion), trifluoroacetic acid, and a reactive acyl halide (CF₃CO). The reactive acyl halide is capable of binding covalently with liver proteins, forming adducts in a process that is similar to that which occurs after exposure to halothane. The resulting liver protein adducts can react with antitrifluoroacetylated protein antibodies, creating a potential for hepatic damage. However, isoflurane-induced hepatotoxicity is rare, which is likely a result of typically rapid excretion and slow metabolism *in vivo*. Certain products such as ethanol and isoniazid act as P450 2E1 inducers, which can increase the metabolism of isoflurane. Conversely, products that inhibit P450 2E1, such as disulfiram (Antabuse®), can be administered to patients recovering from anesthesia to reduce the degree of isoflurane metabolite formation (47).

2.6 BIOLOGICAL MONITORING

Biological monitoring can help physicians, nurses, engineers, industrial hygienists and other environmental health professionals judge the acceptability of workplace exposures (67). The ACGIH (2017) describes biological monitoring as an additional means to assess exposure and health risk to workers. Biological monitoring assesses

biological media, such as the urine, blood, or exhaled air of exposed personnel, for a marker of exposure, which can be used as an indicator of uptake and overall dose. Biological monitoring offers many advantages to the environmental health scientist. It can make possible evaluation absorption from all routes of exposure in the overall exposure assessment, reconstruction of past exposures for which no other exposure data may exist, as well as identify sources of exposure that may be non-occupational in nature. In addition, biological monitoring can help assess the effectiveness of personal protective equipment and standard operating procedures (3). An ideal biological monitoring marker should have several properties. Its concentration should be related to the total exposure dose of the chemical of interest by all routes, it should be non-invasive, specific to the exposure, of high sensitivity, and inexpensive (90).

According to the ACGIH (2017), biological monitoring results are generally compared to a reference, such as the ACGIH Biological Exposure Index (BEI), which can offer guidance for evaluating those results. In general, BEIs represent the levels of the biological marker that is most likely to be observed in workers who have received similar doses (through all routes of exposure) as workers who have had inhalational exposure at the chemical's Threshold Limit Value, Time Weighted Average (TLV-TWA). The BEI marker can be a metabolite formed through biotransformation, a biochemical change characteristic of exposure to that chemical, or the chemical itself. The BEI is generally interpreted as indicating exposure to a level below which most healthy workers should not experience adverse health effects. It is also commonly used to assess body burden, reconstruct past exposure for which there may be little or no exposure data, and detect non-occupational exposures amongst a workforce. A BEI can

also be used as an indicator of the effectiveness of engineering controls (dilution ventilation, scavenging systems, leak control actions), administrative controls (standard operating procedures, work practices), and personal protective equipment. There currently is no BEI for isoflurane. However, this may be due to a lack of adequate available data to support development of an isoflurane BEI. To address these types of data shortages, ACGIH encourages industrial hygienists and other environmental health scientists to collect biological monitoring data along with exposure data (3).

The suitability of exhaled breath to help characterize exposure to many volatile organic compounds has been shown (6; 24; 31; 33; 86; 87). Ghittori et al. (2004) measured toluene in the end-exhaled air of exposed chemical factory workers using Tenax[®] TA thermal desorption tubes. A significant ($p < 0.0001$) correlation was found between environmental air and end-exhaled toluene air. The correlation coefficient was $r = 0.822$ (33). Lourencetti et al. (2010) analyzed exhaled breath of subjects who had bathed in swimming pools and baths for determination of trihalomethanes. Comparisons of trihalomethane levels in exhaled breath before and after bathing showed statistically significant differences ($p < 0.05$) for chloroform, fluorobenzene, bromodichloromethane, dibromochloromethane, and 4-bromofluorobenzene. Only bromoform was not statistically significant (53). Amorim, Carniero, and Cardeal (2008) used solid phase microextraction sampling to measure benzene in the exhaled air of exposed gas station workers and unexposed controls. The study results showed a significant difference ($p < 0.05$) in the mean concentration of benzene in the exhaled breath between the two groups. However, there was large variation in both groups, suggesting a possible lack of homogeneity in the exposed group (6).

Ghimenti et al. (2015) measured the halogenated anesthetic sevoflurane and isopropyl alcohol in the exhaled breath of hospital staff at the conclusion of an 8-hour work day. After collection in a nalophan bag, 250 mL aliquots of exhaled breath were run through a drying tube and then concentrated on glass thermal desorption tubes packed with 250 mg of 60/80 mesh Tenax[®] GR (70% Tenax[®] TA, 30% graphite). Sevofluane concentrations ranged from 1 – 17 ppb, and all breath sample results were below the 2 ppm NIOSH REL for halogenated anesthetics. Data on ambient concentrations of sevoflurane were insufficient for the authors to draw conclusions regarding the correlation between sevoflurane in exhaled breath and ambient concentrations. Isopropyl alcohol concentrations in breath averaged $20 \text{ ppb} \pm 20$, which was far below the NIOSH RELs of 400 ppm (TWA) and 500 ppm (STEL). Isopropyl alcohol was found to have a good correlation ($R^2 = 0.95$, $p < 0.001$) between concentrations in air and in exhaled breath (31).

A study of isoflurane exposure by Prado, Tortosa, Ibarra, Luna, and Peragio (1997) found a correlation between the concentrations of isoflurane in ambient air and in exhaled breath. The researchers measured 167 personal ambient isoflurane exposures and isoflurane in end-exhaled breath in operating room personnel (number of subjects not reported) working in 13 surgical theatres in Spanish hospitals. The ambient isoflurane concentrations ranged from 1.14 to 157.23 mg/m^3 with a geometric mean (GM) of 16.23 mg/m^3 and geometric standard deviation (GSD) of 16.62 mg/m^3 . Isoflurane concentrations in end-exhaled breath ranged from 0.15 to 26.09 mg/m^3 , with a GM of 2.85 mg/m^3 and GSD of 2.51. Both the ambient and end-exhaled concentrations of isoflurane displayed log-normal distributions. A significant ($p = 0.0001$) correlation

between the log of exposure dose (time-weighted average exposure multiplied by the exposure time) and the log of the isoflurane concentration in exhaled air ($R = 0.79$) was observed. This correlation was of sufficient strength to enable the researchers to calculate a biological index that predicted the level of exposure to ambient isoflurane based on the concentration of isoflurane detected in exhaled breath. The calculated biological indices that corresponded to 8 hour exposures to 2 ppm and 10 ppm were 4.4 and 13.6 mg/m³ of isoflurane in end-exhaled breath, respectively (89).

2.7 THERMAL DESORPTION TECHNIQUES

Woolfeden (2013), described the combination of thermal desorption techniques with analysis by GC or GC/MS analysis as being one of the powerful tools for detecting and identifying volatile organic compounds in air. One of thermal desorption's great advantages relative to conventional air sampling methods is increased sensitivity. Air sampling methods that utilize conventional sorbents (such as activated charcoal) rely on liquid solvents to extract the analyte(s) of interest from the sampling media. The extraction solvents are typically flammable and/or toxic. Also, depending on the analyte, liquid extraction can result in lower extraction efficiencies ($\approx 80\%$) than does thermal desorption ($\geq 95\%$). In addition, dilution effects associated with liquid extraction can result in a 1000-fold reduction in sensitivity relative to thermal desorption. This is because the collected molecules are typically desorbed in 1 mL of solvent, but only about 1/1000th of the solvent volume (1 μ L) is usually analyzed. In contrast, thermal desorption preclude the use of solvents and instead relies on the application of heat to the sorbent to stimulate release of adsorbed compounds. And, unlike liquid solvent extraction, virtually all of the desorbed molecules are available for analysis. Other advantages of thermal

desorption methods include media that can be reused for 100 or more analyses, reduced interference since desorption solvent peaks cannot obscure analyte peaks, and the ability to fully automate analysis (116; 118). Thermal desorption tubes have also been shown to be successful for passive sampling of many volatile organic compounds in indoor, outdoor, and occupational environments (13; 35; 58; 93; 98; 106).

2.7.1 Passive Sampling of Isoflurane

Passive sampling operates according to Fick's first law of diffusion, which is described by *Equation 2.1*.

$$M = \frac{DA}{L} C_0 t \quad (2.1)$$

M = amount of isoflurane transported by diffusion (mol)
 D = molecular diffusion coefficient of isoflurane (cm²/s)
 A = cross section of the diffusion path (cm²)
 L = total length of the diffusion path (cm)
 C_0 = average isoflurane concentration in the medium (mol/cm³)
 t = time (s)

The DA/L term is expressed in units of cm³/min, which are the identical units used to describe sample flow rate in active air sampling devices.

As described by Woolfenden (2010 & 2013), traditional passive samplers were generally open-face devices that contained sorbent beds made of various materials such as charcoal. Passive samplers were desirable for several reasons. They were lightweight, did not require external power to function, required minimal training to use properly, and were inexpensive. They could offer good accuracy and precision, and they precluded the need for expensive and complicated sampling pumps. However, passive samplers had limitations, including a requirement for air movement over the sorbent bed in order to maintain a continuous sampling rate. Also, many sorbents were of insufficient strength to retain the compounds that they had collected. Reverse diffusion of those compounds from the sorbent would occur, which led to sample loss. In addition, these samplers relied on liquid desorption and so suffered from dilution effects caused by the desorbing solvent. Since passive sampling rates were lower than active alternatives, longer sampling times were required to ensure collection of sufficient analyte mass to allow

quantification. Development of axial-type samplers that overcame many of these limitations began in the 1970s. Axial samplers are typically tubular, of ¼ inch outer diameter and 5 mm inner diameter (cross-section) and can be constructed from stainless steel, steel coated with an inert substance, or glass. Construction is precisely controlled to leave a 15 mm gap between the face of the sorbent and the outer rim of the tube face, and to ensure a diameter of 5 mm (116; 118). Carefully controlling these dimensions during the manufacturing process enable the use of these tubes for diffusive sampling according to Fick's law described by Equation 2.1.

Several commercially available badge-type samplers have been validated for passive sampling of isoflurane (1; 9; 49; 56). Badge-type samplers typically have higher sampling rates than axial samplers. However, they require liquid solvent extraction and so suffer from dilution effects during analysis. Prado et al. (1993) evaluated three types of thermal desorption tubes (Tenax TA, Chromosorb 102, and Chromosorb 106) for their suitability for use with isoflurane. Chromosorb 106 displayed a breakthrough volume of 45.2 L/g breakthrough volume, which suggested that it could efficiently retain collected isoflurane. Tenax TA and Chromosorb 102 displayed lower breakthrough volumes (0.2 L/g and 7.3 L/g, respectively), suggesting that their strength was unsatisfactory and they were removed from consideration. Chromosorb 106 passive sampling rates were validated in a dynamic flow chamber at five concentrations ranging from 1.27 – 23.13 ppm, three sampling times (90 – 480 minutes), and two relative humidity levels (40% and 98%). The results of the study found that the average sampling rate (corrected to Normal Temperature and Pressure) was $3.43 \text{ ng ppm}^{-1} \text{ min}^{-1}$ (volumetric sampling rate not reported). Average desorption efficiency across the tested concentration range was

99.98% (± 3.9) and storage stability tests showed that the tubes could be stored at room temperature for 2 weeks with little loss of sample. Humidity did not have an observable effect on the collection or recovery of isoflurane (88). Tenax[®] TA tubes have also been validated for passive sampling of isoflurane, and were found to have a sampling rate 2.20 ng ppm⁻¹ min⁻¹ (0.32 mL/min) (38; 57).

2.7.2 Thermal Desorption Tube Selection

Markes International Technical Support document 005 (2014) describes the properties of different sorbents, two of which have been validated for use with isoflurane (Chromosorb 106 and Tenax[®] TA). Also described are two sorbents that are investigated in the studies contained in this dissertation (Carbograph 5TD and Sulficarb).

Chromosorb 106 and Tenax[®] TA have several desirable advantages, including hydrophobicity and inertness, which enables them to be used in humid atmospheres and to sample reactive compounds. However, they also have several disadvantages. They are both porous polymers and have limited maximum desorption temperatures (200°C and 300°C, respectively) relative to graphitized carbon black and carbon molecular sieve alternatives. Tenax[®] TA is classified as a weak sorbent with a surface area of approximately 35 m²/g of sorbent. Tenax[®] TA is too weak to efficiently capture and retain a compound as volatile as isoflurane. Chromosorb 106 is medium-strength with a surface area of approximately 750 m²/g of sorbent. It is relatively “dirty” in nature and requires careful conditioning to reduce artefacts to an acceptable level and has a propensity to self-generate aromatic hydrocarbons in storage. Carbograph 5TD is the strongest of the graphitized carbon black sorbents with a surface area of approximately 100 m²/g of sorbent. Carbograph 5TD bridges the gap between the graphitized carbon

blacks and the high strength carbon molecular sieves. It displays good hydrophobicity and, though mainly non-porous, does exhibit some micropores that allow it to capture compounds of relatively high volatility. Sulficarb is a high surface area material (approximately 1200 m²/g of sorbent) belonging to the carbon molecular sieve sorbent category. These sorbents are high strength and can retain high amounts of water relative to weaker sorbents, which must be accommodated for during sampling and/or analysis. Both Carbograph 5TD and Sulficarb are clean sorbents that display minimal artefacts (59; 117). Affinity for water is an important factor when considering selection of an appropriate thermal desorption tube. This is particularly so when the tube will be used to sample humid air, as in exhaled breath analysis.

Fastyn, Karnacki, Gierczak, and Niedzielski (2005) found that graphitized carbons (such as Carbograph 5TD) and carbon molecular sieves (such as Sulficarb) showed adsorption of water on the polar centers of the sorbents when sampling in atmospheres of 90-95% relative humidity was performed. At high relative humidity levels, carbon molecular sieves also showed micropore volume filling, which reduced sorbent capacity. Removal of the adsorbed water was possible by allowing dry air to pass through the sorbent material (dry purging) prior to analysis. Dry purging of Carbograph 5TD only required a few hundred mL of dry air, while the carbon molecular sieves needed much larger volumes (specific volume not reported) (29).

Sulficarb and Carbograph 5TD have been successfully used in studies as varied as determining the volatile organic compound profiles of cantaloupe melons, detecting *Listeria monocytogenes* on cut melon fruit by identifying changes in the melon's VOC profile, characterizing the volatile and semi-volatile components of aerosols released by

electronic cigarettes, determining the emissions of volatile organic compounds from indoor materials and products, and investigations of the odors of decomposing cadavers (8; 14; 91; 103; 105).

2.8 INFICON HAPSITE® ER PORTABLE GC/MS

Field-portable GC/MS instrumentation is commonly used in military, firefighter, and law enforcement applications and offers investigating personnel a powerful tool enabling on-site, near real-time assessment of VOCs (100; 102). Use of field-portable GC/MS instrumentation can be particularly valuable to military environmental health scientists in austere, deployed military environments since shipping of samples to qualified laboratories and potential analytical hindrances can delay results for several weeks or even months. Additionally, harsh temperatures experienced during shipping can degrade or damage samples, making their results questionable. Portable GC/MS tools can offer good presumptive results and a basis for risk analysis and decision-making without first requiring time-consuming third-party analysis.

Smith and Driscoll (2013) and Martin, Kwak, Fan, Grigsby, and Ott (2015) describe the Inficon HAPSITE® ER as a portable GC/MS system easily transportable by one person to the site where assessment is needed. The HAPSITE® ER utilizes nitrogen as the carrier gas, and vacuum required for the mass spectrometer (quadrupole) is provided by a Non-Evaporative Getter (NEG) chemical vacuum pump and an ion sputter pump. The instrument can be operated on battery power using rechargeable Nickel-Metal-Hydride (NiMH) batteries. The HAPSITE® ER has two methods of sample introduction; a hand-held, whole-air sampling probe and a thermal desorption sampling system. The hand-held probe has historically been the primary method of sample

introduction thermal desorption sampling system, as the thermal desorption sampling system did not exist on earlier iterations of the HAPSITE[®] (61; 100). The hand-held probe attaches to the HAPSITE[®] analytical module by an approximately 4-foot long transfer line which can be heated above ambient temperatures (>40°C) to minimize risk of sample condensation. Organic compounds in the sample air are concentrated on an onboard sorbent trap, which is a small tube containing three sorbents of various strengths. The HAPSITE[®] concentrator does not have the capability to be cooled to sub-ambient temperatures. After sample collection, the concentrator is rapidly heated to desorb the concentrated analyte(s) into the analytical column. In contrast, the thermal desorption sampling system provides heat to desorb analytes that have been collected on glass or steel thermal desorption tubes of much larger dimensions. The desorbed analytes are then collected (focused) on the onboard concentrator in a much smaller sorbent volume. As with the hand-held probe method, the concentrator is then rapidly heated to desorb the collected analytes so that they can be swept into the analytical column by the carrier gas. The U.S. Navy, U.S. Air Force, and others have utilized the HAPSITE[®] for research and to assist with onsite chemical risk assessment in both domestic and deployed environments (20; 37; 46; 50; 62; 76).

Sekiguchi, Matsushita, Yamashiro, Sano, and Seto (2006) used the HAPSITE[®] hand-held probe to sample various chemical warfare agents. Sampling was performed on 1 mg/m³ of the nerve agents sarin (GB) and soman (GD), and 3 mg/m³ of the nerve agent tabun (GA). Mustard gas (HD, 0.5 mg/m³) was also sampled. Good precision of the peak areas (CV = 2.1 – 5.6%) and linearity of the calibration curves (correlation coefficients was not reported) was observed. Limits of Detection for GD, GB, GA, and

HD were 0.2, 0.5, 8, and 0.3 $\mu\text{g}/\text{m}^3$, respectively. Analysis of blank samples indicated carryover of the chemical warfare agents, which was hypothesized to be a result of compound condensation on the interior of the hand-held probe transfer line. Several blanks (GB = 1, GD/GA = 3) were required to eliminate the carryover, though a small peak of HD was still visible after 6 blanks had been run. The authors concluded that the HAPSITE[®] was very useful for rapid identification of the tested chemical warfare agents (94).

Gorder & Dettenmaier (2011) utilized a HAPSITE[®] ER to investigate vapor intrusion by chlorinated volatile organic compounds in residential housing located on Hill Air Force Base near Ogden, Utah. Chlorinated volatile organic hydrocarbons were identified in 42 of 46 homes investigated during the study, with subsurface vapor intrusion verified in two homes. The HAPSITE[®] enabled increased sampling efficiency by offering both a relatively low sensitivity “survey” mode that bypassed the analytical column and routed sample air directly to the mass spectrometer, as well as a Selective Ion Monitoring (SIM) mode, which only scanned areas of the mass spectrum that contained ions belonging to the chemicals of interest. These efficiencies enabled the researchers to determine the locations of interior sources of contamination in 42 homes in less than 2 hours. Based on the success of the study, the U.S. Air Force recommended use of portable GC/MS in future investigations of chlorinated volatile organic compounds (34).

Kwak, Fan, Geier, and Ott (2014) performed a comparison of the hand-held probe and thermal desorption sampling system HAPSITE[®] sample introduction methods during analysis of a standard mixture of EPA Method Toxic Organics (TO)-15 65-component mix. Forty nine of the tested compounds were detected using both introduction methods,

and no compound was detected exclusively by one method. Comparisons of relative peak intensities observed with each method showed that retention times of each component differed slightly between sample introduction methods. This was hypothesized to result from differences in carrier gas pressure between the methods. Also, peak intensities of the two internal standards observed with thermal desorption were of much higher intensity than those observed with the hand-held probe. This was thought to be a result of increased dilution of the internal standard mixture during probe sampling. Peak intensities of the TO-15 compounds (especially those with higher boiling points) were significantly higher with thermal desorption than with the hand-held probe. This was thought to be a result of higher boiling point compounds condensing in the hand-held probe transfer line. A plot of boiling point vs. log of the relative intensities of the TO-15 compounds (thermal desorption intensity/hand-held probe intensity) resulted in a Coefficient of Determination ($R^2 = 0.5518$). This indicated that approximately 55.2% of the variation in peak intensity between the two methods was explained by the boiling points of the TO-15 compounds (50).

Martin et al. (2015) compared the accuracy obtained with the HAPSITE[®] ER thermal desorption sampling system with a bench-top Thermo ISQ GC/MS after analysis of Tenax[®] TA thermal desorption tubes used for passive sampling of EPA Method TO-17 compounds. Overall, the HAPSITE[®] displayed lower sensitivity, resolution, and more rapid saturation than did the bench-top GC/MS model. Twelve of 18 compounds detected with ISQ GC/MS generated uptake rates that were $\pm 25\%$ of the calculated, ideal uptake rate or the uptake rates reported in the literature. The majority of the uptake rates for the 15 compounds detected with the HAPSITE[®] ER were lower than expected in

comparison with the calculated, ideal uptake rate. Only one of the 15 compounds (cyclohexane/3-methylhexane) was within $\pm 25\%$ of its idea uptake rate. It was also observed that HAPSITE[®] accuracy was poor when target compound concentrations were higher than approximately 25 parts per billion (ppb). The HAPSITE[®] was also less sensitive than the ISQ, and was not able to detect compounds with concentrations < 0.1 ppb. This caused the researchers difficulty in detecting the tested compounds in low concentrations samples and in determining the true presence of those compounds in trip blanks. The researchers provided several recommendations to address the HAPSITE[®] ER's limitations, including methods to collect additional sample (longer passive sampling periods, sampling in areas of higher concentration). Full validation of the sampling scenarios most critical to those in the bioenvironmental engineering field were also recommended (61).

Harshman et al. (2017) assessed the performance of the HAPSITE[®] ER's thermal desorption sampling system. Three HAPSITE[®] ER instruments were compared with each other and with a Trace Ultra-ISQ bench-top GC/MS. Significant variability of the standard compounds in the three HAPSITE[®] instruments was observed, with relative standard deviations (RSD) ranging from 17.84 – 49.97%. The bench-top GC/MS displayed better precision, with RSD $< 11.6\%$. Relatively high variability was also observed in peaks that were normalized to internal standards (bromopentafluorobenzene for the HAPSITE[®] ER, and chlorobenzene d-5 and 1,4-difluoribenzene for the bench-top GC/MS). The precision of the internal standard on the HAPSITE[®] was inconsistent and varied over time. On one particular HAPSITE[®], an RSD of 50.55% was observed, while the other two HAPSITE[®] instruments displayed RSD values of $< 10.5\%$. This suggested

that the internal standards used on the bench-top GC/MS accounted for fluctuations in the overall system, while the internal standard in the HAPSITE® did not. The variation was likely due to internal standard addition directly to the onboard concentrator rather than to the thermal desorption tube itself. This indicated that pre-loading thermal desorption tubes with an internal standard would likely give better precision than utilizing the HAPSITE®'s automatically injected internal standard. A significant level ($p = 0.0014$) of carryover of TO-15/TO-17 65 component mix and JP-8 jet fuel was also observed, however, except for dodecane on glass thermal desorption tubes, the carryover was within acceptable limits ($<10\%$) for the U.S. EPA TO-17 standard. Further analysis of carryover determined that thermal desorption tubes displayed a higher level of background artefacts after analysis on the HAPSITE® ER than after analysis on the bench-top GC/MS. This was likely due to incomplete desorption of high boiling point compounds from the thermal desorption tube sorbent caused by insufficient heating during desorption (37).

2.9 CONCLUSION

Isoflurane is one of the most commonly used inhalational anesthetics used in medicine and veterinary science. However, the safety of isoflurane after chronic exposure over a working lifetime has not yet been definitively proven.

Proper implementation of engineering and administrative controls is required to reduce exposures to acceptable levels. Recommended controls include dilution ventilation, use of active and passive waste anesthetic scavenging systems to collect waste anesthetic from the exhaled breath of patients currently under anesthesia, use of key fill vaporizers that minimize the risk of spills during refilling, use of inflatable cuffs

on endotracheal tubes to minimize leakage into the room during anesthesia delivery, use of scavenging masks on recovering patients to collect their exhaled breath, training of workers on prevention of and control of exposures, development of a monitoring program, development of medical surveillance, and written safe standard operating procedures and work practices. However, these controls are not always utilized and, when they are used, they are not always 100% effective.

Utilizing the techniques of thermal desorption, passive sampling, biological monitoring, and field-portable instrumentation techniques described in this dissertation may offer industrial hygienists and other environmental health scientists methods to improve the characterization of worker exposure to isoflurane in a variety of different work environments.

Table 2.1. Occupational Exposure Limits (OEL) for Isoflurane

Organization or Country	OEL (ppm)	Averaging Time
OSHA ¹	Not Established	N/A
ACGIH ¹	Not Established	N/A
NIOSH ¹	Not Established	N/A
Germany ²	Not Established	N/A
European Union ²	Not Established	N/A
U.S. Army ³	2 ppm (Ceiling)	60 minutes
U.S. Navy ⁴	2 ppm (Ceiling)	60 minutes
Cal/OSHA ⁵	2	8 Hours
NIH ⁶	2	8 Hours
Denmark ²	5	8 Hours
UK ²	50	8 Hours
Ireland ²	50	8 Hours
Spain ⁷	50	8 Hours
Netherlands ²	20	8 Hours
Norway ²	2	8 Hours
	4	15 minutes
Switzerland ²	10	8 Hours
	80	15 minutes
Sweden ²	10	8 Hours
	20	15 minutes
Finland ²	10	8 Hours
	20	15 minutes
Abbott Laboratories ⁸	60	8 Hours

¹U.S. Occupational Safety and Health Administration Annotated Table Z-1 (82).

²The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals 141. Isoflurane, Sevoflurane and Desflurane (92).

³U.S. Army TBMED 510, Guidelines for the Recognition, Evaluation, and Control of Occupational Exposure to Waste Anesthetic Gases (110).

⁴U.S. Navy Chemical Stressor Dictionary with Occupational Exposure Limits (112).

⁵California Occupational Safety and Health Administration (Cal-OSHA) (18).

⁶National Institutes of Health (NIH) Waste Anesthetic Gas (WAG) Surveillance Program (75).

⁷Instituto Nacional de Seguridad e Higiene en el Trabajo (INSHT), Límites de Exposición Profesional para Agentes Químicos en España (43).

⁸Abbott Laboratories, Safety Data Sheet for Isoflurane (2).

CHAPTER 3: Analysis of Isoflurane in Humidified Air Using the INFICON HAPSITE® ER Portable Gas Chromatograph/Mass Spectrometer

Edward Benchoff¹, Philip A. Smith², Mary Brueggemeyer¹, Theodore Juarez³

¹Preventive Medicine and Biostatistics Department, Uniformed Services University of the Health Sciences, Bethesda, MD

²Salt Lake Technical Center, U.S. Occupational Safety and Health Administration, Salt Lake City, UT

³Industrial Hygiene Department, Naval Medical Center San Diego, San Diego, CA

ABSTRACT

The INFICON HAPSITE® Extended Range (ER) person-portable gas chromatography/mass spectrometer (GC/MS) instrument was assessed for its ability to quantify isoflurane in warmed, humidified air (as a surrogate for exhaled breath) with an accuracy of $\pm 25\%$. Sample introduction methods included a hand held probe for direct air sampling, and thermal desorption of Carbograph 5TD, Carbotrap 349, and Sulficarb sorbent tubes. Accuracy and precision of the instrument, humidity effects on sampling and analysis, and performance of the internal focusing trap (concentrator) were evaluated. Calibration curves developed using the probe and low humidity isoflurane standards were also assessed for generalizability to thermal desorption methods and high humidity samples. The probe offered the most convenient sampling and greatest overall accuracy for quantifying isoflurane in surrogate breath, displaying an average error of -16% when the instrument was calibrated with low humidity standards. Average error decreased to -8.0% when calibration was performed using high humidity standards. Each method of sample introduction displayed a decrease in instrument response when sampling high humidity surrogate breath. Average decreases in response ranged from approximately -18% (probe and Carbotrap 349), to -33% (Carbograph 5TD), suggesting that a HAPSITE® instrument calibrated using low humidity standards would underestimate the

concentration of isoflurane in exhaled breath. Accuracy improved considerably (absolute error < 4.0%) when each method of sample introduction was individually calibrated using high humidity standards. Breakthrough of the internal concentrator sorbents by isoflurane and other challenge agents, including isooctane, toluene, 1,1,1-trichloroethane, and dichloromethane was observed with thermal desorption sampling approaches. Isoflurane breakthrough ranged between 8.7 – 16.8% across the measured range. Calibration curves were not generalizable between sampling methods nor between thermal desorption tube types. For highest accuracy, the HAPSITE[®] instrument should be calibrated using the exact sample introduction method to be used for sampling exhaled breath (probe is recommended) and with standards created using air of similar temperature and water vapor content as exhaled breath. If thermal desorption is to be used, then calibration should be performed using the specific thermal desorption tube type to be used.

3.1 INTRODUCTION

It is estimated that over 200,000 healthcare professionals in the human and veterinary medicine and dental professions have potential exposure to anesthetic gases and vapors (for simplicity referred to as “gases”) (74; 81). Epidemiological studies associate occupational exposures to anesthetic gases with negative health outcomes such as miscarriages, spontaneous abortions, congenital defects, liver and kidney toxicity, and neurobehavioral changes have been published (21; 22; 31). Some studies have also associated occupational exposure to anesthetic gases with decreased cognitive and motor function (15; 16). Exposures to health care professionals could also have a negative impact on patient safety.

A common inhalational anesthetic in both human and veterinary medicine is 2-chloro-2-(difluoromethoxy)-1, 1, 1-trifluoroethane, more commonly known as isoflurane. It is a colorless and volatile liquid with a vapor pressure of 330 mm Hg at 25°C and a boiling point of 48°C (70). This halogenated ether is used to maintain anesthesia in humans and animals during invasive surgical procedures (92).

Active and passive air sampling methods to estimate personal inhalation exposure to isoflurane have been established (1; 49; 56; 80). Biological sampling may also be used to estimate the dose absorbed by a worker who has been exposed to a variety of chemicals, including isoflurane (3; 33; 53; 86; 87; 89). When used in conjunction with conventional breathing zone air sampling data, analysis of isoflurane in exhaled breath can offer additional information regarding the effectiveness of control measures (67).

Instrumentation based on GC/MS can separate individual VOC components in mixtures with mass spectral information obtained providing qualitative and quantitative data regarding those individual components (66). However, conventional GC/MS equipment found in analytical laboratories is generally very large and can weigh several hundreds of pounds. Such bench top systems are also complex and their effective use is beyond the typical ability of the average industrial hygienist or environmental health scientist. While field-portable GC/MS instruments potentially offer many of the capabilities of larger bench top systems, they are generally much simpler to use, and have a smaller footprint and weight. Some can be easily transported by one or two people to specific areas where VOC analysis is needed, providing investigating personnel a powerful tool for on-site, near real-time risk assessment (102). Technological advances in sampling methods and field-portable analytical instrumentation now offer the ability to

rapidly and reliably screen an environment without having to rely exclusively on traditional laboratory-based methods (101). Specifically, exhaled breath samples could be collected and transported to an instrument for analysis, and/or the instrument itself could be transported to the site of breath sample collection.

The HAPSITE[®] ER portable GC/MS instrument manufactured by INFICON[®], Inc. (East Syracuse, NY) is an example of an instrument that may offer this kind of capability. Additional advantages of the instrument are that users can introduce samples into the instrument using several methods, including through a hand-held direct air sampling probe inlet and through thermal desorption of sorbent tubes. However, no reports were available in the literature regarding the use of portable GC/MS to measure isoflurane in exhaled breath. Therefore, an evaluation was performed to test the capability of the HAPSITE[®] instrument to detect and quantify isoflurane in warmed, humidified air intended to approximate human exhaled breath. The hand-held probe and thermal desorption sample introduction methods were investigated to determine which offered the best accuracy, precision, and linearity of response. The interference of humidity, reproducibility of internal standards used for quantitation, and potential for breakthrough of the sample tube used for thermal desorption and the internal concentrator sorbents were also studied.

3.2 MATERIALS AND METHODS

3.2.1 Analytical Equipment

A HAPSITE[®] ER analytical module (Figure 4.1) was used. This instrument employed a non-polar DB-1ms analytical column of 15 m length with 0.32 mm inner diameter and 1.0 μ m stationary phase film thickness (Agilent Technologies, Santa Clara,

CA), and quadrupole mass analyzer. The high level of vacuum necessary for the proper function of the HAPSITE[®] mass selective detector was provided by a HAPSITE[®] service module for this study. Ultra-pure (99.999%) nitrogen purchased from Roberts Oxygen (Rockville, MD) was used as the carrier gas.

A non-cooled focusing trap (internal concentrator) was employed in this instrument (Figure 3.2). The concentrator contained three sorbents, Carbotrap Y, Carbotrap C, and Carboxen 1018 (in order of increasing strength) to enable collection and focusing of a wide range of volatile organic vapors from whole air and thermally desorbed samples. A hand-held probe sample inlet facilitated introduction of sample air directly into the concentrator (Figure 3.3). Sample introduction using thermal desorption relied on capture of isoflurane from air by one or more sorbents contained inside a stainless steel thermal desorption tube. After sampling, each thermal desorption tube was individually placed into the thermal desorption sampling system module and heated to desorb isoflurane from the tube sorbent(s) (Figure 3.4).

To ensure proper functionality, the mass spectrometer was tuned at the beginning of each day and every 12 hours when in use. To clean out organics from prior analyses or that may have adsorbed between periods of use, the concentrator was heated to high temperature and purged with carrier gas for several minutes at the beginning of each day and on an as-needed basis.

3.2.2 Analytical Method Parameters

HAPSITE[®] Smart IQ software (V1.30) was used to create two analytical methods tailored for either the probe or thermal desorption sampling system. Both methods utilized full scan mode (m/z 46 – 300) and shared the following parameters; the mass

spectrometer inlet membrane was set to 80°C, while the internal valve oven and heated lines used to switch from sample loading to injection/analysis were set to 70°C. The initial column temperature was 40°C held for 0.5 minutes, and was then ramped at 60°C per minute to a maximum temperature of 180°C (held for 0.5 minutes). The total GC analysis time was 10 minutes. A filament delay time of 0.25 minutes allowed oxygen, nitrogen, and carbon dioxide to elute prior to beginning 70 eV ionization. A known volume of 1,3,5-tris(trifluoromethyl)benzene and bromopentafluorobenzene was automatically injected from a pressurized canister (50 ppb and 10 ppm, respectively in nitrogen, Inficon) housed in the analytical module. Parameters specific to the probe method included a 1-minute purge method (126 mL/min), 1-minute sample to the internal concentrator (89 mL/min), and probe transfer line heating (45°C). Programmed parameters specific to the thermal desorption method included an initial thermal desorption sampling system temperature of 40°C, a programmed desorption temperature target of 315°C (ramped at 90°C per minute), and a 10-minute desorption period. To prevent current overload, the HAPSITE[®] ER thermal desorption sampling system is limited by the manufacturer to a maximum temperature of 315°C (37).

3.2.3 Thermal Desorption Tube Selection

Stainless steel thermal desorption tubes (89 mm x 6.35 mm outer diameter, 5 mm inner diameter) were used in this study. Carbon molecular sieve (Sulficarb 70/80 mesh) sorbent was recommended by the thermal desorption tube manufacturer as the most appropriate sorbent for isoflurane sampling. Tubes containing Carbograph 5TD 40/60 mesh sorbent (a graphitized carbon black) were also used to investigate their potential to capture isoflurane. These single-bed tubes were purchased from Markes International

(Llantrisant, UK). Tri-bed Carbotrap 349 tubes containing the graphitized carbon black sorbents Carbopack Y and Carbopack B, and the carbon molecular sieve sorbent Carboxen 1003 (Supelco, Bellefonte, PA) were also used due to the ability of this type of tube to capture VOCs with a wide range of volatilities. All tubes were pre-conditioned according to the tube manufacturers' recommendations prior to use. The classes and characteristics of the sorbents contained in each tube are listed in Table 3.1.

3.2.4 Preparation of Isoflurane Standards

Two groups of static isoflurane atmospheres were prepared, with each comprised of six concentrations that ranged from 0.24 – 6.0 µg/L, a range that was selected based on a study by Prado et al. (1997). These authors measured the concentration of isoflurane in the breath of 167 surgical room workers in a Spanish hospital and observed an average geometric mean (GM) concentration of 2.85 µg/L ($\pm$ 2.51). The median concentration was 3.03 µg/L, with a range of 0.15 – 26.09 µg/L (89).

For one of the groups of standards referred to as “High Humidity”, of isoflurane, a Miller-Nelson HCS-501 temperature and humidity control system (Miller-Nelson Research, Inc., Livermore, CA) warmed and humidified compressed house air to approximately 35°C and 95% relative humidity similar to human exhaled breath (104). This group “Low Humidity” utilized laboratory ambient air (19°C and 62% relative humidity) as the diluent. The absolute humidity of the Low Humidity and High Humidity atmospheres was approximately 10 and 37 mg of water per L of dry air, respectively (113).

3.2.5 Sample Introduction

3.2.5.1 Sample Introduction via Probe Inlet

Sample introduction using the probe involved two steps. First, the sample line was purged with gas from within a sample bag, after the probe was attached to the bag (Figure 3.3). This minimized dilution of the incoming sample with gas that was already present in the sample probe lines. The internal concentrator was bypassed during the purge, with purge gas routed directly to exhaust. The second step consisted of a 1-minute draw through the probe at a flow rate of 89 mL/min where sample air was passed through the internal concentrator to collect isoflurane.

3.2.5.2 Sample Introduction via Thermal Desorption

Isoflurane was loaded from sample bags onto thermal desorption tubes using one stroke of a 100 mL ($\pm 1\%$) Kitagawa AP-20 piston pump (Kitagawa America, Pompton Lakes, NJ). Each tube was then desorbed in the thermal desorption sampling system (Figure 3.4).

3.2.6 HAPSITE[®] Calibration and Analysis

Calibration curves for each sample introduction method (probe inlet, Carbograph 5TD, Carbotrap 349, and Sulficarb) were developed using Low Humidity isoflurane standards. Effects of high humidity on analytical accuracy, precision, and linearity were investigated by comparing instrument response observed during calibration with response resulting from analysis of the High Humidity samples. However, Sulficarb tubes were not used with High Humidity samples due to poor desorption efficiency (discussed later).

Areas of the isoflurane base peak ion (m/z 51) were integrated manually using the HAPSITE[®] ER IQ Plus software (Ver. 2.33). The m/z 51 peaks were not normalized to

TRIS nor BPFB internal standards due to the low peak area precision and resulting poor linearity of normalized calibration curves that resulted with that approach.

3.2.6.1 Generalizability of HAPSITE[®] Calibration Using the Probe Inlet

The probe sample inlet was capable of sampling whole air and offered the simplest, quickest, and most convenient method of calibration. Since this form of calibration may be desirable to HAPSITE[®] users with minimal training and experience in GC/MS, it was investigated whether the relevant calibration curves were generalizable and could be applied to Carbograph 5TD and Carbotrap 349 results while meeting the $\pm 25\%$ level of accuracy sought by OSHA for sampling and analysis methods.

3.2.7 Temporal Humidity Effects on Instrument Response

A comparison was made between probe measurements from the High Humidity 6.0 $\mu\text{g/L}$ sample bag performed on the day of the bag's creation (Day 1) and probe measurements made the following day (Day 2), simulating same-day and next-day analysis of a collected breath sample. The m/z 51 peak areas observed on Day 2 were approximately two times higher than those observed on Day 1 (Table 3.2). Investigation of the sample bag found that the relative humidity in the bag had dropped overnight, from 95% (Day 1) to 52% (Day 2). Additional analyses were performed on Days 3 and 4 to investigate whether additional changes in m/z 51 peak areas occurred, but the results were similar to those seen on Day 2. As a result of these findings and to maximize analytical sensitivity, measurements of the High Humidity samples in this study were performed on Day 2, simulating next day analysis of a collected breath sample.

3.2.8 Performance of the Internal Concentrator

3.2.8.1 Probe Inlet Method

To simulate sample collection using the probe inlet, the internal concentrator was removed from the HAPSITE[®] and allowed to equilibrate for 15 minutes in an Agilent 7890B GC oven at 50 °C. A Carbotrap 349 thermal desorption tube was then attached to the rear of the concentrator. Several different volumes (100, 200, and 300 mL) from the High Humidity 6.0 µg/L isoflurane standard were then drawn through the concentrator using the piston pump. A concentrator cleanout and three blank runs were then performed to ensure that no isoflurane residual carried over into subsequent analyses. The Carbotrap 349 tube was then analyzed for the presence of isoflurane.

The potential for breakthrough of the concentrator by compounds of differing vapor pressures was also explored. A mixture comprised of 2.2 µg/L isooctane (99.71%, Honeywell International, Morris Plains, NJ), 1.5 µg/L toluene (100%, JT Baker, Phillipsburg, NJ), 2.3 µg/L 1,1,1-trichloroethane (99+%, Sigma-Aldrich, St. Louis, MO), and 2.2 µg/L dichloromethane (99.9%, Fisher-Scientific, Fair Lawn, NJ) was created. Volumes of 100, 200, and 300 mL of this mixture were drawn through the concentrator tube and analyzed as previously described.

A Eutech Instruments (Vernon Hills, IL) Model 68900-01 Digi-Sense Temperature Controller and J-type thermocouple was used to measure the temperature of the internal concentrator. The thermocouple was wrapped around the ceramic exterior of the internal concentrator, while making sure to minimize contact with the heating wires. The temperature of the concentrator during each method's "ready" status (just prior to sampling) was 48.6°C. Concentrator temperature decreased to 43.8°C during the probe line purge and to 45.5°C during the thermal desorption cycle. The concentrator

temperature increased to approximately 350°C over approximately 30 seconds during desorption of the concentrator.

3.2.8.2 Thermal Desorption Method

Concentrator performance during thermal desorption was investigated at each of the concentrations used in the study. For each concentration, a 100-mL volume was drawn from the applicable sample bag through a Carbotrap 349 tube, which was then desorbed in the thermal desorption sampling system. A 1-L Tedlar® sample bag was attached to the HAPSITE® exhaust port for the 10-minute duration of each desorption cycle. This sample bag collected the exhaust from triplicate samples desorbed for each concentration and combined them into a composite sample. The six resulting composite samples were then analyzed using the HAPSITE® probe inlet to examine for the presence of isoflurane.

The potential for breakthrough of components of the isooctane, toluene, 1,1,1-trichloroethane, and dichloromethane mixture was also investigated. A 100 mL volume of this mixture was drawn through a Carbotrap 349 tube, which was then thermally desorbed. The exhaust 5-minute and 10-minute desorption cycles were captured and analyzed qualitatively using the method described above.

3.2.8.3 Concentrator Temperature

The temperature of the concentrator was measured during probe and thermal desorption analysis using a Eutech Instruments (Vernon Hills, IL) Model 68900-01 Digi-Sense Temperature Controller and J-type thermocouple.

3.2.9 Statistical Methods

Data were analyzed using Microsoft Excel (2011) and IBM® SPSS® Statistics Software (Version 22) (Armonk, NY) to obtain descriptive statistics (mean, standard deviation, coefficients of variation) and linear least squares regression analyses.

3.3 RESULTS

3.3.1 Precisions of the Internal Standard Peak Areas

Precision of the TRIS and BPFB base peak areas was poor with both the probe and thermal desorption, with CV values ranging from 13 to 27% (Table 3.3). Base peak areas for both internal standards were 3 to 4 times greater with the thermal desorption method than with the probe, a finding also described by Kwak et al (50). Precision of the BPFB base peak (m/z 117) was worse with the probe (CV = 27%) than with thermal desorption (CV = 16%).

3.3.2 Measurement Accuracy, Precision, and Linearity

3.3.2.1 Low Humidity Calibration

Each sample introduction method displayed excellent agreement (slope of 1.002) between the isoflurane concentrations in the Low Humidity standards and those predicted by their respective low humidity calibration curves. Block A of Table 3.4 shows that the accuracy for each sample introduction method was good, with average error ranging from 4.0% to 8.7% across the calibration concentration range. Measurement precision for the probe and Carbotrap 349 was also good, with CV ranging from 0.068 to 4.4% and 1.7 to 9.0%, respectively. Precision for Carbograph 5TD was lower, with CV values of 18% and 26% at the two lowest calibration points. Each method showed good linearity for m/z 51 peak areas plotted against isoflurane concentration, with R^2 values ranging from 0.996

to 0.998. Figure 5 shows that Carbograph 5TD and Carbotrap 349 displayed reduced instrument response relative to the probe method.

3.3.2.2 Effects of Humidity on Analytical Accuracy, Precision, and Instrument Response

Block B of Table 3.4 shows the error and precision observed with the High Humidity samples after calibration to Low Humidity standards. This was analogous to exhaled breath analysis using a HAPSITE® instrument calibrated to isoflurane standards that were created using low humidity ambient lab air as the diluent. This resulted in increased error for each method across the sampled concentration range. The absolute probe inlet error increased to 16%, while the absolute error of Carbograph 5TD and Carbotrap 349 measurements increased to 42% and 16%, respectively. All sample introduction methods displayed a decrease in instrument response during analysis of High Humidity samples. Block C of Table 3.4 shows similar decreases (18.1%) between the probe and Carbotrap 349 across the measured concentration range, with Carbograph 5TD displaying the largest decrease (33%). The results of the ANOVA test indicated that the decrease in instrument response was not statistically significant across sample introduction methods ($p = 0.528$), relative humidity ($p = 0.177$), or with interaction between sample introduction method and relative humidity ($p = 0.937$). However, the observed decreases in instrument response did likely contribute to the lower accuracy and precision observed at the lowest concentration tested ($0.24 \mu\text{g/L}$) for each method. Figure 3.6 illustrates the relative decrease between Low and High Humidity measurements and reduced agreement for each sample introduction method.

The probe inlet offered the greatest instrument response in this area of the study, while Sulficarb offered the poorest (Figure 3.5). Error between the sampled and

predicted isoflurane concentrations for thermal desorption methods increased with increasing isoflurane concentration. Further testing with Sulficarb revealed low desorption efficiency, indicating that a large portion of the isoflurane loaded onto the sorbent was not released during desorption. This retention of isoflurane by the Sulficarb sorbent most likely contributed to the poor instrument response observed and will be discussed in Chapter 4 of this dissertation.

3.3.2.3 Expected Performance of Calibration with High Humidity Standards

Block A of Table 3.5 shows that calibration using High Humidity standards resulted in average errors ranging from 7.2% to 8.0%. Regression slopes for each sample introduction method showed good linearity (R^2 0.994 to 0.997) and showed excellent agreement (slope = 1.002) between the sampled and predicted isoflurane concentrations.

3.3.2.4 Low Humidity Probe Calibration for High Humidity Sampling

Block B of Table 3.5 shows that accuracy of each method decreased when the Low Humidity calibration curve developed using the probe was applied to High Humidity measurements. Absolute error for the probe increased to 16%, while Carbograph 5TD and Carbotrap 349 increased to 36% and 23%, respectively. Figure 3.7 illustrates the poor agreement between sampled and predicted isoflurane concentrations for these data.

3.3.3 Concentrator Performance

No isoflurane breakthrough of the heated concentrator removed from the GC/MS instrument and held at constant temperature by isoflurane was observed during simulated probe sampling of high concentration High Humidity samples. However, isoflurane

breakthrough of the internal concentrator was observed during both 5 and 10-minute thermal desorption cycles. The estimated mass of isoflurane breaking through the concentrator during each 10-minute desorption ranged from 8.7 – 16.7% of the original theoretical mass loaded (Table 3.6). Detection of isoflurane during the 5-minute desorption was qualitative only as the relevant peak area was below that of the lowest calibration curve point. Breakthrough of isooctane, toluene, and dichloromethane were also observed (Table 3.7).

3.4 DISCUSSION

3.4.1 Instrument Response to Internal Standards

Addition of an internal standard to the sample prior to analysis typically provides correction for sample injection size, loss of analyte, and matrix effects that can occur during analysis. However, for an internal standard to properly perform its function, the amount added to each analysis must be highly precise. The difference observed in both TRIS and BPFB base peak areas between the probe and thermal desorption methods was likely due to the higher amount of dilution in the internal standards that occurs during probe sampling. The dilution occurs due to the timing of the internal standard injection during the line purge cycle and is described by Kwak et al (50). Harshman et al. (2017) also noted poor internal standard precision for analysis with a HAPSITE® instrument and believed the most likely cause was addition of the internal standard directly to the onboard concentrator rather than to the tube sorbent (37). The findings observed in this study suggested substantial variation for both internal standards in probe and thermal desorption methods. Poor linearity was observed for normalized peak areas, essentially negating the usefulness of both TRIS and BPFB as internal standards. Users who would

like to use an internal standard for their analysis would likely have better results if they select an appropriate internal standard that performs similarly to isoflurane and spike it into the sample matrix prior to sampling and analysis.

3.4.2 Instrument Response: Probe vs. Thermal Desorption

It is possible that a disparity in the volumes sampled by the probe and by the piston pump contributed to the finding of significant difference in the mean instrument response between probe and thermal desorption methods. The average volume sampled by the probe (89 mL) was 11% lower than the volume drawn through the thermal desorption tubes (100 mL) by the piston pump. As a result, all other things being equal and assuming 100% collection and desorption efficiency, the instrument response observed using thermal desorption should have been higher than the instrument response observed when using the probe. However, in all of the tests conducted, the probe method displayed a higher instrument response. This indicates that the difference in instrument response between the probe and thermal desorption methods would likely have been even higher had the probe sampled an equivalent 100 mL volume (as intended by the manufacturer). Figure 3.5 graphically illustrates the difference in instrument response between the probe and thermal desorption sample introduction methods. Potential causes of the negative bias associated with the thermal desorption methods include:

3.4.2.1 Low Desorption Temperature

Temperature is a critical factor in ensuring complete desorption. Hung, Hung, and Huang (1997) observed that vinyl chloride analysis resulted in negative bias when thermal desorption temperatures were inadequately high to ensure efficient desorption (41). Preliminary interior temperature measurements of thermal desorption tube interiors

taken during this study indicate that the steel tubes did not achieve the desorption temperature target of 315°C, which likely contributed to low desorption efficiency in the stronger sorbents, particularly Sulficarb.

As Kwak et al. (2014) and Harshman et al. (2017) noted (37; 50), the HAPSITE® instrument thermal desorption sampling system was designed for glass tubes. It is possible that thermal conductivity differences between steel and glass create an insurmountable barrier that impedes the desorption system from achieving the necessary temperature to maximize desorption. However, the ability to achieve desired desorption temperatures with steel tubes is highly desirable, particularly when sampling sensitive work processes (such as surgical procedures) that may preclude the use of fragile glass tubes.

3.4.2.2 Concentrator Breakthrough

Breakthrough of isoflurane through the concentrator during thermal desorption results in less isoflurane reaching the detector and likely contributed to the observed decrease in instrument response and sensitivity. Parameters such as concentrator tube temperature, carrier gas flow rate, and total volume of carrier gas passing through the concentrator were investigated but did not appear to be the cause of the breakthrough. It is possible that carrier gas was heated during its transit through the hot thermal desorption tube sorbent and that this heat was transferred to the concentrator sorbent, making collection of highly volatile isoflurane more difficult. Regardless of the cause, concentrator breakthrough should be minimized since it can result in reduced sensitivity and underestimation of exposure. Many bench top thermal desorption systems use Peltier or cryogenic cooling of a concentrator to increase collection efficiency of low boiling

compounds such as isoflurane as these are desorbed from a larger primary sorbent tube. However, Peltier cooling is an energy-intensive capability and cryogenic cooling requires a cryogenic liquid or gas. Neither of these technologies may not be practically suitable for a portable GC/MS system. An alternate solution could be to increase the mass of each type of sorbent in the concentrator, which may help reduce the amount of isoflurane and other compounds from breaking through during thermal desorption.

3.4.2.3 Reduced Instrument Response Due to High Humidity

GC/MS is highly susceptible to interferences resulting from water being introduced into the system (54; 117). The reduced instrument response observed with the High Humidity samples could have been caused indirectly or directly by the large amount of water vapor in the samples. Indirect interference could have occurred by isoflurane vapor solubilizing into the liquid water droplets that condensed on the inside surface of the sample bag, thereby making less isoflurane vapor available for analysis. However, isoflurane's Henry's Law constant of 2.87×10^{-2} atm-m³/mole signifies unfavorable distribution to the aqueous phase (109). It is more likely that direct interference could have occurred by water vapor interfering with the quality of chromatography or proper measurement of isoflurane by the mass spectrometer. Hung et al (1997) found that high humidity negatively affected the chromatographic peak quality of vinyl chloride, making identification and quantitation more challenging (41). Also, the strongest sorbent in the concentrator, Carboxen 1018, is a molecular sieve that is less hydrophobic than the concentrator's two weaker sorbents. It is possible that water vapor from the High Humidity samples passed through the two weaker sorbents but was collected and retained by the Carboxen 1018. The relatively large number of water molecules in a high

humidity sample may have competed more effectively for sorbent binding sites than did isoflurane, forcing isoflurane deeper into the strong Carboxen 1018 sorbent. A portion of the isoflurane might not have efficiently desorbed from the Carboxen 1018 with the limited heating and carrier gas flow available. Future work could more clearly identify a likely mechanism contributing to the lower instrument response observed with high humidity samples.

3.4.2.4 Sorbent Strength

Carbograph 5TD displayed worse agreement with the isoflurane concentrations in the High Humidity samples than did Carbotrap 349. Experiments not described in this paper suggest that Carbograph 5TD achieved 100% desorption efficiency and no isoflurane breakthrough was observed at the tested sample volumes. However, in general, Carbograph 5TD achieved lower instrument response (peak area) and greater error than did Carbotrap 349, suggesting a loss of isoflurane. Considering the high desorption efficiency and the lack of breakthrough, the apparent loss of isoflurane must be due to some other cause. According to the manufacturer, Carbograph 5TD is intended to collect VOCs with boiling points between 50°C and 150°C. The boiling point of isoflurane (48°C), is slightly below this range so it is possible that Carbograph 5TD was capable of capturing isoflurane reasonably well during initial loading, but was too weak to keep the isoflurane strongly adsorbed. Consequently, a portion was lost in the time prior to desorption. This hypothesis can be tested by investigating the breakthrough volume and storage stability, which are recommended for future work.

3.4.3 Performance of the Probe and Thermal Desorption Tubes

Block B of Table 3.4 shows that Carbograph 5TD and Carbotrap 349 both would have underestimated the amount of isoflurane in exhaled breath when calibration was performed using Low Humidity standards. Negative bias in Carbograph 5TD results was particularly high and resulted in error higher than $\pm 25\%$ level of error acceptable to OSHA. Negative bias in Carbotrap 349 was less pronounced and was within the $\pm 25\%$ error criterion. However, both tubes could have led to unacceptable underestimation of the concentration of isoflurane in workers' exhaled breath, especially if the breath contained higher concentrations of isoflurane.

Great improvement in accuracy was observed in all three methods when calibration was performed using isoflurane standards with surrogate exhaled breath as the diluent. HAPSITE[®] instrument users who wish to measure isoflurane in exhaled breath with a high degree of accuracy may want to consider three options. The first is to calibrate the instrument to isoflurane standards created using surrogate (or real) exhaled breath. The second is to calibrate to isoflurane standards created with air of ambient room temperature and relative humidity, but to reduce the amount of water vapor in the exhaled breath sample prior to introducing it to the HAPSITE[®] instrument. One possible alternative to achieve this include routing the sample through a drying tube filled with a substance highly selective in the removal of water (such as Nafion[®] polymer). Another possible alternative is to manually program a dry purge into the analytical method to allow carrier gas to flow through the unheated thermal desorption tube prior to desorption. Dry purging sweeps a portion of the adsorbed water out of the sorbent while by-passing the concentrator, reducing the amount of water introduced to the analytical

column. These options to reduce water introduced into the instrument can be investigated in future work.

Thermal desorption sample introduction methods tested in this study displayed decreased instrument response in comparison with the probe and should not be used for sampling isoflurane in exhaled breath unless the probe is damaged or not available, or the user finds it necessary to utilize a sample volume greater than 100 mL. For example, thermal desorption offers an important advantage over probe sampling since the volume of collected sample air drawn through the thermal desorption tube may be varied by simply adjusting the volume of air pumped through the tube. Variation of the sample volume enables analysis of a wide range of isoflurane concentrations by ensuring that the precise amount of isoflurane collected falls within the range of concentrations used for instrument calibration. This is a desirable advantage since the HAPSITE[®] instrument does not incorporate a split flow capability as exists with many bench top GC/MS instruments. Adjustment of the sample volume is not possible with the probe approach, as the probe only samples a fixed, non-adjustable target volume of approximately 100 mL. Sampling an isoflurane concentration outside the calibration range with the probe would require a very time-consuming recalibration rather than a simple increase to the sample volume as is possible with the thermal desorption tubes and the piston pump used.

3.4.4 Study Limitations

Limitations associated with this study included the use of only a single instrument. It was assumed that the instrument used provided representative internal standard delivery precision and thermal desorption temperatures. Also, verification of the concentrations of the isoflurane standards using a conventional sampling method

(OSHA Method 103) was not performed. Due to equipment limitations, high humidity surrogate exhaled breath could be warmed to approximately 35°C, which was two degrees less than the 37°C average temperature of end-expired air. However, the slight increase in absolute humidity at 37°C (to approximately 43 g of water / L of dry air) would have likely demonstrated similar or even clearer negative bias than was shown with the 36 g of moisture / L of dry air used in this study. Also, measurements for each sample introduction method at each concentration were taken in triplicate due to time limitations and the inability to automate analysis on the instrument used. Increasing the number of replicates would have given the study additional statistical power. Lastly, due to the fragility of glass thermal desorption tubes, stainless steel tubes were used in this study. The steel casing may have negatively impacted the maximum desorption temperatures achieved and could have contributed to the poor results observed with Sulficarb. This is an issue that will be investigated in an upcoming study.

3.5 CONCLUSIONS AND RECOMMENDATIONS

High humidity conditions similar to those found in exhaled breath resulted in reduced instrument response relative to ambient (low humidity) laboratory conditions. If high water content in exhaled breath is not accounted for during calibration, then measurements of isoflurane in exhaled breath will underestimate the concentration of isoflurane by a degree greater than $\pm 25\%$ acceptable to OSHA.

Probe sampling offered the most accurate quantitative results in each of the tested scenarios and is the recommended method for HAPSITE® sampling of isoflurane in exhaled breath using the instrumentation examined. However, Carbograph 5TD and Carbotrap 349 thermal desorption tubes also offered reasonable accuracy under certain

circumstances. HAPSITE[®] instrument users should be aware of the potential for concentrator breakthrough during thermal desorption of volatile substances that could result in sample loss and lowered sensitivity.

Of key importance in the accuracy and precision of each method was how instrument calibration was performed. Calibration curves developed for the probe and thermal desorption methods were found to not be interchangeable. Calibration curves developed using each specific type of thermal desorption tube tested in this study were similarly not interchangeable between different types of tubes. As a result, isoflurane analysis should only be performed using the same sample introduction method (and same tube type) that was used for calibrating the instrument.

Future work could investigate methods to reduce moisture content in the sample introduced into the HAPSITE[®] without affecting the isoflurane concentration. Potential breakthrough of the concentrator during thermal desorption by other volatile compounds could be investigated. Validation of Sulficarb, Carbograph 5TD, and Carbotrap 349 thermal desorption tubes for sampling of isoflurane in air and in exhaled breath could also be performed. Validation should investigate accuracy, precision, breakthrough volume, desorption efficiency, storage stability, and humidity effects. Single bed Sulficarb and Carbograph 5TD sampling tubes can also be used for passive sampling of isoflurane and future work could validate their respective isoflurane uptake rates.

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Figure 3.1. The HAPSITE® ER GC/MS Instrument.
The HAPSITE® ER analytical module (green) sitting atop a service module.



Figure 3.2. Internal concentrator.
The concentrator contained three sorbents of varying strengths (Carbotrap Y, Carbotrap C, and Carboxen 1018). After collecting isoflurane from the whole air sample (probe) or from the thermal desorption cycle, the concentrator is heated to rapidly release the collected isoflurane into the analytical column.



Figure 3.3. Hand-held direct air sampling probe.
The probe is drawing sample air from a 5-L Tedlar® bag.



Figure 3.4. The HAPSITE thermal desorption sampling system (TDSS).
The TDSS pictured in place on the upper surface of the HAPSITE analytical module.

Table 3.1. Thermal desorption tube sorbent characteristics

Tube Type	Number of Sorbent Beds	Sorbent Type(s)	Sorbent Strength	Approximate Analyte Volatility Range ⁴	Recommended Desorption Temperature
Carbograph 5TD ¹	Single bed	Graphitized Carbon Black	Medium/Strong	n-C _{3/4} to n-C _{6/7} BP 50 to 150°C n-C ₃ to n-C ₈	350°C
Sulficarb ²	Single bed	Carbonized Molecular Sieve	Very Strong	BP -30 to 150°C	360°C
Carbotrap 349 ³	Tri-bed	1. Carbopack Y – Graphitized Carbon Black	1. Weak/Medium	n-C ₉ to n-C ₁₄ ⁵	330°C
		2. Carbopack B – Graphitized Carbon Black	2. Medium	n-C ₅ to n-C ₁₂ ⁵	
		3. Carboxen 1003 – Carbon Molecular Sieve	3. Very strong for small molecules	n-C ₂ to n-C ₅ ⁵	

¹Tube specifications obtained from Markes International Thermal Desorption Technical Support Document 5 (TDTS 5) (59).

²Tube specifications obtained from Markes International Thermal Desorption Accessories and Consumables catalog (2013/2014)(60).

³Tube specifications obtained from Perkin-Elmer Lab Products and Services webpage (84).

⁴Approximate analyte volatility range relative to n-alkanes.

⁵Information obtained from Jochman (2014) (45).

Table 3.2. Temporal effects on MS peak area for isoflurane.

Data were obtained using the probe.

Replicate Sample	Mean <i>m/z</i> 51 Peak Area (Day 1)	Mean <i>m/z</i> 51 Peak Area (Day 2)	% Change
1	9,838,969	17,346,836	176%
2	9,838,969	19,789,049	201%
3	9,838,969	20,443,320	208%
4	9,838,969	19,448,605	198%
5	9,838,969	20,254,099	206%
Mean	9,838,969	20,048,675	198%

Table 3.3. Instrument Response to Internal Standards.

	Probe		Thermal Desorption	
	TRIS Peak Area (<i>m/z</i> 213) n = 20	BPFB Peak Area (<i>m/z</i> 117) n = 20	TRIS Peak Area (<i>m/z</i> 213) n = 20	BPFB Peak Area (<i>m/z</i> 117) n = 20
Mean	271,578	1,683,238	1,154,208	7,667,573
Standard Deviation	39,878	448,487	145,598	1,232,676
CV	15%	27%	13%	16%

Table 3.4. Accuracy, precision and difference in sensitivity between low¹ and high² humidity measurements.

Isoflurane Concentration (µg/L)	Block A ³						Block B ⁴						Block C ⁵		
	Low Humidity Standard Low Humidity Calibration (n = 3)						High Humidity Sample Low Humidity Calibration (n = 3)						Difference in Instrument Response High Humidity vs. Low Humidity (n = 3)		
	Probe		Carbograph 5TD		Carbotrap 349		Probe		Carbograph 5TD		Carbotrap 349		Probe	Carbograph 5TD	Carbotrap 349
	Error (%)	CV (%)	Error (%)	CV (%)	Error (%)	CV (%)	Error (%)	CV (%)	Error (%)	CV (%)	Error (%)	CV (%)			
0.24	8.6	2.3	-6.1	26	12	1.7	-3.2	1.2	-71	48	-4.1	18	-12%	-71%	-17%
0.59	1.5	3.4	10	18	-3.0	4.9	-9.1	1.6	-45	20	-17	3.2	-12%	-69%	-17%
1.5	-2.4	4.4	-8.4	7.2	-6.0	8.9	-20	1.0	-33	7.5	-24	5.7	-24%	-32%	-24%
3.0	-2.5	1.3	5.7	2.3	-5.9	1.8	-21	3.9	-30	7.2	-14	4.5	-25%	-48%	-9.1%
4.5	-3.4	0.81	0.22	5.3	5.4	9.0	-19	3.3	-31	4.0	-18	4.4	-20%	-44%	-28%
6.0	2.7	0.068	-0.70	4.1	-0.76	8.8	-12	0.29	-25	3.5	-12	5.7	-16%	-32%	-13%
Avg	4.0	2.0	8.7	10	6.4	5.8	16	1.9	42	15	17	6.9	-18%	-33%	-18%

¹Low humidity $\cong$ 10 mg of moisture / L dry air. High humidity $\cong$ 37 mg of moisture / L dry air.

²Sulficarb results were not included in this table due to poor sensitivity described in Figure 5.

³Block A results were calculated using the respective low humidity calibration curve developed for each individual method. Block A contains the error between the sampled and predicted isoflurane concentrations and precision between triplicate measurements observed during calibration of each sample introduction method using low humidity standards.

⁴Block B shows the resulting error and precision observed when the low humidity calibration curves developed for each sample introduction method were applied to high humidity measurements.

⁵Block C describes the difference in instrument response (sensitivity) observed between the low and high humidity samples within each sample introduction method.

Table 3.5. Expected performance of High Humidity measurements.

Isoflurane Concentration (µg/L)	Block A ¹						Block B ²					
	High Humidity Sample High Humidity Calibration (n = 3)						High Humidity Sample Low Humidity Probe Calibration (n = 3)					
	Probe		Carbograph 5TD		Carbotrap 349		Probe		Carbograph 5TD		Carbotrap 349	
	Error (%)	CV (%)	Error (%)	CV (%)	Error (%)	CV (%)	Error (%)	CV (%)	Error (%)	CV (%)	Error (%)	CV (%)
0.24	15	1.2	17	16	12	13	-3.2	1.2	-26	15	-13	13
0.59	7.8	1.6	4.9	14	-3.0	1.0	-9.1	1.6	-35	13	-25	1.0
1.5	-5.3	1.0	2.2	6.6	-11	5.7	-20	1.0	-37	6.6	-31	5.7
3.0	-6.4	3.9	-0.44	6.7	1.2	1.7	-21	3.9	-39	6.7	-22	1.7
4.5	-4.4	3.3	-4.4	3.8	-3.6	4.4	-19	3.3	-42	3.8	-25	4.4
6.0	-4.7	0.29	2.8	3.4	2.8	5.7	-12	0.29	-37	3.4	-20	5.7
Avg	8.0	1.9	7.7	8.3	7.2	5.2	16	1.9	36	8.2	23	5.2

¹Block A describes the expected accuracy and precision when each sample introduction method is calibrated using the high humidity samples. Accuracy and precision both improve considerably in comparison with calibration performed using low humidity standards (Block B of Table IV).

²Block B describes the accuracy and precision that would result if the HAPSITE[®] instrument user were to measure high humidity samples after calibrating to the low humidity isoflurane standards using the probe.

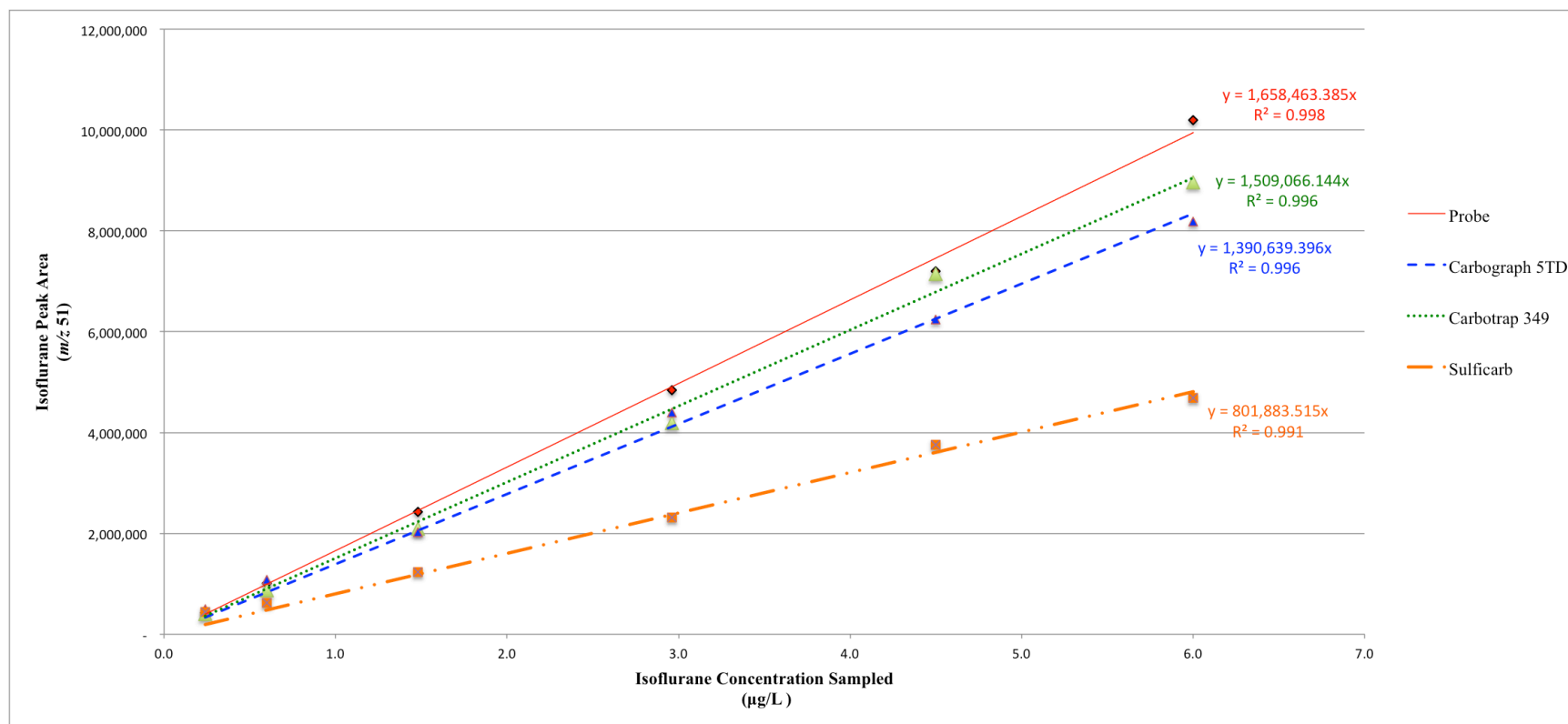


Figure 3.5. Instrument response observed during analysis of low humidity standards.

Decreased instrument response was observed for Carbograph 5TD, Carbotrap 349, and Sulficarb relative to the probe method. Instrument response observed with Sulficarb was less than 50% of that observed with the probe for all but the lowest calibration point. Testing not discussed in this paper identified low desorption efficiency, which very likely contributed to the low instrument response resulting from the use of Sulficarb illustrated above. Similar relative decreases in instrument response were also observed in high humidity samples.

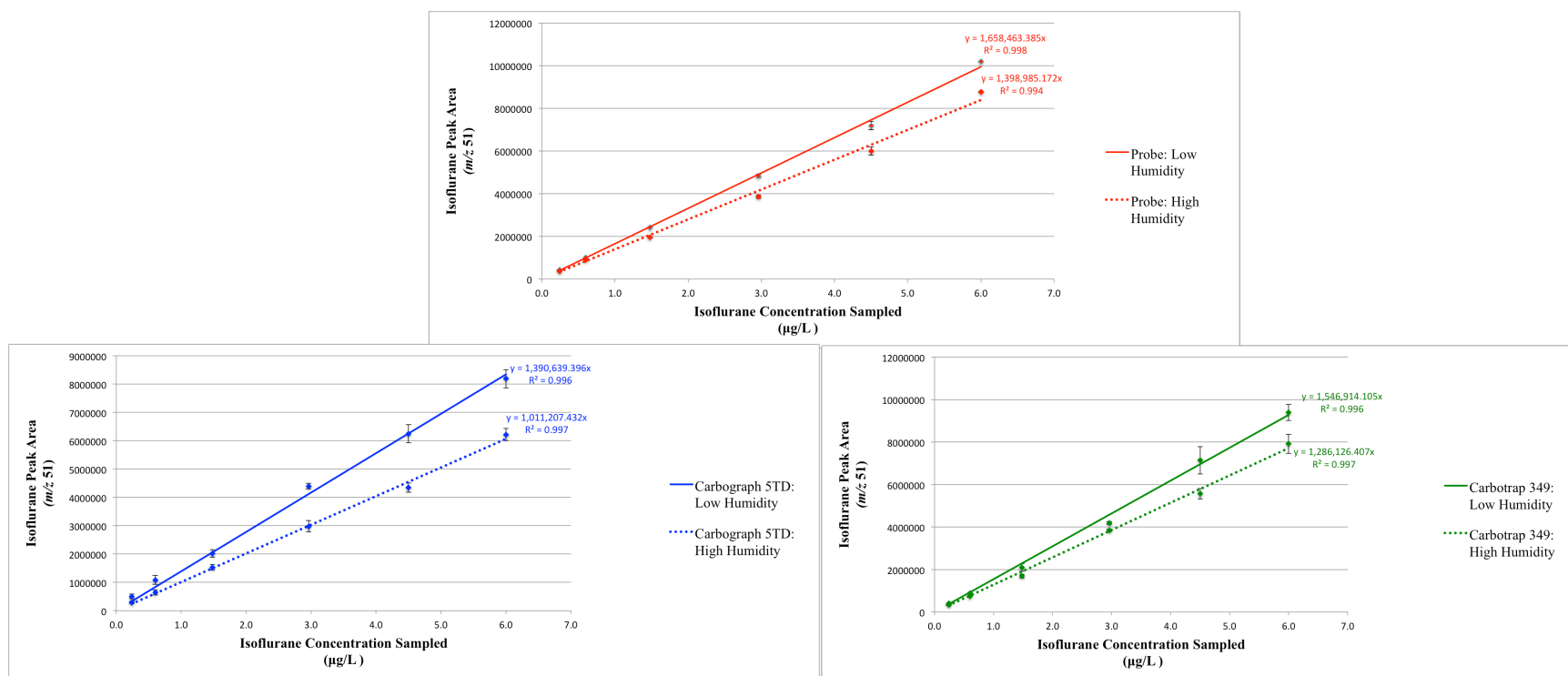


Figure 3.6. Instrument Response: Low Humidity vs. High Humidity.

Decrease in instrument response between low humidity isoflurane standards (solid lines) and high humidity samples (dashed lines).

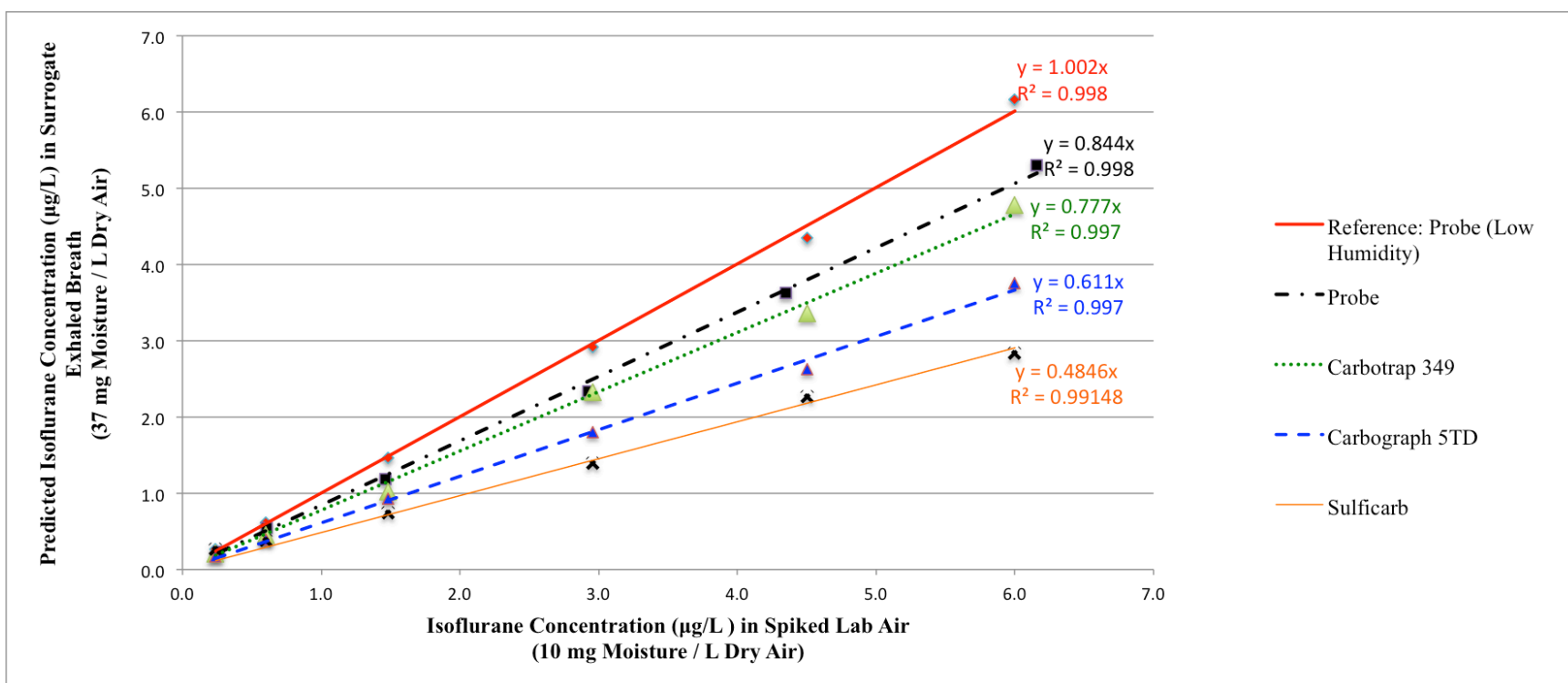


Figure 3.7. Agreement between sample introduction methods.

Agreement between predicted isoflurane concentrations in high humidity samples after calibration with the probe and low humidity isoflurane standards.

Table 3.6. Isoflurane average % breakthrough of the internal concentrator during thermal desorption (10-minute desorption).

Sample Bag Concentration	Composite Sample Data Avg % Breakthrough per Analysis ¹	
	Low Humidity Standards (n = 3)	High Humidity Samples (n = 3)
0.24 µg/L (0.031 ppm)	Detected-Not Quantified ²	Detected-Not Quantified ²
0.60 µg/L (0.078 ppm)	Detected-Not Quantified ²	Detected-Not Quantified ²
1.5 µg/L (0.19 ppm)	12.8	16.7
3.0 µg/L (0.39 ppm)	11.9	11.1
4.5 µg/L (0.58 ppm)	10.0	8.9
6.0 µg/L (0.78 ppm)	9.5	11.3

¹Calculated by dividing the average mass breakthrough per analysis by the sum of the masses of isoflurane loaded onto the three tubes.

²Instrument response was below the lower range of the calibration curve.

Table 3.7. Qualitative results of the ¹internal concentrator breakthrough by selected challenge agents.

Test Agent	Concentration	Composite Sample Data (n = 3)			
		Isoflurane Mass Loaded On Tube (µg)	Simulated Probe Method ²	5-Minute Thermal Desorption	10-Minute Thermal Desorption
Isoflurane	6.0 µg/L (0.78 ppm)	0.60	None Detected	Detected	See Table 3.6
Isooctane	2.2 µg/L (0.58 ppm)	0.22	None Detected	Detected	Detected
Toluene	1.5 µg/L (0.40 ppm)	0.15	None Detected	Detected	Detected
1,1,1-Trichloroethane	2.3 µg/L (0.60 ppm)	0.23	None Detected	None Detected	Detected
Dichloromethane	2.2 µg/L (0.55 ppm)	0.22	None Detected	Detected	Detected

¹Concentrator removed from the GC/MS instrument and held at constant 50°C during sampling as described in the text.

Chapter 4: Performance of Carbotrap 349, Carbograph 5TD, and Sulficarb Thermal Desorption Tubes on the INFICON HAPSITE® ER Portable Gas Chromatograph/Mass Spectrometer

4.1 INTRODUCTION

Woolfenden (2010 & 2013) described thermal desorption as an increasingly common technique that is becoming more heavily used relative to conventional sampling and analysis methods that rely on solvent extraction. Thermal desorption captures the sorbate(s) of interest from workplace or environmental air by active or passive sampling using a steel or glass tube that contains one or more sorbents. After sampling, heat is then applied to the tube to promote desorption of the collected sample. Flow of an inert carrier gas, such as ultra pure nitrogen or helium, passes through the tube and sweeps the desorbed sorbate into an instrument (usually a gas chromatograph) where chemical compounds may be separated and quantified. Thermal desorption offers several advantages over conventional solvent desorption of air sampling media, such as up to 1000-fold higher sensitivity, increased safety, capability for reuse of the sampling devices, and potential for the automated analysis (117; 118).

Thermal desorption tube sorbents rely mainly on physical adsorption and can be manufactured from a diverse variety of materials, including glass beads, porous polymers, graphitized carbons, and molecular sieves. The ideal sorbent must interact strongly enough with the sorbate(s) of interest to ensure efficient capture, but also must not be so strong that its strength precludes efficient desorption during analysis. The retentive strength of a sorbent is its ability to collect, retain, and release specific sorbates and is dependent on the nature and strength of forces between the sorbent material and the sorbate. Sorbent strength is generally described qualitatively as “weak”, “medium”,

or “strong” and is generally measured by retention or breakthrough volume (36; 117). Eide, Simmons, and Hendricks (2010) and Harper (1993) describe the considerations used by OSHA and others for determining breakthrough volume of actively sampled media. Sample air is drawn through the sampling media sorbent at a flow rate suitable for the samplers, at a concentration of 2 times the target concentration, and with an absolute humidity of about 16 g of water per liter of air (approximately 80% relative humidity at 22°C). The breakthrough volume is reached when the effluent from the tube reaches a concentration of 5% of the concentration entering the tube (28; 36). The collection efficiency for a sorbent/sorbate pair can depend on various factors, including the vapor concentration, polarity, temperature and humidity of the ambient air, interfering compounds that compete for sorbent binding sites, sampler flow rate, and geometry of the sorbent bed (36). Some stronger sorbents, such as a carbon molecular sieve, utilize structural micropores to selectively capture molecules of defined size (97). Carbon molecular sieves have a stronger affinity for water than other sorbents and sampling in high humidity conditions can reduce their sorptive capacity by a factor of 10 (118).

For quantitative analysis, the sorbate must also be efficiently desorbed from the sorbent. Cao & Hewitt (1993) defined desorption efficiency as the ratio of sorbate mass recovered from a sorbent to the known mass of sorbate that was collected. Considered in these terms, desorption efficiency can help describe how efficiently a sorbate can overcome, through the application of heat and inert carrier gas, the various forces that physically bind it to a specific sorbent. Desorption efficiency is a function of sorbate volatility and sorbent adsorption capacity and should be as high as possible in order to maximize analytical sensitivity (19). As a result, it is important to carefully match the

sorbent/sorbate pair with the conditions encountered in the sampling and analytical environments (19; 117).

A common inhalational anesthetic in both human and veterinary medicine, 2-chloro-2-(difluoromethoxy)-1, 1, 1-trifluoroethane, more commonly known as isoflurane, is a colorless, volatile liquid with a vapor pressure of 315 mm Hg at 25°C and a boiling point of 48°C (71). Isoflurane is a halogenated ether commonly administered to maintain anesthesia in humans and animals during invasive surgical procedures (92).

This study supplements work described in Chapter 3 by further investigating the ability of several types of thermal desorption tubes to efficiently release isoflurane when analyzed on a Inficon HAPSITE® ER GC/MS instrument. Sorbent and instrument parameters investigated included desorption flow rate, desorption efficiency, and ability to achieve the target desorption temperature.

4.2 Materials and Methods

4.2.1 Analytical Equipment

4.2.1.1 Analysis Using the HAPSITE®ER GC/MS

The HAPSITE® ER instrument includes a gas chromatograph with a non-polar DB-1MS analytical column of 15 m length x 0.32 mm inner diameter and 1.0 µm thick stationary phase (Agilent Technologies, Santa Clara, CA), and a quadrupole mass analyzer to filter ions by mass to charge ratio (m/z) as these are produced in the instrument's ion source. The initial column temperature was 40°C held for 0.5 minutes, and was then ramped at 60°C per minute to a maximum temperature of 180°C (held for 0.5 minutes). The total GC analysis time was 10 minutes. A filament delay time of 0.25 minutes was used to allow oxygen, nitrogen, and carbon dioxide to elute prior to

ionization. Known volumes of 1,3,5-tris(trifluoromethyl)benzene (TRIS, 50 ppb) and bromopentafluorobenzene (BPFB, 10 ppm) internal standards (IS) were automatically injected from a pressurized canister housed in the analytical module. However, due to low precision in the mass of internal standard injected during each analysis described in Chapter 3, isoflurane peak areas were not normalized to either internal standard.

Parameters included an initial thermal desorption sampling system temperature of 40°C, a target desorption temperature of 315°C (ramped at 90°C per minute), and a 10-minute desorption period. For comparison purposes, tests were also performed using a 5-minute desorption period. During desorption, carrier gas flowed through the thermal desorption tube at an approximate flow rate of 25 mL/min, which was set by the instrument manufacturer. Also for comparison purposes, the desorption efficiency tests were repeated on a laboratory GC/MS instrument combined with a laboratory thermal desorption system.

4.2.1.2 Analysis Using the Agilent 7890B / 5977B GC/MS Instrument

An Agilent 7890B bench-top gas chromatograph with a 5977B mass selective detector (Agilent Technologies, Santa Clara, CA), in conjunction with a Markes Unity 2 thermal desorption unit and Series 2 ULTRA autoloader (Markes International, Llantrisant, UK) were used to assess the desorption efficiencies of Carbograph 5TD and Sulficarb. The results were compared with the desorption efficiencies achieved with the HAPSITE[®] instrument. The Agilent/Markes system was also used to determine isoflurane retention efficiency for Sulficarb and Carbograph 5TD thermal desorption tubes.

The laboratory gas chromatograph contained a HP-5ms column (30 m length x 250 μm inner diameter x 0.25 μm film thickness). Analytical parameters were as follows: the column flow rate was 1.287 mL/min, initial column temperature was 35°C held for 1.5 minutes and then ramped at 40°C per minute to a maximum temperature of 315°C (held for 3 minutes). The total gas chromatography (GC) analysis time was 10.6 minutes. A filament delay time of 0.25 minutes was used. Peak areas were manually integrated using Agilent Enhanced Chem Station software (Ver. F.01.03.2357). Ultra high purity helium (99.999%) purchased from Roberts Oxygen (Rockville, MD) was used as the Agilent carrier gas.

4.2.1.3 Markes Unity 2 Parameters

The Unity 2 cold trap had the ability to achieve sub-ambient temperatures and was programmed to attain –10°C during desorption of the thermal desorption tubes. The focusing trap temperature was then flash heated at the highest rate possible (approximately 100°C/sec) to a maximum temperature of 300°C (held for 3 minutes) to ensure full desorption of the isoflurane collected on the trap. From a thermal desorption tube. The temperature of the transfer line to the gas chromatograph was set to 150°C. The desorption flow rate was 10 mL/min, while the desorb split and trap split flow rates were both 50 mL/min, giving a total split ratio of 239:1. Desorption temperature was 300°C with a 6-minute desorption time.

4.2.2 Thermal Desorption Tube Selection

Stainless steel and glass thermal desorption tubes were used in this study. Stainless steel tubes (89 mm x 6.35 mm outer diameter (5 mm inner diameter) packed with Tenax[®] TA (60/80 mesh), Sulficarb (40/70 mesh), and Carbograph 5TD (40/60

mesh) were purchased from Markes International. The Sulficarb tubes contained an average of 0.302 mg of sorbent ($n = 20$, range = 0.299 – 0.304 g), while the Carbograph 5TD tubes contained an average of 0.301 g of sorbent ($n = 20$, range 0.297 – 0.304 g). The average mass of sorbent in steel Tenax TA tubes was 0.201 g ($n = 18$). Stainless steel multi-sorbent Carbotrap 349 tubes were purchased from Supelco (Bellafonte, PA). The sorbent masses in the Carbotrap 349 tube were unavailable. Glass thermal desorption tubes (89 mm x 6.35 mm outer diameter (4 mm inner diameter) packed with Tenax[®] TA (35/60 mesh), Sulficarb (40/70 mesh) and Carbograph 5TD (40/60 mesh) were purchased from Markes International. The masses of Tenax[®] TA, Sulficarb and Carbograph 5TD contained in the glass thermal desorption tubes were 0.200 g, 0.300 g and 0.250 g, respectively (114). Each tube was pre-conditioned using a TC-20 sorbent tube conditioner (Markes International) according to the tube manufacturers' recommendations prior to use. Though Tenax[®] TA is classified as a weak sorbent and is not intended to capture and hold a compound as volatile as isoflurane, it has been investigated for sampling of isoflurane (44). Tenax[®] TA was included in several tests conducted in this for comparison purposes. The classes of sorbents contained in each tube type are listed in Table 4.1.

4.2.3 Preparation of Isoflurane Atmospheres

A Miller-Nelson HCS-501 temperature and humidity control system (Miller-Nelson Research, Inc., Livermore, CA) warmed and humidified compressed house air to 35°C and 95% relative humidity to approximate human exhaled breath (104). A volume of 10.6 L of this air was injected into a 10-L SamplePro FlexFilm[®] air sample bag (SKC, Inc., Eighty Four, PA). A stock isoflurane atmosphere of 148 µg/L was made by using a

Hamilton 700 series 10 μL ($\pm 1\%$) microsyringe to inject 1.0 μL of isoflurane (99.99%, Baxter International, Deerfield, IL) into the 10-L bag, which was allowed to cool and equilibrate for one to two hours. Volumes ranging from 8.0 – 200.0 mL were drawn from the stock atmosphere bag and injected into 5-L Tedlar[®] bags. The 5-L sample bags were then filled to a total volume of 5.2 L with warmed, humidified air (35°C and 95% relative humidity) and allowed to cool and equilibrate for at least one hour. The final concentrations of the High Humidity samples ranged from 0.24 – 6.0 $\mu\text{g/L}$, which were based on prior a study by Prado et al. (1997) of isoflurane in the exhaled breath of surgical personnel (89). The absolute humidity of the surrogate exhaled breath was approximately 37 g of water per m^3 of dry air.

4.2.4 HAPSITE[®] Instrument Calibration and Analysis

Accuracy, precision, and linearity of Carbotrap 349, Carbograph 5TD and Sulficarb using the HAPSITE[®] instrument were previously described in Chapter 3.

4.2.5 Sample Loading onto Thermal Desorption Tubes

A Kitigawa (Tokyo, Japan) AP-20 100-mL piston pump was used to load the isoflurane standards onto each thermal desorption tube.

4.2.6 Determination of Desorption Flow Rate and Volume

After loading, the tubes were desorbed in the thermal desorption sampling system of the HAPSITE[®] instrument. During desorption, the tube bed was nominally heated at 90°C per minute while carrier gas flowed through the tube at a flow rate of approximately 25 mL/min. The target desorption temperature programmed for each tube was 315°C.

The flow rate of carrier gas through the thermal desorption tube was measured using a Bios Defender 510 primary flow meter (Mesa Labs, Lakewood, CO). A direct measurement of the carrier gas flow rate through the desorption tubes was only possible if the rear thumbwheel assembly of the thermal desorption sampling system was left unattached from the back of the thermal desorption tube (Figure 4.1). During testing, it was found that leaving the rear thumbwheel assembly unattached decreased the flow of carrier gas through the tube to < 1 mL/min. To address this, and to estimate the flow rate of the carrier gas flowing through the tube during the thermal desorption process as accurately as possible, the rear thumbwheel assembly was properly installed and desorption flow rate was measured indirectly through the exhaust port of the instrument.

4.2.7 Desorption Efficiency

Desorption efficiency tests were performed to characterize the ability of each type of thermal desorption tube to release collected isoflurane. Desorption efficiency tests performed on the HAPSITE[®] were conducted at only two concentrations of isoflurane (3.0 µg/L and high range 6.0 µg/L). These were the middle and highest isoflurane concentrations tested in this study. Desorption efficiency was calculated using a method based on work performed by Cao & Hewitt (1993), who defined desorption efficiency as the ratio of the mass of analyte recovered from the sorbent during thermal desorption to the mass of analyte added to the sorbent (Equation 4.1) (19).

$$D.E._i (\%) = \frac{A_{i,1} - A_{Bi}}{A_{i,1} + A_{i,2} - A_{Bi}} \quad (4.1)$$

$D.E._i$ = desorption efficiency for isoflurane

$A_{i,1}$ = area count of isoflurane from the first desorption of the spiked tube

$A_{i,2}$ = area count of isoflurane from the second desorption of the spiked tube

A_{Bi} = area count of isoflurane from the adsorbent blank

A 100-mL volume of air was drawn from the 3.0 µg/L and 6.0 µg/L isoflurane standards through each type of thermal desorption tube. Each tube was then desorbed in the HAPSITE[®] thermal desorption sampling system. The two desorption cycles described in the Cao & Hewitt (1993) protocol were performed. The Cao & Hewitt method was modified to include a third desorption to ensure that no additional isoflurane was detectable. In cases where isoflurane was detectable after the third desorption, such as with Sulficarb, the *m/z* 51 peak area from the third desorption was added to the numerator and denominator of Equation 4.1. The resulting desorption efficiency was then reported with a “less than” (<) symbol in order to provide a conservative estimate of the desorption efficiency and to reflect the fact that a portion of the isoflurane likely remained in the sorbent after the third desorption.

To determine whether the low desorption efficiencies observed with the Sulficarb tubes were a function of the HAPSITE[®] system, or resulted from Sulficarb being an overly strong sorbent inappropriate for isoflurane, this test was repeated using the Agilent/Markes laboratory instrumentation.

4.2.8 Thermal Desorption Tube Breakthrough

Each thermal desorption tube type was assessed for sorbent breakthrough at the highest isoflurane concentration used in the study (6.0 µg/L). Breakthrough was tested using a Carbotrap 349 tube in series with the sampling tube being evaluated. A calibrated (0.05567 L/min) SKC Airchek Sampler Pump (Model 224-44XR) was then used to draw up to 1 L of air from a Tedlar gas sampling bag containing the highest concentration of isoflurane (6.0 µg/L, with an absolute humidity of 17.5 mg of water per liter of air at 21°C and 95% relative humidity through the tube pair. The backup tube was then

analyzed to determine the m/z 51 peak area. If isoflurane was observed on the back tube then isoflurane was considered to have broken through the front tube. The mass of isoflurane in the backup tube would then be divided by the volume of air that had passed through the tube to determine whether the concentration was $\geq 5\%$ of the inlet concentration.

4.2.9 Thermal Desorption Tube Temperature Measurement

The ability of the HAPSITE® ER thermal desorption sampling system to achieve the maximum desorption temperature of 315°C was investigated. Temperature profiles for each type of tube were constructed by measuring (in triplicate) the temperature of the sorbent inside during thermal desorption. Measurements were made using a Eutech Instruments (Vernon Hills, IL) Model 68900-01 Digi-Sense Temperature Controller and J-type thermocouple. Prior to measurement, each thermal desorption tube was installed into the HAPSITE® thermal desorption sampling system with the thumbwheel assembly left unattached from the back end of the thermal desorption tube. The thermocouple was then inserted into the tube, with the tip buried in the sorbent (Figure 4.1) to characterize the temperature profile over the 10-minute desorption cycle. Temperature readings were recorded every 15 seconds.

4.2.10 Statistical Methods

Data were analyzed using Microsoft Excel (2011) and IBM® SPSS® Statistics Software (Version 22) (Armonk, NY). A two way Analysis of Variance (ANOVA) test was conducted to compare the main effects of tube casing material (steel, glass) and sorbent type and the interaction effect of tube casing material and tube type (hollow, and single-sorbent Sulficarb, Carbograph 5TD, and Tenax TA tubes) on the average steady-

state desorption temperature. Carbotrap 349 was not included in the statistical analysis because it was not a single-sorbent tube. A Tukey Honest Significant Difference (HSD) test was performed to determine which levels in tube casing material and tube type differed.

4.3 RESULTS

4.3.1 Determination of Desorption Flow Rate and Volume

The average flow rate of carrier gas through the thermal desorption tube during desorption on the HAPSITE[®] was 25.3 mL/min. This resulted in a total desorption volume of 253 mL over a 10-minute desorption period.

4.3.2 Breakthrough of Thermal Desorption Tube Sorbent

Isoflurane quickly broke through the Tenax[®] TA sorbent, resulting in a breakthrough volume of 14 mL (0.07 L/g of sorbent). No isoflurane breakthrough of the Sulficarb, Carbograph 5TD, or Carbotrap 349 tubes was identified after a total of 1.1 L had been sampled. Further efforts to identify the breakthrough volumes for these sorbents under the temperature and humidity parameters common to exhaled breath were not performed since it is unlikely that a larger volume of exhaled breath would be collected and analyzed. However, a breakthrough evaluation described in Chapter 5 utilized a concentration of 100 ppm and an absolute humidity of 15.3 mg of water per liter of air (21.7°C, 80.4% relative humidity). These parameters were selected by following the OSHA-recommended breakthrough volume validation protocol of identifying the volume at which 5% breakthrough occurs with a concentration two times the target (which was 50 ppm in that study) and an absolute humidity of approximately 15.7 mg of water per liter of air (22.2°C, 80.3% relative humidity) (28). The resulting

breakthrough volumes for Sulficarb and Carbograph 5TD under these conditions were determined to be 35 L (110 L/g of sorbent) and 5.8 L (19 L/g of sorbent), respectively. The breakthrough volume for Carbotrap 349 was determined to be 29 L. The masses of sorbent contained in the Carbotrap 349 tube were unknown, so the breakthrough volume per unit mass of sorbent was not calculated. These findings are discussed in greater detail in Chapter 5.

4.3.3 Desorption Efficiency

Glass and steel Carbograph 5TD tubes achieved 100% desorption efficiency on both the HAPSITE[®] ER and Agilent/Markes instruments at all isoflurane loading levels. Carbotrap 349 achieved an average desorption efficiency of 98% and 99% on the HAPSITE[®] ER at loading levels of 0.3 µg and 0.6 µg, respectively, and 100% on the Agilent/Markes system. Sulficarb achieved poor desorption efficiency (68% to 84%) on the HAPSITE[®] ER. However, Sulficarb achieved 100% desorption efficiency on the Agilent/Markes system at all loading levels. Desorption efficiency results for each type of tube are described in Table 4.2.

4.3.3.1 HAPSITE[®] ER Results

Tubes (glass and stainless steel) containing Carbograph 5TD tubes achieved a mean desorption efficiency of 100%. Stainless steel Carbotrap 349 tubes contained three sorbents of varying strengths (Carbopack Y (weak/medium), Carbopack B (medium), and Carbotrap 1003 (very strong))(84). Carbotrap 349 achieved a mean desorption efficiency of approximately 98 – 99%. Stainless steel and glass Sulficarb tubes had average desorption efficiencies of <71% and <84%, respectively.

4.3.3.2 Agilent 7980B / 5977B Results

Desorption efficiency tests for Sulficarb, Carbograph 5TD, and Carbotrap 349 tubes were repeated with analysis performed on the Agilent 7980B / 5977B with the Markes Unity 2 and Series 2 ULTRA autoloader. Desorption efficiencies for the three types of tubes were 100% (Table 4.2).

4.3.4 Desorption Temperature Profiles

The desorption temperature profile for each type of thermal desorption tube tested is illustrated in Table III and Figure 4.2. It is important to note that the desorption temperatures described in Table III and Figure 4.2 may overestimate the actual temperatures achieved during a typical desorption on the HAPSITE® ER. This is because the method of sorbent temperature measurement used in this study required leaving the thumb-wheel unattached from the rear of the tube (Figure 4.1.B and 4.1.C). When in this configuration, the carrier gas flow rate through the tube decreased from 25 mL/min to 0.9 mL/min, which likely limited heat transfer from the sorbent to the carrier gas. This could have resulted in increased heating of the thermal desorption sampling system and higher temperatures inside the thermal desorption tubes than would normally be achieved during a typical analysis.

4.3.4.1 Hollow Tubes

The interior temperatures of hollow glass and steel thermal desorption tubes were measured for comparison purposes. The hollow glass tube achieved the highest steady-state temperature of all tubes tested (344.3°C). This was the only tube to meet (and exceed) the thermal desorption sampling system's nominal maximum desorption temperature of 315°C. The hollow steel tube achieved an average steady-state

temperature of 303.2°C (12% lower than the hollow glass tube), which was the highest steady-state temperature of all steel tubes tested. The results were expected since the hollow tubes have a lower thermal mass than sorbent-laden tubes.

4.3.4.2 Sorbent-Filled Tubes

The data in Table 4.3 show that none of the sorbent-filled tubes (glass or steel) were able to achieve the 315°C maximum temperature permitted by the HAPSITE[®] thermal desorption sampling system. Additionally, most tubes, with the exception of the glass tube containing Tenax TA, were not able to achieve the maximum desorption temperature recommended by the manufacturer.

Two way ANOVA test results indicated that all effects were significant at the .05 significance level (Table 4.5). The main effect for tube casing resulted in an F statistic of $F(1,16) = 17,419.7$, $p < .001$, indicating a significant difference between the average steady-state desorption temperature achieved by tubes constructed of glass (Mean = 304.9°C, 95% CI = 304.5°C – 305.3°C) and tubes constructed of stainless steel (Mean = 271.8°C, 95% CI = 271.4°C – 272.2°C). The main effect for sorbent type resulted in an F statistic of $F(3, 16) = 11,820.9$, $p < .001$, indicating a significant difference between at least one sorbent and the others tested. The interaction effect between the tube casing and sorbent type factors was also significant, with an F statistic of $F(3, 16) = 5,255.9$, $p < .001$. A Tukey HSD multiple comparisons post-hoc test indicated that the average steady-state desorption temperatures were significantly different between all of the tested sorbent types, averaging across tube casing materials (Table 4.6).

4.4 DISCUSSION

4.4.1 Breakthrough of Thermal Desorption Tube Sorbent

Rapid breakthrough of isoflurane through the Tenax TA sorbent was expected, since Tenax[®] TA is classified as a ‘weak’ sorbent and is not intended to capture low boiling point compounds. The results clearly verify that Tenax TA is too weak to efficiently capture isoflurane. Breakthrough results for Sulficarb, Carbograph 5TD, and Carbotrap 349 suggest that these sorbents can efficiently capture isoflurane at a concentration at the high end of the range that may be expected in exhaled breath. A well-defined breakthrough volume was not identified for either of these three tube types. However, the breakthrough volume is not a critical parameter in this context, since it is unlikely that a volume of actual exhaled breath greater than or equal to 1.1 L would be needed or available for testing.

4.4.2 Desorption Efficiency

Desorption efficiency can be affected by several factors, including desorption temperature, desorption flow rate, and sorbent strength. Users of the HAPSITE[®] ER instrument are able to select the specific desired maximum desorption temperature (up to a maximum of 315°C) and desorption time. Higher desorption temperatures and/or longer desorption times may be necessary when analyzing compounds with low vapor pressure/high boiling points or when higher strength sorbents are utilized.

4.4.2.1 Desorption Temperature

Heating of the thermal desorption tube sorbent increases the vibrational energy of the adsorbed isoflurane to the point where it can overcome the Van der Waals forces that bind it to the sorbent. Insufficient heating may not impart enough energy to allow

efficient release of the adsorbed isoflurane, particularly if the sorbent used is of high strength. The instrument manufacturer (Inficon) has observed that glass thermal desorption tubes used with the HAPSITE® ER thermal desorption sampling system achieved maximum desorption temperatures of approximately 300°C, while steel tubes achieved maximum temperatures of approximately 200°C (37; 50). This study corroborated those findings by showing that the HAPSITE® thermal desorption system was able to heat thermal desorption tubes that were constructed of glass more efficiently than those that were constructed of stainless steel. It is possible that thermal conductivity differences between steel and glass create an insurmountable barrier for the thermal desorption sampling system to heat steel tubes as efficiently as those made of glass. However, the results of this study indicate that the HAPSITE® was unable to heat any of the tested sorbent-filled tubes (glass or steel) to the target desorption temperature or to the desorption temperature recommended by the tube manufacturer. It is important to note that the low carrier gas flow rate observed during temperature measurement (0.9 mL/min) likely resulted in higher desorption temperatures than those normally achieved during typical desorption. The higher carrier gas flow rate (25 mL/min) achieved during typical desorption would likely carry additional heat away from the hot sorbent, thereby lowering the actual desorption temperatures from what was measured during this study.

The desorption temperature profiles constructed for each type of tube suggest that the average steady-state desorption temperature is affected not only by the tube casing material (glass, steel), but also by the sorbent type. This may be due to differences in the specific heat capacities of each sorbent, which could cause variation in the amount of energy required to heat an equivalent mass of each sorbent to the same target desorption

temperature. Regardless of the cause, variation in the desorption temperature due to tube casing material and sorbent type is important to keep in mind when selecting sorbents for use with the HAPSITE® ER, particularly since this instrument has been shown to not achieve its maximum desorption temperature.

4.4.2.2 Desorption Flow Rate

Desorption flow rate is also an important factor in overall desorption efficiency since carrier gas flowing through the desorption tube acts to sweep isoflurane from the hot sorbent into the analytical system. A low flow rate may not be efficient in sweeping the desorbed isoflurane into the concentrator. The HAPSITE® desorption flow rate during typical desorption was measured to be approximately 25 L/min, which is typical for this type of application (118). Though the HAPSITE® flow rate itself is not directly adjustable by the user as it is on many bench top systems, desorption time is adjustable. This enables a user to increase the total volume of carrier gas that has swept through the sorbent by increasing desorption time. Increasing desorption flow rate or overall desorption volume may increase desorption efficiency in some cases, provided that the increase in flow or volume does not increase the risk of concentrator breakthrough. It is possible that increasing the total carrier gas volume flowing through the thermal desorption tube by increasing the carrier gas flow rate or desorption time might have helped sweep additional isoflurane from the sorbent and into the analytical system. However, this could have resulted in a non-proportional increase (or even a net decrease) in instrument response due to loss of sample loss through increased breakthrough of the onboard concentrator.

4.4.2.3 Sorbent Strength

Table II shows that the desorption efficiencies of both Carbograph 5TD and Carbotrap 349 ranged between 98% and 100%. The desorption efficiency of both glass and steel Sulficarb desorption tubes was low, ranging between <69% and <71%. Sulficarb is classified as a strong molecular sieve in which sorbate capture occurs through a combination of van der Waals forces and increased surface area created by well-defined micropores within the sorbent (45). The steel Sulficarb tubes used in the study achieved an observed average steady-state desorption temperature of 285°C, which was approximately 79% of the desorption temperature recommended by the manufacturer. The very high surface area of Sulficarb offers a large number of binding sites for adsorption, and it is very likely that the heating and carrier gas flow that the HAPSITE[®] ER was capable of were not sufficient to enable efficient release of isoflurane.

Regardless of the causal factor(s), the underlying question was whether the low desorption temperatures observed with the HAPSITE[®] ER thermal desorption sampling system negatively affected the desorption efficiency of the types of tubes tested in this study. Carbograph 5TD and Carbotrap 349 tubes (glass and steel) did not appear to be greatly affected by the low desorption temperatures since they achieved desorption efficiencies >98%. This was likely due to the relatively weak sorbents onto which isoflurane was bound with these tubes. However, this was not the case with glass and steel Sulficarb tubes. Glass Sulficarb tubes achieved a higher desorption temperature (303.8°C) and desorption efficiency (<84%) than their steel counterpart (285.0°C and <71%, respectively), suggesting that low desorption temperature negatively impacted desorption efficiency of the high sorbent strength Sulficarb material. Based on the results

observed in this study, the HAPSITE® thermal desorption sampling system is not capable of heating Sulficarb to the temperature required to efficiently desorb isoflurane. As a result, Sulficarb is not recommended for analysis of isoflurane if analysis is performed using the HAPSITE® ER instrument.

To quantify the magnitude of this effect, future studies could investigate the desorption efficiency of isoflurane from Sulficarb at various desorption temperatures. Future work could also assess the ability of equivalent masses of different sorbents to achieve target desorption temperatures.

4.5 STUDY LIMITATIONS

There were limitations associated with this study. With the equipment available, the surrogate exhaled breath could not be reliably heated to a temperature higher than 35°C, a value at the lower end of the temperature range of typical exhaled breath (55). This resulted in the surrogate breath having a lower absolute humidity than actual exhaled breath (37.7 mg of water per L of air vs. 43.6 mg of water per L of air). Also, the method of measuring sorbent temperature during desorption required that the rear mesh screen be removed from the interior of the thermal desorption tube and deep insertion of the thermocouple into the sorbent. This effectively destroyed the desorption tube. Due to the limited number of tubes available for the study, the triplicate temperature measurements were performed on only one tube of each type. It is possible that desorption temperatures achieved by the measured tubes were not representative of all tubes of that respective type. Similarly, only one HAPSITE® ER instrument was utilized in the study, and it is possible that its performance was not representative of all HAPSITE® ER instruments.

4.6 CONCLUSIONS AND RECOMMENDATIONS

Sulficarb collected isoflurane efficiently. However, the sorbent strength of Sulficarb appears to be too high to allow for acceptable desorption efficiency at the maximum temperature and desorption flow rate that the HAPSITE[®] achieved. As a result, Sulficarb is not recommended for use with the HAPSITE[®] ER instrument. Carbograph 5TD offered efficient collection and desorption of isoflurane under the test conditions. Carbotrap 349 was the only tri-bed sorbent tube tested in this study. It offered efficient collection and desorption of isoflurane under the test conditions. Of the three types of tubes tested on the person-portable GC/MS instrument used in this study, it offered the best performance.

The study findings suggest that the Inficon HAPSITE[®] ER thermal desorption sampling system did not provide equivalent heating to thermal desorption tubes constructed of steel and glass, nor to those containing different types of sorbents. This could have inhibited the ability of the HAPSITE[®] to provide suitable conditions for efficient desorption of isoflurane from sorbents that might otherwise seem appropriate for its efficient capture (such as Sulficarb). As the use of breakable glass tubes would not be advisable in most areas where isoflurane is used, it is recommended that users desiring to quantify isoflurane in exhaled breath use the hand-held probe instead of thermal desorption tubes. However, if the probe is unavailable or thermal desorption methods must be used for some other reason (i.e. for increased sample volume greater than the 100 mL volume the probe can offer), then it is recommended that the HAPSITE[®] ER user exercise great care in sorbent selection and consider using a multi-bed sorbent tube (such as Carbotrap 349) rather than a single bed tube that contains a strong sorbent that might

normally be recommended for capture of isoflurane (such as Sulficarb). In light of this recommendation, it is also recommended that the selected thermal desorption tubes be refrigerated after sampling or that analysis be performed as soon as possible to minimize the risk of sample degradation.

4.7 ACKNOWLEDGMENTS

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Table 4.1. Thermal desorption tube sorbent characteristics

Tube Type	Number of Sorbent Beds	Sorbent Type(s)	Sorbent Strength	Approximate Analyte Volatility Range ⁴	Recommended Desorption Temperature
Tenax [®] TA ¹	Single Bed	Porous Polymer	Weak	n-C ₇ to n-C ₃₀ BP 100 to 450°C	Up to 300°C
Carbograph 5TD ¹	Single bed	Graphitized Carbon Black	Medium/Strong	n-C _{3/4} to n-C _{6/7} BP 50 to 150°C n-C ₃ to n-C ₈	350-400°C
Sulficarb ²	Single bed	Carbon Molecular Sieve	Very Strong	BP -30 to 150°C	Up to 360°C
Carbotrap 349 ³	Tri-bed	1. Carbopack Y – Graphitized Carbon Black	1. Weak/Medium	n-C ₉ to n-C ₁₄ ⁵	330°C
		2. Carbopack B – Graphitized Carbon Black	2. Medium	n-C ₅ to n-C ₁₂ ⁵	
		3. Carboxen 1003 – Carbon Molecular Sieve	3. Very strong for small molecules	n-C ₂ to n-C ₅ ⁵	

¹Tube specifications obtained from Markes International Thermal Desorption Technical Support Document 5 (TDTS 5) (59).

²Tube specifications obtained from Markes International Thermal Desorption Accessories and Consumables catalog (2013/2014)(60)

³Tube specifications obtained from Perkin-Elmer Lab Products and Services webpage (84).

⁴Approximate analyte volatility range relative to n-alkanes.

⁵Information obtained from Jochman et al. (2014) (45).

Table 4.2. Desorption efficiencies of Carbograph 5TD, Sulficarb, and Carbotrap 349 (low concentration isoflurane standards).

Tube Type	Tube Casing	HAPSITE® ER ¹ (n = 3)		HAPSITE® ER ² (n = 3)		Agilent 7980B / 5977B Markes Unity 2 / ULTRA ³ (n = 3)	
		Avg Desorption Efficiency (%)	CV (%)	Avg Desorption Efficiency (%)	CV (%)	Avg Desorption Efficiency (%)	CV (%)
Carbograph 5TD	Stainless Steel	100	N/A	100	N/A	100	N/A
Carbograph 5TD	Glass	100	N/A	100	N/A	100	N/A
Sulficarb	Stainless Steel	<68	10	<71	8.8	100	N/A
Sulficarb	Glass	<83	1.6	<84	0.41	100	N/A
Carbotrap 349	Stainless Steel	98	0.27	99	0.58	100	N/A
Carbotrap 349	Glass	Not tested	N/A	Not tested	N/A	Not tested	N/A

¹Each tube was loaded with 100 mL from a 3.0 µg/L isoflurane standard (0.30 µg total loading). A 10-minute desorption period was used.

²Each tube was loaded with 100 mL from a 6.0 µg/L isoflurane standard (0.60 µg total loading). A 10-minute desorption period was used.

³Each tube was loaded with 50 mL from a 31 µg/L isoflurane standard (1.5 µg total loading). A 6-minute desorption period was used.

Table 4.3. Temperature profile data for tested thermal desorption tubes.

Tube, Sorbent	Maximum Recommended Desorption Temp (°C)	Avg Steady-State Temp Achieved¹ (°C)	% of Maximum Recommended Desorption Temp	% HAPSITE Max Desorption Temperature (315°C)
Steel, Hollow	N/A	303.2	N/A	95.9%
Glass, Hollow		344.3		109%
Steel, Carbograph 5TD	360°C ²	241.3	67.0%	76.5%
Glass, Carbograph 5TD		303.3	84.3%	96.3%
Steel, Sulficarb	360°C ²	285.0	79.2%	90.5%
Glass, Sulficarb		303.8	84.4%	96.4%
Steel, Carbotrap 349	330°C ³	285.3	86.5%	90.6%
Steel, Tenax [®] TA	320°C ²	238.3	74.5%	75.6%
Glass, Tenax [®] TA		287.0	89.7%	91.1%

¹The average steady-state temperatures were determined by averaging the desorption temperatures achieved between the 5.0 minute and 10-minute points in the desorption period.

²Recommended desorption temperature obtained from Markes International Thermal Desorption Accessories and Consumables (2013/2014)(60).

³Tube specifications obtained from Perkin-Elmer Lab Products and Services webpage (84).

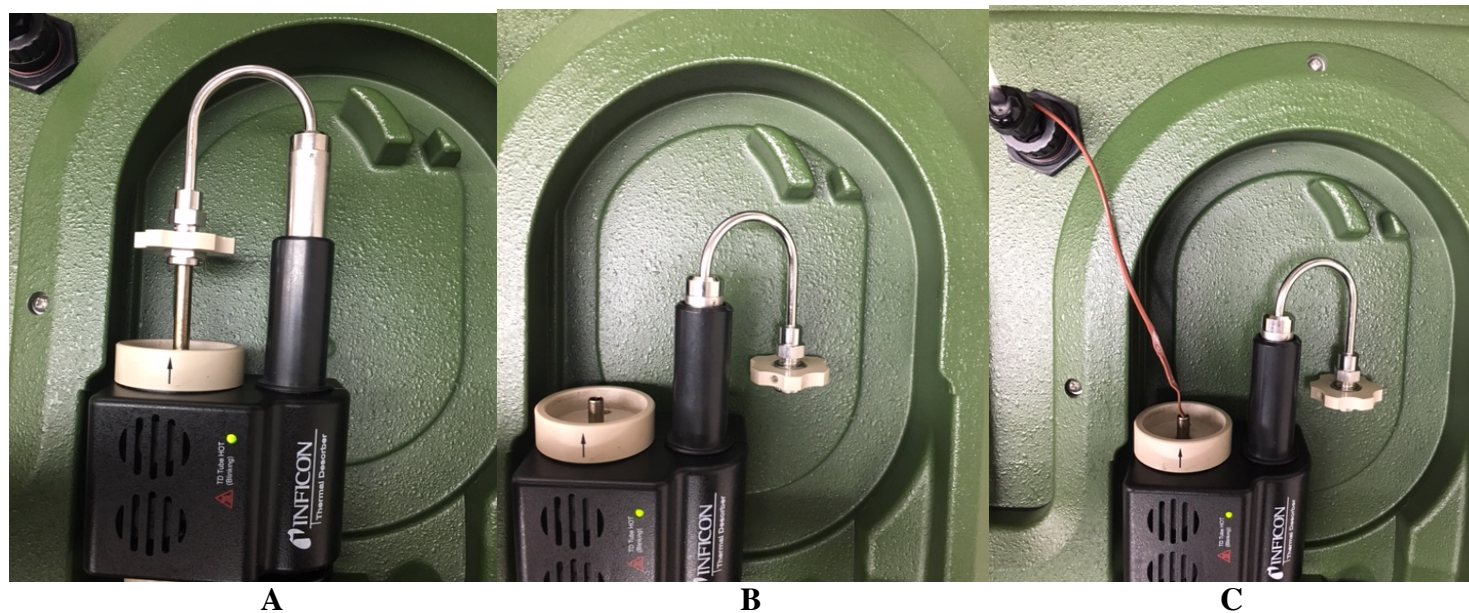


Figure 4.1. Temperature measurement in the HAPSITE® ER's thermal desorption sampling system (TDSS).

Photo A shows the tube being loaded into the TDSS with the thumb wheel properly attached to the rear of the tube. Photo B shows the tube fully loaded with the thumb wheel detached in preparation for temperature measurement. Photo C shows the system during temperature measurement, with the J-type thermocouple inserted into the sorbent through the rear of the tube.

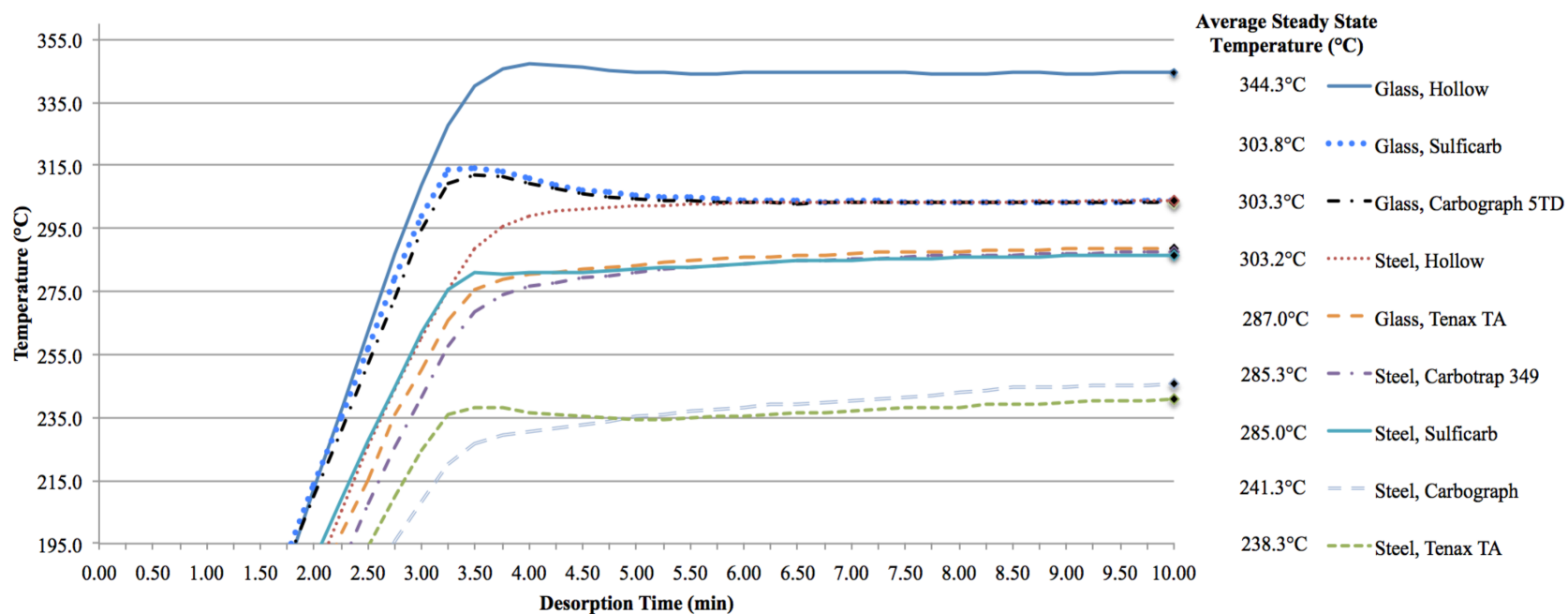


Figure 4.2. Desorption temperature profiles of tested thermal desorption tubes.

The average steady state temperatures were derived by averaging the measured temperatures between the 5.00 to 10.00 desorption times.

Table 4.4. Desorption tube average steady-state desorption temperature.

Temperatures are broken down by casing material, sorbent type, and effect of interaction between tube casing material and sorbent type. The HAPSITE's maximum desorption temperature was 315°C.

	Sorbent Type	Mean (°C)	% of Recommended Desorption Temp	95% Confidence Interval (°C)
Glass and Steel Combined	Sulficarb	294.9	82%	294.4 – 295.5
	Carbograph 5TD	272.3	78%	271.8 – 272.8
	Tenax TA	262.4	87%	261.8 – 262.9
	Hollow (no sorbent)	323.8	N/A	323.2 – 324.3
Steel	Sulficarb	285.0	79%	258.8 – 284.3
	Carbograph 5TD	241.3	69%	240.6 – 242.1
	Tenax TA	237.7	79%	237.0 – 238.5
	Hollow (no sorbent)	303.2	N/A	302.5 – 304.0
Glass	Sulficarb	304.9	85%	304.2 – 305.7
	Carbograph 5TD	303.3	87%	302.5 – 304.0
	Tenax TA	287.0	96%	286.2 – 287.7
	Hollow (no sorbent)	344.3	N/A	343.5 – 345.0

Table 4.5. Statistical analysis of average steady-state desorption temperature.

Statistical test results for two way ANOVA. Data is broken down by tube casing material and sorbent type.

Source	Type III Sum of Squares	Degrees of Freedom	Mean Square	F Statistic	p
Tube Material	6571.5	1	1995519.3	5289745.0	<.001
Sorbent Type	13378.0	3	6571.5	17419.7	<.001
Tube Material * Sorbent Type	5948.3	3	1982.8	5256.0	<.001
Error	6.036	16	0.377		

Table 4.6. Tukey Honest Significant Difference Multiple Comparisons post hoc test.
The Tukey HSD Multiple Comparisons post-hoc test compared the mean differences in average steady-state desorption temperature. The mean difference is significant at the .05 level.

Sorbent Type	Sorbent Type	Mean Difference (°C)	Std. Error (°C)	p	95% Confidence Interval	
					Lower Bound (°C)	Upper Bound (°C)
Sulficarb	Carbograph 5TD	22.6	0.354	<.001	21.6	23.7
	Tenax TA	32.6	0.354	<.001	31.6	33.6
	Hollow	-28.8	0.354	<.001	-29.8	-27.8
Carbograph 5TD	Sulficarb	-22.6	0.354	<.001	-23.7	-21.6
	Tenax TA	9.94	0.354	<.001	8.92	11.0
	Hollow	-51.5	0.354	<.001	-52.5	-50.4
Tenax TA	Sulficarb	-32.6	0.354	<.001	-33.6	-31.6
	Carbograph 5TD	-9.94	0.354	<.001	-11.0	-8.93
	Hollow	-61.4	0.354	<.001	-62.4	-60.4
Hollow	Sulficarb	28.8	0.354	<.001	27.8	29.8
	Carbograph 5TD	51.4	0.354	<.001	50.4	52.5
	Tenax TA	61.4	0.354	<.001	60.4	62.4

Chapter 5: Partial Validation of Sulficarb and Carbograph 5TD Thermal Desorption Tubes for Passive Sampling of Isoflurane

5.1 INTRODUCTION

Passive sampling of chemical contaminants in air can offer acceptable accuracy and precision and is a well-known alternative to active sampling (35). Many passive samplers have been validated for long-term studies (≥ 8 hrs) of chemical contaminants in environmental and workplace air (51). Passive sampling in short-term studies (≤ 4 hrs) can allow for a more accurate characterization of peak exposures that may not be detectable with long-term sampling, though validation of the passive samplers must first be performed (35; 51). Passive air sampling capitalizes on Fick's first law of diffusion rather than sampling pumps that actively draw contaminant-laden air through a sorbent to collect the analyte of interest. Fick's first law of diffusion is described by *Equation 5.1*.

$$M = \frac{DA}{L} C_0 t \quad (5.1)$$

- M = amount of isoflurane transported by diffusion
- D = molecular diffusion coefficient of isoflurane (cm^2/s)
- A = cross section of the diffusion path (cm^2)
- L = total length of the diffusion path (cm)
- C_0 = isoflurane concentration in the medium (mol/cm^3)
- t = time (s)

The DA/L term is expressed in units of cm^3/min , which are the identical units used to describe sample flow rate in active air sampling devices. Passive techniques have been proven to be a successful alternative to traditional active sampling for gases and volatile vapors and validation of commercially available badge-type samplers has been conducted (9; 13; 49; 56). Prado et al. (1993) also validated Chromosorb 106 thermal desorption tubes for passive sampling of isoflurane (88). Górecki & Namieśnik (2002) described several advantages of passive samplers including their ability to be constructed

of small size, of light weight, and convenient to wear. Passive samplers generally require less training to be used effectively, and are less expensive than the use of sampling trains required by active sampling methods. They also do not require time-consuming pre/post sampling calibration, which can result in additional productivity, efficiency, and cost-savings for passive sampling. Additionally, passive samplers are convenient for long-term studies since they require no electricity, batteries, or other sources of electrical power (35). This also makes them advantageous for use in austere military settings. Passive diffusion methods also avoid the need to wear the cumbersome pumps that active sampling techniques require (52). This study partially validated a method that used Sulficarb and Carbograph 5TD thermal desorption tubes to passively sample isoflurane.

5.2 MATERIALS AND METHODS

5.2.1 Analytical Equipment

An laboratory gas chromatograph (7890B, Agilent Technologies, Santa Clara, CA) with a 5977 mass selective detector (Figure 5.1, Agilent Technologies) was used in this study in conjunction with a Markes Unity 2 thermal desorption unit and Series 2 ULTRA autoloader (Figure 5.2, Markes International, Llantrisant, UK) (Figure 5.2).

The gas chromatograph contained a HP-5ms column (30 m length x 250 μ m inner diameter x 0.25 μ m film thickness). Analytical parameters were as follows: the column flow rate was 1.287 mL/min, initial column temperature was 35°C held for 1.5 minutes and then ramped at 40°C per minute to a maximum temperature of 330°C (held for 3 minutes). The total gas chromatography (GC) analysis time was 10.6 minutes. A filament delay time of 0.25 minutes was used. Peak areas were manually integrated using

Agilent Enhanced Chem Station software (Ver. F.01.03.2357). Ultra high purity helium (99.999%) purchased from Roberts Oxygen (Rockville, MD) was used as the carrier gas.

5.2.1.1 Markes Unity 2 Parameters

The thermal desorption system cold trap was capable of achieving sub-ambient temperatures and was programmed to attain -10°C during desorption of the thermal desorption tubes. The focusing trap was then flash heated at the highest rate possible (approximately $100^{\circ}\text{C}/\text{sec}$) to a maximum temperature of 300°C (held for 3 minutes) to ensure full desorption of the isoflurane collected on the cold trap from a thermal desorption tube. The transfer line temperature to the gas chromatograph was set to 150°C . The desorption flow rate was 5 mL/min, while the desorb split and trap split flow rates were both 180 mL/min, giving a total split flow of 5,210:1. The desorption temperature was 300°C with a 6-minute desorption time.

5.2.2 Chemicals

Isoflurane (99.99%) was purchased from Baxter International (Deerfield, IL). Ultra high purity helium (99.999%) was purchased from Roberts Oxygen (Rockville, MD) and used as the Agilent carrier gas. High purity nitrogen (99.99%) used as the cryogenic gas and for operating valves on the Markes Unity II was purchased from Roberts Oxygen (Rockville, MD).

5.2.3 Thermal Desorption Tube Selection

Stainless steel (89 mm x 6.35 mm outer diameter (5 mm inner diameter) Sulficarb and Carbograph 5TD thermal desorption tubes were used in this study. Both types of tubes were purchased from Markes International. Sulficarb tubes contained an average of 0.3017 mg of sorbent ($n = 20$, range = 0.299 to 0.304 g), while Carbograph 5TD tubes contained an average of 0.3012 g of sorbent ($n = 20$, range 0.297 to 0.304 g). Each tube was pre-conditioned using a TC-20 sorbent tube conditioner (Markes International) according to the tube manufacturers' recommendations prior to use. Passive sampling was performed with a diffusion cap (Markes International) installed on the front section of the tube and a brass storage cap installed on the rear section of the tube. After sampling, each groups of tubes was capped with DiffLok caps (Markes International) and loaded into the Markes Series 2 ULTRA autoloader for analysis.

5.2.4 Isoflurane Concentration Range of Interest

The target concentration for this study was 50 ppm. The validation concentration range of interest was 15 – 125 ppm (approximately 0.3x – 2.5x the target concentration).

5.2.5 Calibration of Agilent 7890B / 5977B Instrument

A 7-point calibration curve was generated using static atmospheres of isoflurane combined with ambient laboratory air contained in Tedlar[®] bags. Isoflurane-laden air was drawn from a bag and through a thermal desorption tubes using a Kitigawa (Tokyo, Japan) AP-20 100 mL piston pump. The isoflurane masses used to generate the calibration curve ranged from 0.395 – 11.5 μg . The calibration curve had good linearity ($R^2 = 0.99967$, Figure 5.3) and good accuracy, with an average error of 2.5% across the calibration range (Table 5.1).

5.2.6 Development of the Dynamic Flow Chamber

A dynamic flow chamber was constructed using a Supelco Model 1062 Vacuum Bag Sampling Pump case (Figure 5.4). A Miller-Nelson HCS-501 temperature and humidity control system (Miller-Nelson Research, Inc., Livermore, CA, Figure 5.4) was used to control the flow rate, temperature, and relative humidity of the diluent air entering the chamber. Freshly mixed isoflurane in air was routed from the Miller-Nelson instrument through an inlet port drilled in the right side of the chamber. A 6-inch diameter plastic, flexible pipe was attached to an exit port in the bottom of the chamber in order to keep the interior of the chamber at atmospheric pressure irrespective of flow rate. A Harvard Apparatus Pump 11 Elite syringe pump was used to inject isoflurane into the system. Compressed house air served as the diluent and was plumbed into the Miller-Nelson instrument. An Aalborg (Orangeburg, NY) GFM-57 Mass Flow Meter (0-200 L/min) was used to verify the diluent flow rate. Syringe pump feed rates necessary to achieve the target isoflurane chamber concentrations were calculated according to Equation 5.2 (77).

$$C = \frac{(24.45)(10^6)\left(\frac{T_{Chamber}}{298\text{ K}}\right)\left(\frac{760\text{ mmHg}}{P_{Chamber}}\right)(F_{Rate})(\rho_{iso})}{(Q_{Air})(MW)} \quad (5.2)$$

- C = desired isoflurane concentration (ppm)
- V_{Mol} = molar volume of isoflurane (L/mol)
- $T_{Chamber}$ = temperature of the chamber (K)
- $P_{Chamber}$ = atmospheric pressure of the chamber (mmHg)
- F_{Rate} = syringe pump feed rate ($\mu\text{L}/\text{min}$)
- Q_{iso} = density of isoflurane (g/mL)
- Q_{Air} = diluent air flow rate (L/min)
- MW = molecular weight of isoflurane (g/mol)

Temperature, pressure, and relative humidity inside the chamber were measured during sampling using a TSI (Shoreview, MN) VelociCalc Model 9545 air velocity meter, which was placed inside the chamber and monitored during all sampling events.

5.2.7 Validation of Isoflurane Concentrations in Dynamic Flow Chamber

To verify that the diluent air flow rate and syringe advance rates recommended by *Equation 5.2* achieved the desired isoflurane concentrations (15 – 125 ppm), validation was performed using conventional active air sampling with SKC Model 226-81A Anasorb 747 (70/140) sampling tubes (SKC, Inc., Eighty-Four, PA). Validation air sampling followed guidance contained in OSHA Method 103, Enflurane, Halothane, and Isoflurane (80). SKC Airchek Sampler Pumps (Model 224-44XR) were used in the chamber validation study. Active air sampling was performed by connecting the Anasorb 747 tubes to brass sampling ports that were installed in the lid of the chamber. Three thermal desorption tubes of each type were placed inside the chamber to passively sample during active air sampling (Figure 5.5). Two pumps sampling the 50 and 75 ppm theoretical concentrations had post-sampling flow rates that varied by > 10% from pre-sampling flow rates. These sampling tubes were analyzed but were later discarded due to very low results. Two other pumps stopped completely during sampling of the 125 ppm theoretical concentration and their data was discarded. After sampling, the Anasorb 747 tubes were packaged in an insulated cooler with several gel ice packs and shipped overnight to an AIHA-accredited laboratory for analysis. The results of the passive and OSHA 103 method were compared to determine the agreement in chamber concentrations predicted by the thermal desorption tube calibration curve and the Anasorb 747 tubes.

5.2.8 Method Validation Tests

Method validation tests were selected based on Eide, Simmons, and Hendricks'. (2010) Validation Guidelines for Air Sampling Methods Utilizing Chromatographic Analysis, which is utilized by OSHA for method development (28).

5.2.8.1 Sampling Rates and Sampler Capacity

The isoflurane sampling rates for each type of tube were estimated at six validated concentrations ranging from 14 – 119 ppm, and two humidity levels (20% and 80%) over a sampling time interval of 15 minutes. The experimental design to determine sampling rates for each type of tube is described in Table 5.4. Due to time limitations, the full 16-run factorial validation of passive sampling rate variation by Eide (2004), which accounts for variations in sampling rate resulting from variations in analyte concentration, relative humidity, interferences, sampling time, face velocities, and sampler orientation, was not performed (27).

The sampler capacity for each type of tube was estimated by measuring the sampling rate at one concentration (93 ppm) over increasing time intervals (15 – 120 minutes). Sampler capacity was defined as the sampling time period at which a decrease in the average preliminary sampling rate of $\geq 10\%$ was observed. The preliminary sampling rate was determined by averaging the six values for the first two sampling times (15 and 25 minutes). The average sampling rates for both methods were converted to equivalent sampling rates at normal temperature and pressure (NTP) conditions of 760 mmHg and 298.2 K in accordance with Eide et al. (2010) using *Equation 5.3* (28):

$$R_{NTP} = R_{SS} \left(\frac{T_{NTP}}{T_{SS}} \right)^{3/2} \left(\frac{P_{SS}}{P_{NTP}} \right) \quad (5.3)$$

R_{NTP} = sampling rate at NTP conditions
 R_{SS} = sampling rate in the dynamic flow chamber
 T_{SS} = temperature (K) in the dynamic flow chamber
 T_{NTP} = 298.2 K
 P_{SS} = pressure in the dynamic flow chamber
 P_{NTP} = 760 mmHg

5.2.8.1.1 Isoflurane Sampling Rates During 15-Minute Sampling

The parameters for each sampling rate test are described in Table 5.4. The average isoflurane sampling rate of each tube type over a 15-minute sampling period was measured at 6 chamber concentrations (14, 25, 41, 69, 93, and 119 ppm). To determine the effect of humidity on sampling rate, each concentration was tested in triplicate at two relative humidity (RH) levels (20% and 80%). The average ($\pm$ SD) environmental conditions (temperature, barometric pressure, and relative humidity) for the 20% RH Sulficarb tests were 21.7°C ($\pm$ 0.12), 766.1 mmHg ($\pm$ 0.37), and 20.4% ($\pm$ 0.20), respectively. The average environmental conditions for the 80% RH Sulficarb tests were 21.8°C ($\pm$ 0.25), 757.9 mmHg ($\pm$ 0.56), and 81.2% ($\pm$ 0.58), respectively. The average ($\pm$ SD) environmental conditions (temperature, barometric pressure, and relative humidity) for the 20% RH Carbograph 5TD tests were 21.7°C ($\pm$ 0.12), 766.0 mmHg ($\pm$ 0.38), and 20.4% ($\pm$ 0.16), respectively. The average environmental conditions for the 80% RH Sulficarb tests were 21.8°C ($\pm$ 0.24), 758.0 mmHg ($\pm$ 0.56), and 80.5% ($\pm$ 0.78), respectively.

5.2.8.1.2 Sampling Rate Over Increasing Time Intervals and Sorbent Capacity

The average sampling rate of each tube type was measured over increasing time intervals (15, 30, 45, 60, 90, and 120 minutes) until a 10% decrease from the average

preliminary sampling rate was observed. The average preliminary sampling rates for Sulficarb and Carbograph 5TD were obtained by averaging the sampling rates of the 15, 30, 45, and 60 minute sampling times. The preliminary sampling rates for Sulficarb and Carbograph 5TD were calculated to be 0.54 mL/min and 0.53 mL/min, respectively. Each time interval was sampled in triplicate. The concentration of isoflurane used in this test was 93 ppm. The average temperature, pressure, and RH for these tests were 21.8°C (± 0.26), 751.5 mmHg (± 0.26), and 80.2% (± 0.41), respectively.

5.2.8.2 Sorbent Breakthrough

A test was performed to determine the point of breakthrough of the Sulficarb and Carbograph 5TD thermal desorption tube sorbents. For comparison, the test was also performed using a Carbotrap 349 thermal desorption tube. Two tubes of each tube were connected (front-to-back in series). A calibrated SKC Airchek Sampler Pump (Model 224-44XR) drew sample air of 93 ppm (703 mg/m³) concentration from the interior of the dynamic flow chamber at a flow rate of approximately 50 mL/min. Each test was performed at an absolute humidity of approximately 15.4 mg of water per L of air (average temperature 21.7°C (± 0.12), average pressure 743.5 mmHg (± 2.0), average relative humidity 80.6% (± 0.0050). The sampling times ranged from 20 – 390 minutes. After sampling, the backup tube was analyzed to determine the m/z 51 peak area. If analysis of the back tube showed an m/z 51 and m/z 149 peak (isoflurane's second most prevalent 70 eV electron ionization ion) at a retention time between 1.2 and 1.9 minutes then isoflurane would be assumed to have broken through the front tube sorbent. The mass of isoflurane in the backup tube would then be divided by the volume of air that had passed through the tube during the sampling period to estimate the concentration of

isoflurane in the effluent air. Testing was halted when the effluent concentration was calculated to be $\geq 5\%$ of the inlet concentration.

5.2.8.3 Sorbent Retention - Reverse Diffusion

This test investigated the propensity for post-sampling diffusion of the collected isoflurane from the Sulficarb and Carbograph 5TD sorbents. Ten tubes of each type were placed in the dynamic flow chamber to passively sample an isoflurane atmosphere of 703 $\mu\text{g/L}$ (93 ppm) for 15 minutes. Five of the tubes were then removed and analyzed. The injection of isoflurane into the diluent air stream was halted and the chamber lid was opened and allowed to purge for 3 minutes. When purging was complete, the remaining 5 tubes were placed back in the chamber to sample isoflurane-free diluent air for 60 minutes. The second group of 5 tubes was then analyzed. The masses of isoflurane recovered from both groups of tubes were then compared.

5.2.8.4 Desorption Efficiency

The desorption efficiency of Sulficarb and Carbograph 5TD tubes was assessed using 6 static isoflurane atmospheres contained within 5 L Tedlar[®] bags. The concentrations ranged from 4 ppm (31 $\mu\text{g/L}$) to 157 ppm (1,184 $\mu\text{g/L}$). A volume of 50 mL was drawn from each bag through the Sulficarb and Carbograph 5TD tubes, which loaded between 1.5 – 5.9 μg of isoflurane. Desorption efficiency was calculated using a method based on work performed by Cao and Hewitt (1993), who defined desorption efficiency as the ratio of the mass of analyte recovered from the sorbent during thermal desorption to the mass of analyte added to the sorbent (*Equation 5.4*) (19).

$$D.E._i (\%) = \frac{A_{i,1} - A_{Bi}}{A_{i,1} + A_{i,2} - A_{Bi}} \quad (5.4)$$

$D.E._i$ = desorption efficiency for isoflurane

$A_{i,1}$ = area count of isoflurane from the first desorption of the spiked tube

$A_{i,2}$ = area count of isoflurane from the second desorption of the spiked tube

A_{Bi} = area count of isoflurane from the adsorbent blank

Each spiked tube was desorbed twice in the Aglient / Markes system. The results were then entered into *Equation 5.4* to determine the estimated desorption efficiency.

5.2.8.5 Storage Stability

The stability of isoflurane on Sulficarb and Carbograph 5TD sorbents after storage at ambient and refrigerated temperatures was investigated. Eighteen tubes of each type were spiked with 56 µg of isoflurane by drawing 100 mL from the 0.565 µg/L bag (20.1°C, 95% relative humidity). Three tubes of each type were analyzed immediately. The remaining tubes were tightly capped, and stored. This test was performed twice, once with storage at ambient temperature (in a lab drawer) and again with storage in a refrigerator at 4°C. The remaining tubes were analyzed in groups of three every 3-5 days over a 15 day period.

5.2.9 Statistical Methods

Data was analyzed using Microsoft Excel (2011) and IBM® SPSS® Statistics Software (Version 22) (Armonk, NY). A two way Analysis of Variance (ANOVA) with Between and Within Factors test was conducted to investigate the effects of relative humidity (between factor) and tube type (within factor) on isoflurane uptake rate during 15 minute sampling.

5.2.9.1 Sampling Rate Variation

Sampling rate variation for each type of thermal desorption tube was calculated by calculating the average CV for each type of tube across both relative humidity levels (20% and 80%) and the six tested isoflurane concentrations. The total number of measurements used to calculate the average CV for each type of tube was 36.

5.2.9.2 Standard Error of Estimate for Storage

The Standard Error of Estimate for Storage was calculated using the variation observed during the storage stability test and *Equation 5.5* (28).

$$S_{Y \cdot X(STO)} = \sqrt{\frac{\sum(Y_{OBS} - Y_{EST})^2}{n - k}} \quad (5.5)$$

$S_{Y \cdot X(STO)}$ = standard error of estimate from storage
 Y_{OBS} = observed response
 Y_{EST} = estimated response from regression curve
 n = total number of data points
 k = 2 for a linear equation

5.2.9.3 Standard Error of the Estimate for the Diffusive Samplers

The overall Standard Error of Estimate for the Diffusive Samplers was calculated using *Equation 5.6* (28).

$$S_{EE} = \sqrt{S_{Y \cdot X(STO)}^2 + V_{SR}^2} \quad (5.6)$$

S_{EE} = overall standard error of estimate
 $S_{Y \cdot X(STO)}$ = standard error of the estimate from storage
 V_{SR} = sampling rate variation

5.3. RESULTS

5.3.1 Calibration Data

The 7-point isoflurane calibration curve had excellent linearity, with an R^2 value = 1.000 (Figure 5.1). The accuracy of the predicted isoflurane mass was also good, with an average error across the calibration range of 2.5% (Table 5.1). The extended 10-point calibration curve (Figure 5.1) used in the sampling capacity test also had excellent linearity ($R^2 = 0.999$).

5.3.2 Dynamic Flow Chamber Validation

The results of the chamber validation tests are displayed in Table 5.2 and Figure 5.6. Agreement and linearity was good between passive sampling using Sulficarb (slope = 0.952, $R^2 = 0.993$) and Carbograph 5TD (slope = 0.953, $R^2 = 0.992$).

5.3.3 Method Validation Tests

5.3.3.1 Sampling Rate and Sampling Capacity

5.3.3.1.1 Sampling Rate Over a 15-minute Sampling Period

Sulficarb had a slightly higher average sampling rate over the tested concentration range at both 20% and 80% relative humidity than did Carbograph 5TD (Table 5.3). The overall average sampling rates for Sulficarb and Carbograph 5TD across the 20% and 80% relative humidity levels were $4.4 \text{ ng ppm}^{-1} \text{ min}^{-1} \pm 0.064$ ($0.58 \text{ mL/min} \pm 0.0073$) and $4.2 \text{ ng ppm}^{-1} \text{ min}^{-1} \pm 0.17$ ($0.54 \text{ mL/min} \pm 0.022$), respectively. Sulficarb measurements achieved higher precision ($1.5\% \pm 0.88$) than did Carbograph 5TD ($4.2\% \pm 2.5$) across both of the tested relative humidity levels. The sampling rate variability (V_{SR}) for Sulficarb and Carbograph 5TD was 6.9% and 9.1%, respectively.

The results of the two way ANOVA indicated that there was a significant difference in the mean corrected sampling rates between Sulficarb and Carbograph 5TD tube types ($p = 0.006$). Relative humidity level did not have a significant effect on the mean sampling rate of either tube type ($p = 0.157$). The interaction of relative humidity and tube type on mean sampling rates was also not significant ($p = 0.875$). Tables 5.5 and 5.6 contain the results of statistical analysis.

5.3.3.1.2 Sampling Rate and Sampling Capacity Over a 120-minute Sampling Period

The average sampling rates for both Sulficarb and Carbograph 5TD were reasonably constant up to a sampling time of approximately 60 minutes (Figure 5.7). At the 60 minute mark, the average sampling rate for Sulficarb was 0.52 mL/min for and 0.53 mL/min For Carbograph 5TD. The sampling rates of both Sulficarb and Carbograph 5TD had decreased more than 10% (denoted by red line limits) from their preliminary average sampling rates by the 90-minute mark.

5.3.4 Sorbent Breakthrough

Figure 5.8 illustrates the breakthrough volumes observed for Sulficarb, Carbograph 5TD, and Carbotrap 349 during this study. Sulficarb displayed the highest breakthrough volume of 35 L (110 L/g of sorbent). The breakthrough volumes for Carbograph 5TD and Carbotrap 349 were 5.8 L (19 L/g of sorbent) and 29 L, respectively. The masses of the three sorbents contained in the Carbotrap 349 tubes were unknown, so the breakthrough volume per unit mass of sorbent was not calculated.

5.3.5 Retention of Isoflurane - Reverse Diffusion

Tables 5.7 and 5.8 describe the results of the reverse diffusion test, with both Sulficarb and Carbograph 5TD achieving an average recovery of 98.0%.

5.3.6 Desorption Efficiency

Sulficarb and Carbograph 5TD both achieved average desorption efficiencies of >99.9% over the tested concentrations (Table 5.9).

5.3.7 Storage Stability

Figure 5.10 and Table 5.10 describe the stability of isoflurane on Sulficarb and Carbograph 5TD. Sulficarb tubes that were stored at ambient laboratory temperature (approximately 20°C) had a mean recovery of 99% (± 5.9), CV = 5.9%, and Standard Error of Estimate for Storage of 5.9%. Sulficarb tubes that were refrigerated during storage had a mean recovery of 98% (± 1.9), CV = 1.8%, and Standard Error of Estimate for Storage of 1.5%.

Carbograph 5TD tubes displayed similar, but more pronounced results. Tubes that were stored at ambient laboratory temperature had a mean recovery of 82% (± 25), CV = 21%, and Standard Error of Estimate for Storage of 7.9%. Carbograph 5TD tubes that were refrigerated had a mean recovery of 98% (± 1.7), CV of 2.7%, and Standard Error of Estimate for Storage of 1.8%.

5.4 DISCUSSION

5.4.1 Dynamic Flow Chamber Validation

The overall agreement between the passive thermal desorption tubes and the active Anasorb 747 tubes was very good. However, it appears that opening of the

chamber lid to install/extract the thermal desorption tubes resulted in some dilution of the isoflurane atmosphere. This is thought to have resulted in the negative bias of both passive and active samples at higher chamber concentrations (Table 5.2). Though the overall linearity and agreement between the OSHA 103 active samples and the passive thermal desorption tube samples was very good, there was relatively high error at the 50 and 75 ppm theoretical concentrations. Opening and closing of the chamber lid at the beginning of sampling could have contributed to this error. Another contributing factor could include the removal of several OSHA 103 validation data points (due to sampling pump problems), which decreased the number of replicate samples that each average concentration was based on. Inaccurate laboratory results would have affected the sampling rate calculations since the chamber concentration is an integral part of the sampling rate equation. Future work with this chamber should investigate using drilled access ports through which thermal desorption tubes can be inserted into the chamber without the need for opening the chamber lid. Re-validation of the chamber in this configuration should also be performed.

5.4.2 Sampling Rate and Sampling Capacity

Humidity did not have a significant effect on the sampling rate of either sorbent type. However, Sulficarb has a stronger affinity for water than Carbograph 5TD, so it is possible that sorption of water at higher absolute humidity levels than those tested could produce a difference in the sampling rates. This could occur as a result of competition for binding sites by water, which could force isoflurane deeper into the sorbent. This would effectively lengthen the diffusion path and lower the sampling rate by a

proportionate amount. The effect of higher absolute humidity levels could be investigated in future work.

There was a significant difference in the sampling rates between Sulficarb and Carbograph 5TD sorbents. The average sampling rates across both of the tested relative humidity levels were approximately 5.5% higher for Sulficarb than for Carbograph 5TD. A possible reason for the differences could be due to the higher strength Sulficarb sorbent being a more effective zero sink than the weaker Carbograph 5TD. The higher sampling rate observed with Sulficarb would enable collection of a greater mass of isoflurane per unit time, which could increase analytical sensitivity, though the difference between Sulficarb and Carbograph 5TD in the overall isoflurane mass collected during short-term, 15-minute sampling may not be significant. This could be verified during a field evaluation.

The sampling capacities of Sulficarb and Carbograph 5TD under the test conditions were similar. The sampling rates of both sorbents displayed a decrease in preliminary sampling rates that was greater than 10% at approximately the same sampling time (90 minutes). The 60-minute sampling time was the longest period that contained all of the data points within the 95% confidence intervals. In accordance with Eide, Simmons, and Hendricks (2010), the maximum sampling time should therefore be kept to less than 48 minutes (80% of 60 minutes) in order to minimize the risk of reaching sampler capacity and to maximize quantitative capture of isoflurane (28).

Similar to results described by Martin et al. (2015), the sampling rates observed in this study are presented as a validation of the overall passive sampling method rather than as fully validated sampling rates for Sulficarb and Carbograph 5TD thermal desorption

tubes. The distinction is important since full validation of the sampling rate variation using the 16-run factorial test described by Eide (2004) was not performed (27). Full validation is recommended for future work.

5.4.3 Sorbent Breakthrough

Sulficarb had a breakthrough volume (35 L (110 L/g)) that was approximately 6 times higher than that of Carbograph 5TD (5.8 L (19 L/g)). This indicates that Sulficarb has a greater ability to adsorb isoflurane at a given concentration, temperature, and humidity level. The breakthrough volume of Carbotrap 349 (29 L), which was determined for comparative purposes, was approximately 20% less than that of Sulficarb and 5 times greater than Carbograph 5TD. This was expected since Carbotrap 349 contains very high strength Carboxen 1003 (a carbon molecular sieve) as one of its three sorbents.

5.4.4 Retention of Isoflurane - Reverse Diffusion

The mean recoveries of Sulficarb and Carbograph 5TD observed in the first retention test were identical (98%). This suggested that both sorbents retained most of the isoflurane they had collected over the 15-minute sampling period and that approximately 2% had diffused back off from the sorbent during the 60-minute sampling of uncontaminated air. According to Eide et al. (2010), the 98% mean recoveries of both tubes met the OSHA mean recovery requirement of $\geq 90\%$ (28). This level of recovery verified that both tubes could be used to sample isoflurane for a time period up to the safe sampling time of 48 minutes described in paragraph 5.3.2.

5.4.5 Storage Stability

Storage temperature appeared to affect the stability of isoflurane on Sulficarb and Carbograph 5TD. Sulficarb samples stored at ambient laboratory temperature (approximately 20°C) had a similar overall mean recovery (98.7% vs. 98.1%, respectively) over the 15-day study period. However, the precision obtained with ambient storage was relatively poor. This effect was much more pronounced with Carbograph 5TD. (CV =20.7% vs. CV = 2.4% with refrigeration), and ambient temperature storage of these tubes also led to a marked decrease in the amount of isoflurane recovered.

The results of the storage stability tests signify that isoflurane collected on Sulficarb had reasonably good stability when stored at 20°C. However, for best results Sulficarb should be refrigerated to 4°C between the time of sampling completion and the time of analysis. Stability of Carbograph 5TD was relatively poor when samples were stored at ambient laboratory temperature. Mean recovery from Carbograph 5TD was within 75% criterion for tubes stored under ambient and refrigerated temperatures. However, the 95% confidence limit of the tubes stored at ambient temperature exceeded the $\pm 25\%$ criterion. As a result, it is not recommended that isoflurane be stored on unrefrigerated Carbograph 5TD tubes.

Keeping samples cold during shipping is challenging. An ideal sorbent should offer good stability of the collected analyte even when samples are stored at ambient temperatures. As a result, Sulficarb is the recommended sorbent for isoflurane sampling. However, this recommendation is made with the presumption that analytical conditions (such as desorption temperature) are capable of fully desorbing the isoflurane from the

high strength Sulficarb sorbent. If this is not the case (such as when using a HAPSITE[®] ER with a thermal desorption sampling system), then the weaker Carbograph 5TD with either refrigerated storage or analysis soon after sampling may be the more appropriate choice.

5.5 STUDY LIMITATIONS

The data for several Anasorb 747 tubes used during the chamber validation were not used due to sampling pump problems. This resulted in two theoretical chamber concentrations (50 ppm and 75 ppm) being validated in duplicate and one theoretical concentration (125 ppm) being validated with only one replicate. Additionally, thermal desorption tubes were placed into the dynamic flow chamber through the chamber lid. Though the tube mounting system could be placed into the chamber in approximately one second, opening of the lid still caused some dilution of the chamber atmosphere. Drilling additional sampling ports into the side of the chamber for passive sampling using thermal desorption tubes would be an effective way of minimizing the impact of the chamber atmosphere during sampling. Only two storage temperatures were investigated in this study. Future work could investigate isoflurane stability on these sorbents at temperatures that would more closely approximate temperatures in typical military deployment environments.

5.6 CONCLUSIONS AND RECOMMENDATIONS

Sulficarb displayed equal or superior performance to Carbograph 5TD in each of the tests performed in this study. As a result, Sulficarb is the recommended type of tube for passive sampling of isoflurane. Results of the storage stability tests indicate that Sulficarb does not necessarily require refrigeration, though stability and precision

improve if stored at cold temperatures prior to analysis. Carbograph 5TD showed unacceptable stability with storage at ambient temperatures and, if used, must be refrigerated if analysis is to be delayed.

The recommendation for use of Sulficarb is made with the presumption that the analytical conditions (such as desorption temperature) are capable of fully desorbing isoflurane from the high strength Sulficarb sorbent. If this is not the case, such as with the INFICON HAPSITE[®] ER (discussed in Chapter 3), then users may choose to consider Carbograph 5TD as a reasonable alternative. This would require those users to fully understand and accommodate for the identified weaknesses of Carbograph 5TD.

Opportunities for future work include full validation of the method, including determining the overall sampler variation, Limit of Detection, Limit of Quantification, and other tests outlined by Eide et al. (2010) and a field study on an exposed population (28).

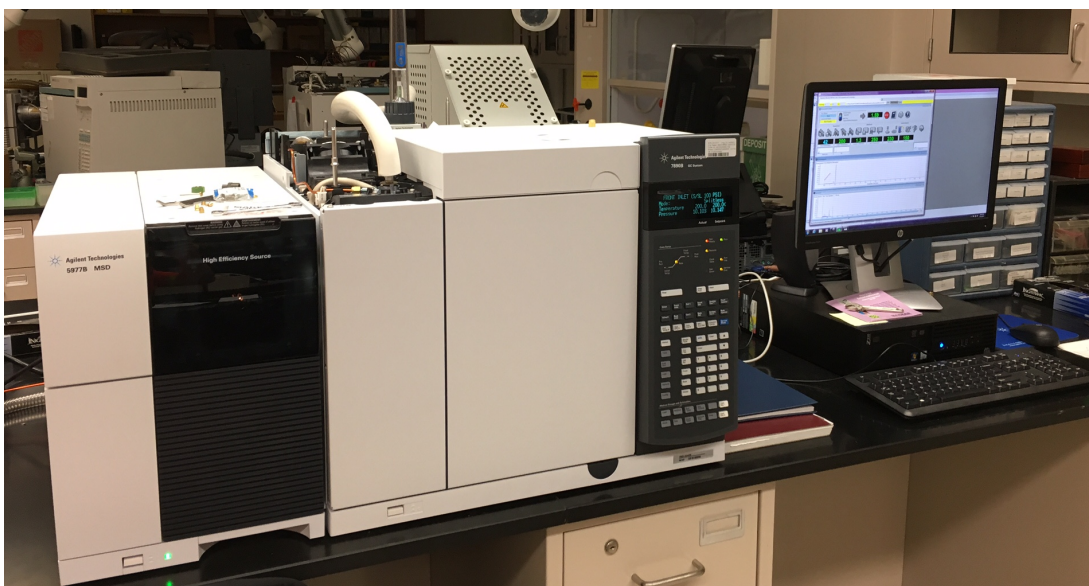


Figure 5.1. Agilent 7890B / 5977B Gas Chromatograph / Mass Spectrometer.

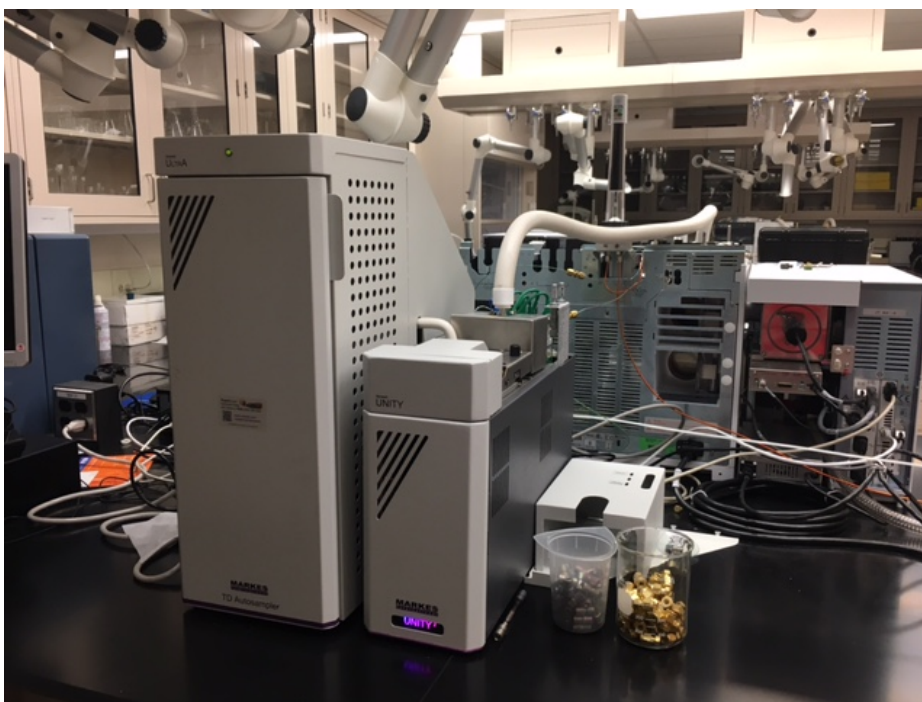


Figure 5.2. Markes Unity 2 and Series 2 ULTRA Thermal Desorption Autosampler.

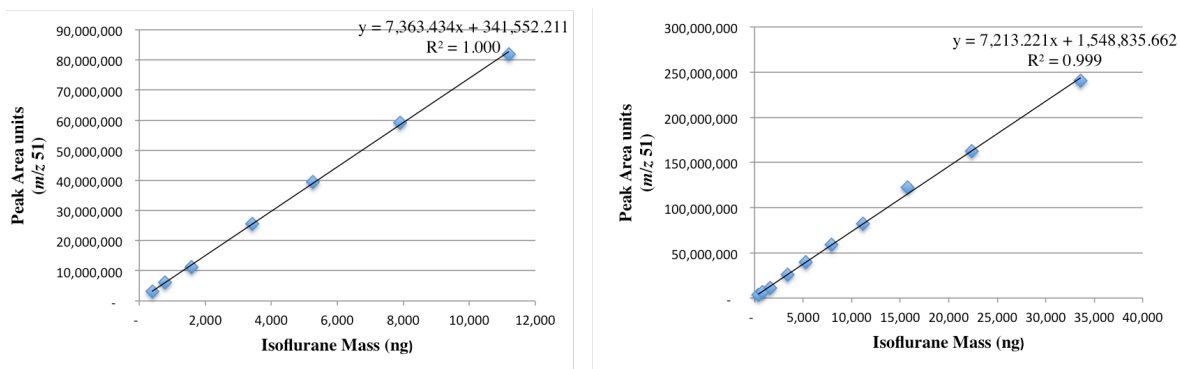


Figure 5.3. Seven and Ten Point calibration curves for Agilent 7890B / 5977B.

The 7 and 10-point calibration curves achieved excellent linearity ($R^2 = 1.000$ and $R^2 = 0.999$, respectively) over the calibration ranges.

Table 5.1. Calibration curve data for Agilent 7890B / 5977B.

Good accuracy of the predicted isoflurane mass was displayed, with an average error of 2.5% over the calibration range. Analysis of each theoretical mass was performed in triplicate.

Theoretical Isoflurane Mass (ng)	Peak Area (m/z 51)	Predicted Isoflurane Mass (ng)	Error (%)
395	3,173,264	385	-2.6
790	6,251,122	803	1.6
1,580	11,321,610	1,491	-5.6
3,423	25,725,926	3,447	0.7
5,265	39,448,517	5,311	0.9
7,898	59,317,829	8,009	1.4
11,189	82,026,588	11,093	-0.9
Average Error:			2.5

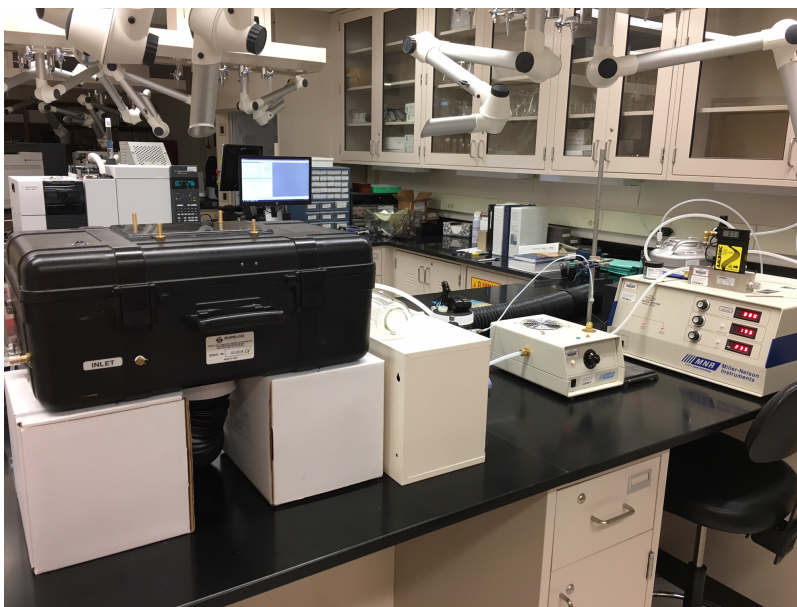


Figure 5.4. Dynamic flow chamber and Miller-Nelson temperature, humidity, and flow control system.

The dynamic flow chamber used in this study was constructed from a Supelco Model 1062 Vacuum Bag Sampling Pump case. The figure also illustrates the main system that controls the temperature, humidity, and flow rate of the incoming diluent air (right) and the solvent generator system (middle). Liquid isoflurane is injected into the heated solvent generator at a precise rate by the syringe pump (not shown), where it is volatilized and introduced into the diluent air stream.

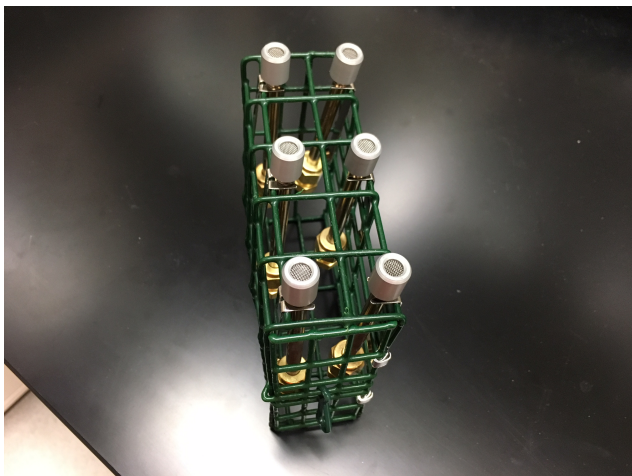


Figure 5.5. Thermal desorption tube mounting system.

The thermal desorption tube mounting system is shown holding three thermal desorption tubes of each type (Sulficarb and Carbograph 5TD). The tubes have the passive sampling caps installed. The mounting system was capable of holding from 1 – 6 tubes of each type.

Table 5.2. Chamber Validation: Average Agreement of Thermal Desorption Tubes (Passive Sampling) and Anasorb 727 Tubes (OSHA 103)

Chamber Theoretical Concentration	Chamber Concentration (Sulficarb) (n = 3)			Chamber Concentration (Carbograph 5TD) (n = 3)			Chamber Concentration (OSHA Method 103)	Number of OSHA 103 Samples ¹
ppm	ppm	CV (%)	Error ² (%)	ppm	CV (%)	Error ² (%)	ppm	CV (%)
15	15	0.089	7.1	13	5.6	-6.2	14	5.4
25	25	0.30	0.0	23	6.7	-9.7	25	8.2
50	46	1.9	12.2	46	2.3	12	41	4.6
75	75	0.13	8.7	71	4.5	3.0	69	1.3
100	92	1.7	-1.1	88	5.4	-5.3	93	2.8
125	115	2.0	-3.4	114	0.98	-4.3	119	N/A
			$\bar{x} = 6.9$			$\bar{x} = 7.5$		

¹The number of OSHA Method 103 samples are not in triplicate across all chamber concentrations due to unforeseen issues with several of the sampling media and two sampling pumps. As a result, several samples were compromised and were not included in the analysis.

²Error between the passive and OSHA Method 103 sampling results.

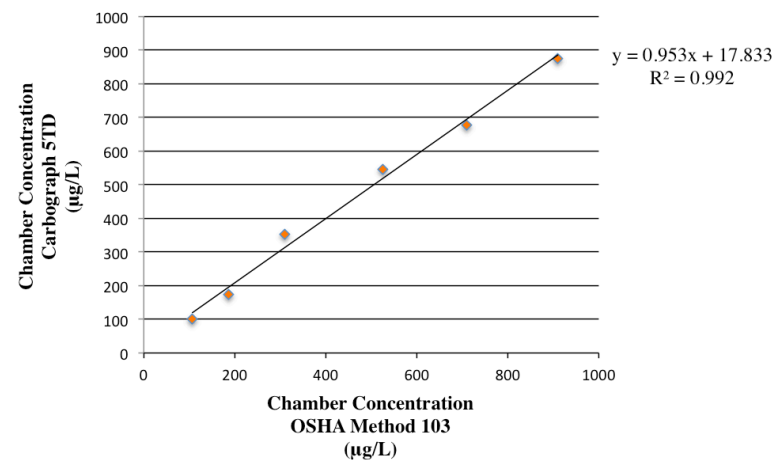
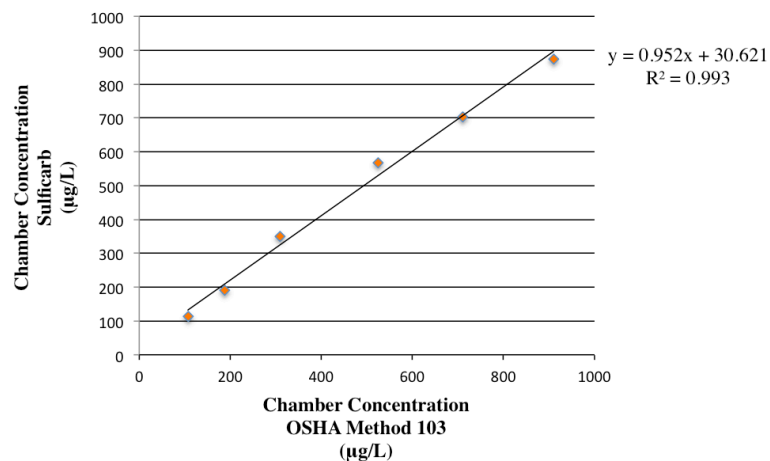


Figure 5.6 Chamber Validation: Agreement of Thermal Desorption Tubes (Passive Sampling) and OSHA Method 103 (Active Sampling).

Sulficarb displayed good agreement (Slope = 0.952) between the chamber concentrations predicted by passive sampling with thermal desorption tubes (with analysis on the Agilent 7890B / 5977B) and the results of the OSHA 103 method sampling with Anasorb 747 sampling media. Carbograph 5TD also displayed good agreement (Slope = 0.953).

Table 5.3. Thermal Desorption Tube Sampling Rates During 15-minute Sampling (20% and 80% Relative Humidity)

The difference in corrected mean sampling rates of Sulficarb and Carbograph 5TD was significant ($p < 0.001$).

Humidity had no significant effect on sampling rates ($p = 0.124$). The interaction of relative humidity and tube on mean was also not significant ($p = 0.672$).

		Sulficarb (n = 3)				Carbograph 5TD (n = 3)			
Chamber Concentration (ppm)		Average Predicted Mass Collected (ng)	Average Sampling Rate ¹ (ng ppm ⁻¹ min ⁻¹)	Average Sampling Rate ¹ (mL/min)	CV (%)	Average Predicted Mass Collected (ng)	Average Sampling Rate ¹ (ng ppm ⁻¹ min ⁻¹)	Average Sampling Rate ¹ (mL/min)	CV (%)
20% RH	14	821	4.0	0.53	0.47	751	3.7	0.48	8.0
	25	1,500	4.1	0.54	0.62	1,386	3.8	0.49	7.0
	41	2,962	4.9	0.64	2.2	2,898	4.8	0.63	1.2
	69	4,472	4.4	0.58	0.72	4,206	4.3	0.56	6.2
	93	5,879	4.3	0.57	2.3	5,293	3.9	0.51	5.0
	119	7,337	4.2	0.56	1.3	7,075	4.1	0.54	1.0
		Average:	4.3 ± 0.056	0.57 ± 0.0052	1.3 ± 0.82	Average:	4.1 ± 0.18	0.53 ± 0.024	4.7 ± 3.0
80% RH	14	892	4.3	0.57	0.62	885	4.3	0.56	3.1
	25	1,558	4.2	0.55	2.7	1,424	3.8	0.50	6.3
	41	3,033	5.0	0.65	1.6	2,829	4.7	0.61	3.5
	69	4,566	4.5	0.58	1.9	4,550	4.5	0.58	2.8
	93	5,992	4.4	0.57	0.44	5,679	4.1	0.54	5.7
	119	7,406	4.2	0.55	2.5	7,111	4.0	0.53	0.95
		Average:	4.4 ± 0.072	0.58 ± 0.0094	1.6 ± 0.93	Average:	4.2 ± 0.16	0.55 ± 0.020	3.7 ± 2.0
		Overall Average:	4.4 ± 0.064	0.58 ± 0.0073	1.5 ± 0.88	Overall Average:	4.2 ± 0.17	0.54 ± 0.022	4.2 ± 2.5

Table 5.4. Experimental Design of the Passive Sampling Tests.

Passive sampling tests were run at 6 different concentrations of isoflurane, two different relative humidity levels, one sampling period, and one sampler orientation.

Run Number	Isoflurane Concentration (ppm)	Average Temperature (°C)	Average Pressure (mm Hg)	Relative Humidity (%)	Sampling Time (min)	Face Velocity (cm/s)	Sampler Orientation
1	14	21.7	766.7	20.4	15	11	Vertical
2	25	21.8	766.3	20.1	15	6.1	Vertical
3	41	21.8	766.1	20.1	15	4.1	Vertical
4	69	21.8	765.8	20.5	15	4.1	Vertical
5	93	21.8	765.8	20.5	15	4.1	Vertical
6	119	21.5	765.8	20.5	15	4.1	Vertical
7	14	22.0	758.7	81.0	15	11	Vertical
8	25	22.1	758.2	81.5	15	6.1	Vertical
9	41	22.0	758.2	82.2	15	4.1	Vertical
10	69	21.6	757.7	80.5	15	4.1	Vertical
11	93	21.6	757.3	81.0	15	4.1	Vertical
12	119	21.6	757.3	81.0	15	4.1	Vertical

Table 5.5. Estimated Marginal Means for Sulficarb and Carbograph 5TD Sampling Rates.

	Tube Type	Relative Humidity (%)	Mean (ng ppm⁻¹ min⁻¹)	95% Confidence Interval
Tube Type	Sulficarb	-	4.4	4.3 – 4.5
	Carbograph 5TD	-	4.2	4.0 – 4.3
Relative Humidity (RH)	20% RH	-	4.2	4.1 – 4.3
	80% RH	-	4.3	4.2 – 4.4
Tube Type * RH (Interaction)	Sulficarb	20%	4.3	4.2 – 4.5
		80%	4.4	4.3 – 4.6
	Carbograph 5TD	20%	4.1	3.9 – 4.2
		80%	4.2	4.1 – 4.4

Table 5.6. Two Way ANOVA Results for Sulficarb and Carbograph 5TD Sampling Rates.

Statistical test results for two way ANOVA. Data is factored by tube type and relative humidity (RH) level.

Source	Type III Sum of Squares	Degrees of Freedom	Mean Square	F Statistic	p
Corrected Model	1.177	3	0.392	3.344	0.024
Intercept	1308.502	1	1308.502	11150.222	<.0.000
Tube Type	0.934	1	0.934	7.958	0.006
RH	0.240	1	0.240	2.048	0.157
Tube Type * RH	0.003	1	0.003	0.025	0.875
Error	7.980	68			
Total	1317.659	72			
Corrected Total	9.157	71			

a. $R^2 = 0.129$ (Adjusted $R^2 = 0.090$).

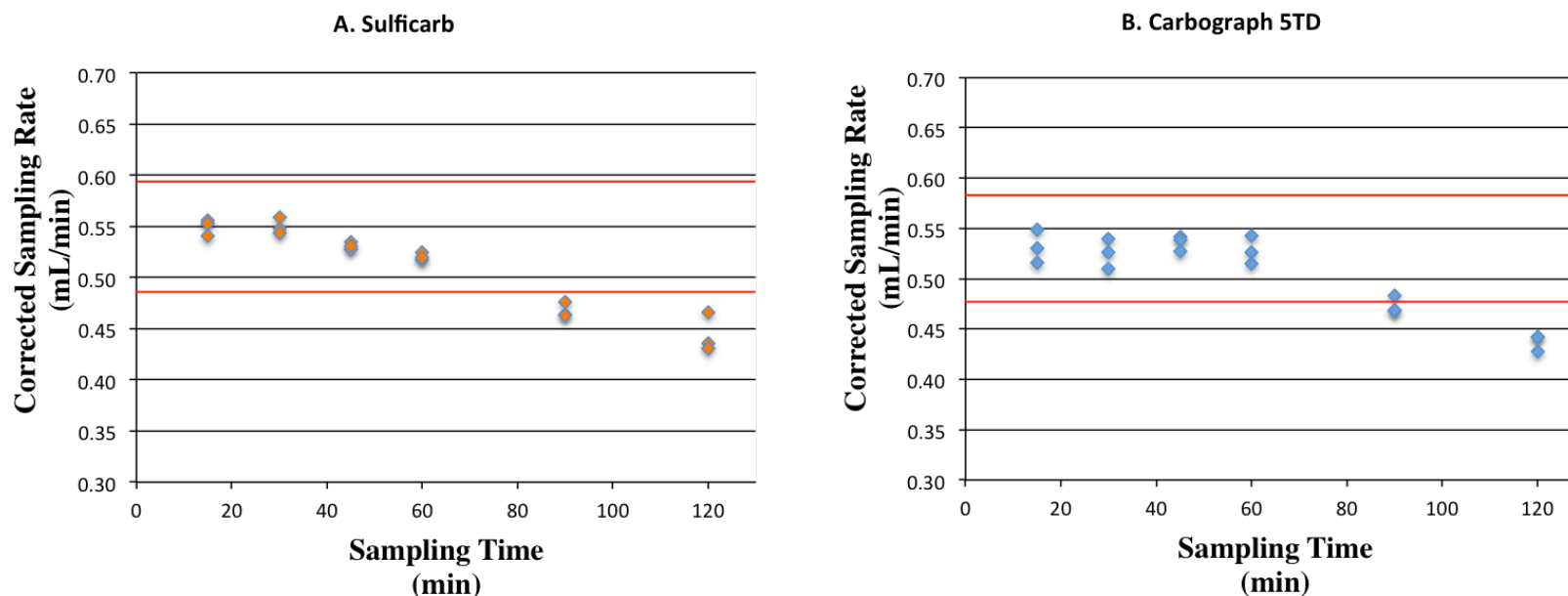


Figure 5.7. Sulficarb and Carbograph 5TD Sampling Rates and Sampler Capacity During 120-minute Sampling.

Sampling rates were relatively constant up to approximately 60 minutes. The sampling rate decreased more than 10% from the preliminary sampling rate (bottom red line) at the 90-minute mark. Prior to performing this sampling rate test, the calibration curve was extended to a 10-point curve for the 120-minute sampling rate test (see Figure 5.3). This was done in order to verify that any apparent decrease in the sampling rate was not due to saturation of the detector, and so that the mass of isoflurane loaded onto the thermal desorption at the higher sampling times (60, 90, and 120 minutes) did not exceed the curve's upper limit. Linearity of the extended calibration curve was excellent ($R^2 = 0.999$). These tests were performed at an isoflurane concentration of 93 ppm, an average temperature of 21.8°C, average pressure of 751.5 mm Hg, average relative humidity of 80.2%, and average face velocity of 6.1 cm/s.

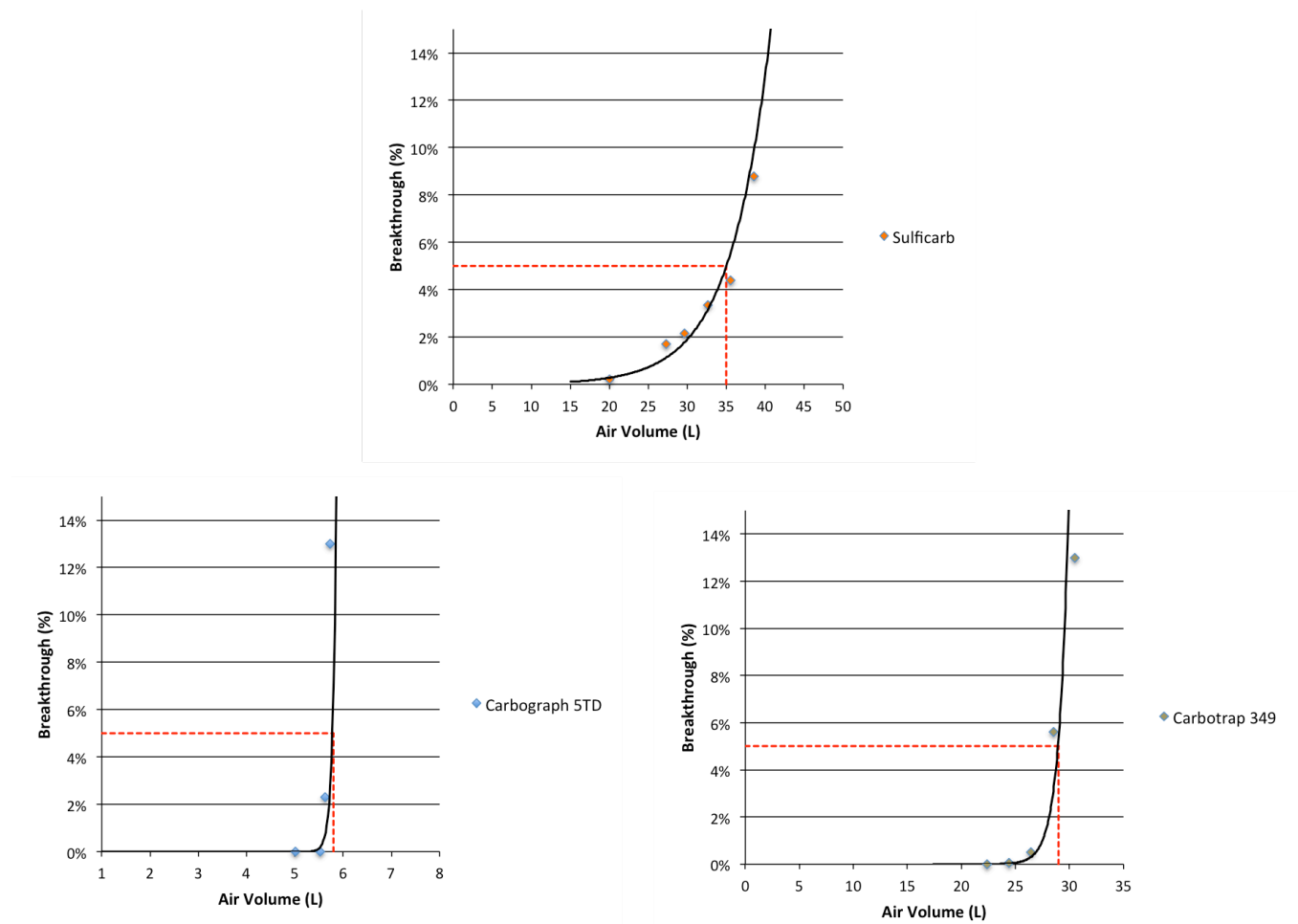


Figure 5.8. Sorbent Breakthrough.

Sulficarb thermal desorption tubes displayed the highest breakthrough volume of 35 L. The breakthrough volumes of Carbograph 5TD and Carbotrap 349 were 5.8 L and 29 L, respectively. The tests were performed using a dynamic isoflurane concentration of 93 ppm and an absolute humidity of 15.3 mg of water per liter of dry air (21.7°C, 80% relative humidity).

Table 5.7. Reverse Diffusion of Isoflurane from Sulficarb.

	Replicate #	Sampling Time (min)	Peak Area (m/z 51)	Predicted Isoflurane Mass (ng)	Mean Recovery (%)
Isoflurane Sampling	1	15	42,720,388	5,755	98.0%
	2	15	43,544,819	5,867	
	3	15	42,010,292	5,659	
	4	15	42,472,280	5,722	
	5	15	43,009,866	5,795	
		Mean	42,758,500	5,761	
		Std Deviation	767,973	104.3	
		CV %	1.8%	1.8%	
Sampling of uncontaminated air	1	60	42,164,429	5,680	
	2	60	41,000,267	5,522	
	3	60	42,076,737	5,668	
	4	60	41,747,144	5,623	
	5	60	42,320,769	5,720	
		Mean	41,861,869	5,643	
		Std Deviation	525,314	76	
		CV %	1.3%	1.3%	

Table 5.8. Reverse Diffusion of Isoflurane from Carbograph 5TD.

	Replicate #	Sampling Time (min)	Peak Area (m/z 51)	Predicted Isoflurane Mass (ng)	Mean Recovery (%)
Isoflurane Sampling	1	15	43,672,807	5,885	98.0%
	2	15	38,631,514	5,200	
	3	15	42,581,683	5,736	
	4	15	40,244,182	5,419	
	5	15	40,209,202	5,414	
		Mean	41,067,878	5,531	
		Std Deviation	2,026,631	275	
		CV %	4.9%	5.0%	
Sampling of uncontaminated air	1	60	41,447,144	5,582	
	2	60	43,022,879	5,796	
	3	60	37,546,970	5,053	
	4	60	40,142,510	5,405	
	5	60	39,117,712	5,266	
		Mean	40,255,443	5,421	
		Std Deviation	2,104,867	286	
		CV %	5.2%	5.3%	

Table 5.9. Desorption Efficiency of Sulficarb and Carbograph 5TD.

Both Sulficarb and Carbograph 5TD achieved excellent desorption efficiency at the tested isoflurane mass loadings.

Type of Tube	Isoflurane Mass Loaded (µg)	Desorption Efficiency (%)
Sulficarb	1.5	100
	3.0	100
	6.1	100
	12.5	99.9
	28.0	99.9
	59.3	99.9
Carbograph 5TD	1.5	100
	3.0	100
	6.1	100
	12.5	100
	28.0	100
	59.3	99.8

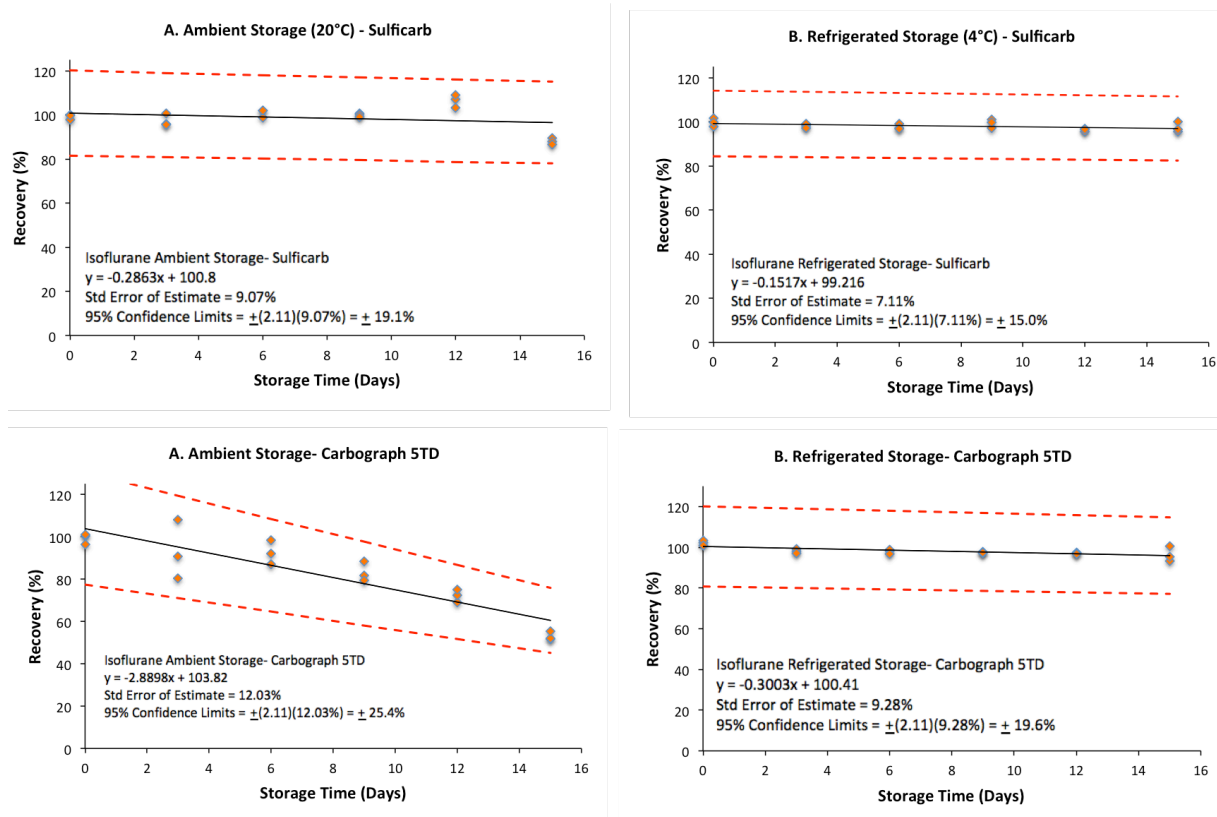


Figure 5.9. Stability of Isoflurane on Sulficarb and Carbograph 5TD Sorbents

The overall mean recovery of Sulficarb tubes stored at ambient laboratory temperature and those that were refrigerated were similar (98.7% and 98.1%, respectively). However, tubes stored at ambient laboratory temperatures experienced poorer precision than those that were refrigerated (SEE = 9.07% vs. 7.11%, respectively). The overall mean recovery of Carbograph 5TD tubes stored at ambient laboratory temperature and those that were refrigerated different considerably (82.2% and 98.2%, respectively). Tubes stored at ambient laboratory temperatures experienced a steady decline in recovery after Day 6 and much worse precision (SEE 12.0% vs. 18.2%) over the entire 15 days. Due to small sample size ($n = 18$), the critical t value for a 95% confidence interval with 17 degrees of freedom was used in calculating the 95% confidence limits (denoted in red).

Table 5.10. Storage Stability Test Results.

Results describe % recovery of the spiked isoflurane mass.

		Sample Number					
	Sorbent Type	Storage Time (Days)	1	2	3	Average	
Ambient Temperature Storage (20°C)	Sulficarb	0	100%	97.8%	99.8%	99.3%	
		3	94.8%	100%	95.6%	97.1%	
		6	102%	98.7%	102%	101%	
		9	101%	100%	99.1%	99.9%	
		12	107%	109%	103%	106%	
		15	89.6%	88.0%	86.6%	88.1%	
		Overall Average:					98.6%
		Standard Error of Estimate for Storage:					5.88%
	Carbograph 5TD	0	96.2%	100%	101%	99.2%	
		3	80.5%	90.8%	108%	93.2%	
		6	87.1%	98.2%	91.9%	92.4%	
		9	81.6%	79.4%	88.2%	83.0%	
		12	68.9%	72.4%	75.0%	72.1%	
		15	51.8%	52.0%	55.2%	53.0%	
		Overall Average:					82.2%
		Standard Error of Estimate for Storage:					7.87%
Refrigerated Storage (4°C)	Sulficarb	0	102%	97.7%	99.8%	99.7%	
		3	98.2%	99.0%	97.1%	98.1%	
		6	99.2%	97.1%	97.0%	97.8%	
		9	97.3%	97.0%	97.3%	99.4%	
		12	96.7%	95.5%	96.4%	96.2%	
		15	100%	95.5%	96.4%	97.3%	
		Overall Average:					98.1%
		Standard Error of Estimate for Storage:					1.71%
	Carbograph 5TD	0	102%	103%	101%	102%	
		3	98.2%	99.0%	97.1%	98.1%	
		6	98.0%	98.9%	96.6%	97.8%	
		9	97.2%	97.7%	97.3%	97.4%	
		12	97.5%	97.1%	96.6%	97.0%	
		15	101%	93.3%	95.4%	96.5%	
		Overall Average:					98.1%
		Standard Error of Estimate for Storage:					1.84%

Chapter 6: Conclusion and Future Studies

The overall hypotheses of this dissertation were 1) that the INFICON HAPSITE[®] Extended Range (ER) portable GC/MS could successfully quantify isoflurane anesthetic in surrogate exhaled breath with an accuracy of $\pm 25\%$, and 2) that passive sampling of isoflurane using thermal desorption Sulficarb and Carbograph 5TD thermal desorption tubes could successfully quantify isoflurane anesthetic in ambient air with an accuracy of $\pm 25\%$.

6.1 ANALYSIS OF ISOFLURANE IN HUMIDIFIED AIR USING THE INFICON HAPSITE[®] ER PORTABLE GAS CHROMATOGRAPH/MASS SPECTROMETER

The HAPSITE[®] ER was capable of quantifying isoflurane in surrogate exhaled breath within the desired accuracy limits. Two methods of sample introduction (hand-held, whole air sampling probe and thermal desorption sampling system used with Sulifcarb, Carbograph 5TD, and Carbotrap 349 thermal desorption tubes) were investigated. The absolute humidity level of the calibration had a large effect on the overall accuracy of each method. The hand-held probe had the greatest accuracy (8.0 and 16% average error) when calibration was performed using low and high humidity isoflurane standards, respectively. Carbotrap 349 had the best accuracy of the thermal desorption tubes tested, with average error of 23% when calibrated using low humidity standards, and average error of 7.7% when calibrated to high humidity standards. Agreement between the probe and thermal desorption methods was poor and calibration curves developed for each method were not generalizable to the other. It was discovered that sample loss occurred because of isoflurane breakthrough of the onboard concentrator, which likely contributed to negative bias observed with the thermal

desorption tubes. The amount of breakthrough ranged from 8.7 – 17% of the original mass loaded. This was likely a result of the high volatility of isoflurane and the fact that the HAPSITE[®] concentrator could not achieve sub-ambient temperatures, which is a capability that would likely increase the concentrator's collection efficiency. Specific aim (1) from Chapter 1 was accomplished during this study.

Several recommendations were made based on the results of this study.

- (1) Calibration should be performed using isoflurane standards that are of the same approximate absolute humidity (35 g of water per L of air) as is found in exhaled breath.
- (2) The hand-held probe is the recommended sample introduction method for sampling isoflurane in highly humidified air.
- (3) If thermal desorption methods must be used (if more than the 100-mL allowed by the sample probe is needed, for example), then use of Carbotrap 349 thermal desorption tubes is recommended.
- (4) Future work could investigate whether other highly volatile substances of concern also breakthrough the tri-sorbent concentrator.

6.2 EVALUATION OF CARBOTRAP 349, CARBOGRAPH 5TD, AND SULFICARB THERMAL DESORPTION TUBES FOR SAMPLING ISOFLURANE IN HUMIDIFIED AIR USING THE INFICON HAPSITE[®] ER PORTABLE GAS CHROMATOGRAPH/MASS SPECTROMETER

This study conducted a more in-depth analysis of the performance of Sulficarb, CarboGraph 5TD, and Carbotrap 349 thermal desorption tubes when used with the HAPSITE[®] ER. Validation included desorption flow rate, desorption efficiency, ability to achieve the target desorption temperature, and retention efficiency/breakthrough.

Desorption temperature varied significantly by tube casing material (glass vs. steel) and

sorbent type ($p < 0.001$). None of the sorbent-filled tubes tested achieved the target desorption temperature of 315°C. The inability to attain the desired desorption temperature negatively affected efficient release of isoflurane from Sulficarb, resulting in poor desorption efficiency of 71%. Carbotrap 349 and Carbograph 5TD achieved good desorption efficiency (98 – 99%). Specific aim (2) was accomplished.

Several recommendations were made.

- (1) The sorptive properties of Sulficarb were too strong for the HAPSITE® ER thermal desorption sampling system to overcome with the amount of heating that was available. As a result, Sulficarb is not recommended for use with the HAPSITE® ER when sampling isoflurane.
- (2) Because of the variation in steady-state desorption temperature observed with different type of thermal desorption tubes, users should consider using a multiple bed sorbent such as Carbotrap 349 rather than a strong single bed sorbent when sampling isoflurane. A single bed sorbent tube containing a slightly weaker sorbent (such as Carbograph 5TD) than what would otherwise be recommended for isoflurane (such as Sulficarb) is another alternative. However, users would have to be aware of and accommodate for the weaker sorbent's limitations.

6.3 CHAPTER 5: VALIDATION OF SULFICARB AND CARBOGRAPH 5TD THERMAL DESORPTION TUBES FOR PASSIVE SAMPLING OF ISOFLURANE

Sulficarb and Carbograph 5TD were partially validated for short-term (15-minute) passive sampling of isoflurane concentrations ranging from 14 – 119 ppm. Validation included determination of sampling rates, humidity effects, desorption efficiency, breakthrough volume, reverse diffusion, and storage stability. Sulficarb and Carbograph

5TD had an average sampling rate of approximately $4.4 \text{ ng ppm}^{-1} \text{ min}^{-1} \pm 0.064$ ($0.58 \text{ mL/min} \pm 0.0073$) and $4.2 \text{ ng ppm}^{-1} \text{ min}^{-1} \pm 0.17$ ($0.54 \text{ mL/min} \pm 0.022$), respectively. Sampling rate was not significantly affected by humidity level. Both types of tubes achieved excellent desorption efficiency (99 – 100%). The higher breakthrough volume of Sulficarb (35 L, 119 L/g of sorbent) suggested that it retained isoflurane more efficiently than Carbograph 5TD (5.8 L, 19 L/g of sorbent). Sulficarb showed reasonably good stability when stored under ambient (20°C) temperatures. Carbograph showed poor stability when stored under ambient temperature. Both types of tubes showed good stability when stored under refrigeration (4°C). Specific aim (3) from Chapter 1 was accomplished.

Several recommendations were made.

- (1) Sulficarb is the recommended type of tube for passive sampling of isoflurane.
This recommendation is made under the presumption that the analytical system is capable of efficiently desorbing the isoflurane from Sulficarb sorbent.
- (2) The accuracy and precision of both types of tubes improve considerably when the tubes were refrigerated after sampling. Refrigeration is recommended for Sulficarb and required for Carbograph 5TD unless analysis is performed very soon after sampling.
- (3) Future work could include a field evaluation of both Sulficarb and Carbograph 5TD on an exposed population (surgical, anesthesia, and/or veterinary personnel).

6.4 SUMMARY

The methods and techniques investigated in these studies could help provide better characterization of isoflurane exposure and dose in exposed personnel. It is important to note that no one tool or method is the best to use at all times and under all conditions. For example, thermal desorption methods are very convenient and useful and can provide good accuracy and precision. However, peculiarities with the HAPSITE® ER system (non-cooled concentrator, low thermal desorption temperatures, etc.) likely hindered thermal desorption methods from reaching full efficiency. As a result, the hand-held probe displayed superior performance for sampling isoflurane in surrogate exhaled breath. Similarly, the results of this study found that Sulficarb was an inappropriate sorbent for use with the HAPSITE® ER. However, Sulficarb displayed very good performance during passive sampling and when analysis was performed using an Agilent GC/MS. These results emphasize the important role industrial hygienists and other environmental health scientists have in choosing appropriate exposure assessment tools and methods. Environmental health scientists must have a sound understanding of the limitations and weaknesses of their equipment to achieve the most accurate and valid characterization of exposure.

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