Validation of 5 Angstrom Molecular Sieve Thermal Desorption Tubes for Passive Sampling of Nitrous Oxide

by

Graham S. Clark

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[redacted] MAJ Jon Sharp DEPARTMENT OF PREVENTIVE MEDICINE & BIOSTATISTICS Committee Chair	X	—
[redacted] CDR Edward Benchoff DEPARTMENT OF PREVENTIVE MEDICINE & BIOSTATISTICS Thesis Advisor	X	—
[redacted] LTC William Whitaker DEPARTMENT OF PREVENTIVE MEDICINE & BIOSTATISTICS Committee Member	X	—

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APPROVAL FOR THE MASTER OF SCIENCE IN PUBLIC HEALTH THESIS IN THE DEPARTMENT OF PREVENTIVE MEDICINE AND BIOSTATISTICS

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Master of Science in Public Health
May 28, 2019

THESIS AND ABSTRACT APPROVED:

DATE:

[redacted] 5/28/19
MAJ Jon Sharp
DEPARTMENT OF PREVENTIVE MEDICINE & BIOSTATISTICS
Committee Chair

[redacted] 5/28/19
CDR Edward Benchoff
DEPARTMENT OF PREVENTIVE MEDICINE & BIOSTATISTICS
Thesis Advisor

[redacted] 5/28/2019
LTC William Whitaker
DEPARTMENT OF PREVENTIVE MEDICINE & BIOSTATISTICS
Committee Member

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I have been fortunate these past two years. The workload for school has been tremendous, but I have been able to spend time with my family. While I worked on my thesis, many Soldiers, Sailors, and Airmen served in locations around the world, unable to be close to family and friends. I humbly dedicate this work to them, and trust that they come home safely.

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Graham S. Clark

August 6th, 2019

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ABSTRACT

Validation of 5 Angstrom Molecular Sieve Thermal Desorption Tubes for Passive Sampling of Nitrous Oxide

CPT Graham Clark, MSPH, 2019

Thesis directed by: CDR Edward Benchoff, Division Director for Occupational and Environmental Health Sciences, Preventive Medicine and Biostatistics

Background: Nitrous oxide (N_2O) is used in many areas of medicine, and is the most commonly used inhalational anesthetic in dentistry (5). Current sampling options for nitrous oxide are limited and may not provide the sensitivity necessary to adequately characterize exposures, particularly those of short duration. The purpose of this study was to validate the use of stainless steel thermal desorption (TD) tubes containing 5 angstrom (Å) molecular sieve sorbent for passive sampling of N_2O during short exposures (30 minutes) of concentrations of up to 400 ppm.

Methods: The method validation followed protocol set forth by OSHA for the development of industrial hygiene sampling and analytical methods. A dynamic flow exposure chamber coupled with a Miller-Nelson HCS-501 flow, temperature, and humidity control system created nitrous oxide concentrations ranging between 10 - 400 ppm and relative humidity of 80% and 20% (absolute humidity reported per test). Sampling time was 30 minutes. Exposed tubes were analyzed using gas

chromatography/mass spectrometry (GC/MS). Specific tests investigated the detection limit of the overall procedure, the reliable quantitation limit, sampling rate and capacity, accuracy and precision, reverse diffusion, desorption efficiency, low humidity effects, and effects of storage.

Results: Molecular sieve 5Å sorbent has a variable sampling rate similar to other passive samplers, but is averaged to be 1.27 ng ppm-1 min-1 between 50 ppm and 150 ppm for a 30 minute sampling period. The sorbent does not hold nitrous oxide well in storage and experiences a 10% decrease in the mass of nitrous oxide retained on spiked samples after 6 days of refrigerated and 3 days of non-refrigerated storage, respectfully. Also, over 10% of nitrous oxide underwent reverse diffusion after 20 minutes of exposure to uncontaminated air. Molecular sieve 5Å sorbent is very hydrophilic, which is believed to cause a significantly reduced sampling rate when exposed in low humidity and to low concentrations.

Conclusion: 5Å molecular sieve TD tubes may not offer an ideal alternative to direct reading instruments or conventional passive dosimeters. If they are used to sample for nitrous oxide ensure that environmental conditions such as temperature, pressure, and humidity are recorded in order to estimate the effect on sampling rate. Furthermore the tubes should be analyzed as soon as possible after sampling, preferably within 3 days of sampling.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: Introduction

1.1 STATEMENT OF PURPOSE

The purpose of this study was to validate the use of thermal desorption (TD) tubes containing 5 angstrom (Å) molecular sieve sorbent for passive sampling of nitrous oxide (N_2O). The validation method followed protocol set forth by the Occupational Safety and Health Administration (OSHA) for the development of industrial hygiene sampling and analytical methods where applicable. This study validated the sampling and analytical methods for use of 5Å molecular sieve TD tubes with a short sampling time (30 minutes) and over a concentration range of 10 - 400 ppm. This method has the potential to enable industrial hygienists and other environmental health scientists to obtain exposure data for short sampling periods and to identify excursion events (short periods of high exposure) without the use of bulky and expensive direct reading instruments. Validation of this method will enhance nitrous oxide exposure assessment programs where nitrous oxide is utilized as an anesthetic gas during dental procedures.

1.2 BACKGROUND

1.2.1 Introduction to Nitrous Oxide

Nitrous oxide is a colorless gas that has a slightly sweet odor (21). It has many uses, but medically it is used as an inhalable analgesic and anesthetic. Nitrous oxide is used in many areas of medicine; including dentistry and veterinary practices, in the emergency room, and in labor and delivery rooms (26). Its wide use can be attributed to not only its analgesic and anesthetic effects, but also its noninvasive mechanism of delivery and ability to reduce anxiety (28).

While there is no U.S. federal regulatory exposure limit for nitrous oxide promulgated by OSHA, the National Institutes of Occupational Safety and Health (NIOSH) and the American Conference of Governmental Industrial Hygienists (ACGIH) have developed non-regulatory exposure limit recommendations. The exposure limit recommended by NIOSH is 25 parts per million by volume (ppmv) averaged over the time of exposure (42). ACGIH's Threshold Limit Value (TLV) recommends that worker exposure be kept below 50 ppmv for a time-weighted average (TWA) of 8 hours (2) ACGIH has not established a short-term exposure limit (STEL) or ceiling limit for nitrous oxide, and therefore these limits fall under ACGIH's "3/5 Rule." According to ACGIH, the 3/5 rule is "[t]ransient increases in workers' exposure levels may exceed 3 times the value of the TLV-TWA level for no more than 15 minutes at a time,… and under no circumstances should they exceed 5 times the value of the TLV-TWA level." These limits, also called excursion limits, are therefore 150 ppmv for a STEL and 250 ppmv for a ceiling limit. Recommendations for limits on nitrous oxide exposure vary by country, as seen in Figure 1.1.

Country	TWA/ppm	Short-term Peak/ppm
Australia	25	
Austria	100	400 (15 min)
Canada	25 (Ontario)	200 (Alberta)
	50 (Quebec)	
	100 (Alberta)	
Denmark	50	
United Kingdom	100	
Estonia	100	
Finland	100	
Germany	100	200 (15 min)
	50 (local recommendations)	
The Netherlands	80	
Norway	50	
New Zealand	25	
Spain	50 (daily exposure)	
Sweden	100	500 (15 min)
Switzerland	100	200 (15 min)
South Africa	100	
United States of America	25 (NIOSH)	75 (Washington)
	50 (ACIH, California, Washington)	

ACIH = American Conference of Industrial Hygienists; NIOSH = National Institute for Occupational Safety and Health; ppm = parts per million; TWA = time-weighted average.

Data supplied by BOC Ltd., Guildford, United Kingdom.

Figure 1.1 Time-weighted Average and Short-Term Peak Concentrations for Nitrous Oxide in Different Countries (54).

1.2.2 Health Effects of Nitrous Oxide

Nitrous Oxide has been in use for nearly 150 years and was originally used for pain reduction in laboring women(50). As use of nitrous oxide increased, a series of studies in the 1960's, 1970's, and 1980's showed that occupational exposure was associated with serious chronic health effects, such as reproductive health effects (such as reduced fertility), spontaneous abortions, and teratogenic effects (11). These studies also reported an increased risk for liver and kidney disease (37). However, recent advocates, such as those seen in the studies by Sanders et al (2008) and Rooks (2007), have found

many of these studies to be inadequate and call for further research on the biological effects of nitrous oxide.

1.2.2.1 Chronic Health Effects of Nitrous Oxide

1.2.2.1.1 Carcinogenicity & Genotoxicity

According to the ACGIH, nitrous oxide is not classified as a carcinogen (2). Its carcinogen category, A4, designates that the evidence regarding carcinogenicity is inconclusive. Other organizations, such as the US Environmental Protection Agency and the International Agency for Research on Cancer, have not assessed nitrous oxide for carcinogenicity (21). It is well known that nitrous oxide oxidizes cobalamin (vitamin B_{12}), which then interferes with methionine synthase's ability to act as an enzyme in numerous cellular activities, such as DNA and RNA synthesis (54; 66). While this action does effect DNA production, and thus makes nitrous oxide genotoxic, it is not believed to be mutagenic. Further studies on the ramifications of methionine synthase obstruction are required to update nitrous oxide's carcinogenic classification.

1.2.2.1.2 Reproductive Health Effects

Several early epidemiologic studies involving nitrous oxide and other waste anesthetic gases reported an increased risk of spontaneous abortions (11; 59). Some researchers have been critical of these studies, stating that the conclusions reached are unlikely as a result of reporting bias, poor survey response, lack of nitrous oxide exposure data, confounding variables, and inadequate controls (11; 54; 60). However, it is believed that there is an increased risk for spontaneous abortions at high occupational exposure levels (>1000 ppm). Although these levels of exposure do not generally occur in modern

practices if adequate general and local exhaust (scavenging) ventilation systems are available (60).

Teratogenicity has been reported in rats exposed for 24 hours to 75% nitrous oxide, or 750,000 ppm (36). Another study by Vieira et al. (1980) showed that rats exposed continuously for 19 days at 1000 ppm, caused teratogenic effects as demonstrated by measurements such as difference in litter size and fetal crown rump measurements. Vieira et al. also showed there were no congenital abnormalities when exposed to all concentrations below 1,000 ppm (61). These results are similar to the human risk of spontaneous abortion, where it is unlikely that occupational exposures will ever reach those necessary to potentially cause teratogenic effects. However, as always with animal studies, the application of these findings to humans may be inappropriate due to the physiological differences, therefore making it unknown if human fetuses are affected by nitrous oxide exposure.

1.2.2.1.3 Hepatic and Renal Effects

In 1980, Cohen et al. reported relative risks for liver disease and kidney disease of 1.7 and 1.2, respectively, for dentists and chair-side assistants occupationally exposed to inhalational anesthetics (13). This study is widely cited, but subsequent studies have not reported liver or kidney disease as a result of nitrous oxide exposure. Cohen et al. contradicted the results of a 1976 study by Salo & Vapaavuori that showed no hematologic abnormalities after nitrous oxide exposures of up to 860 ppm (53). Another study in 1983 by DeZotti et al. reported that there was no significant difference in liver function between personnel exposed to nitrous oxide and personnel that were not exposed (16). These results are in line with other chronic health effects of exposure to nitrous

oxide, where occupational exposure is unlikely to occur at levels required to achieve negative health outcomes with ventilation and scavenging systems in place.

1.2.2.2 Acute Health Effects of Nitrous Oxide

Healthcare workers exposed to inhalational anesthetics may also experience acute cognitive effects that might lead to decreased job performance. In the mid-1970s, studies were conducted to test for perceptual, cognitive, and motor skill degradation as a result of exposure to nitrous oxide and halothane. In 1975, Bruce & Bach suggested a significant decrease in performance on a divided attention audiovisual task test when human test subjects were exposed to 500 ppm of nitrous oxide for as little as 5 minutes (9). Then, in 1976, Bruce & Bach showed a decrease in performance on audiovisual tasks for four out of seven subjects exposed to a nitrous oxide concentration of 50 ppm for two hours (10). The National Institute's of Health Hazardous Substances Data Base reports that concentrations of 50 ppm can reduce dexterity, cognition and motor and audiovisual skills (21). However, this report does not include an estimate of exposure time at which these effects are likely to occur.

1.3 PUBLIC HEALTH RELEVANCE

1.3.1 Prevalence of Nitrous Oxide Use

Nitrous oxide is reportedly used in many areas of medicine, to include dental clinics, operating rooms, and emergency rooms (26). In dentistry, N_2O is the most commonly used inhalation anesthetic, and 97% of pediatric dentists use nitrous oxide in their clinics (5; 63). Additionally, nitrous oxide is used in veterinary medicine, though its application is less common in recent years (25).

Nitrous oxide is also used for management of labor pain. In the United States, its use in this capacity is extremely limited, with only about 1% of women using nitrous oxide for labor pain management (17). However, it is used extensively in several other countries. In the United Kingdom, approximately 60% of women in labor are given N_2O for pain management (12). It is also given to approximately 50% of laboring women in Australia, Finland, and Canada (4; 49). Recent literature is supporting more use within labor and delivery departments in the United States (12; 14; 49; 54).

1.3.2 Occupational Exposure and Control

Current general ventilation and local exhaust scavenging systems can reduce the occupational exposure of nitrous oxide to levels that are not likely to cause adverse health outcomes (49; 50; 54; 60). While general ventilation, or air provided and exhausted throughout the room or building, has been shown to reduce exposure magnitude it does not typically reduce exposures to acceptable levels when used alone. Therefore, the addition of a scavenging system is often necessary (29). A scavenging system, according to NIOSH, is " simply defined [as] a means to collect and remove excess gases to prevent them from being vented back into the operating room (38)." An example of a scavenging system is displayed in figure 1.2. Currently, active scavenging systems that utilize a vacuum source other than the patient's exhalation have replaced passive scavenging systems (systems that only use the exhalation of the patient to remove nitrous oxide) (47). The use of scavenging systems can be the most important measure in controlling waste anesthetic gases (38). For instance, Rowland et al. (1992; 1995) showed that females occupationally exposed to nitrous oxide in unscavenged rooms had a reduced ability to conceive, but those in scavenged rooms did not experience similar effects (51; 52).

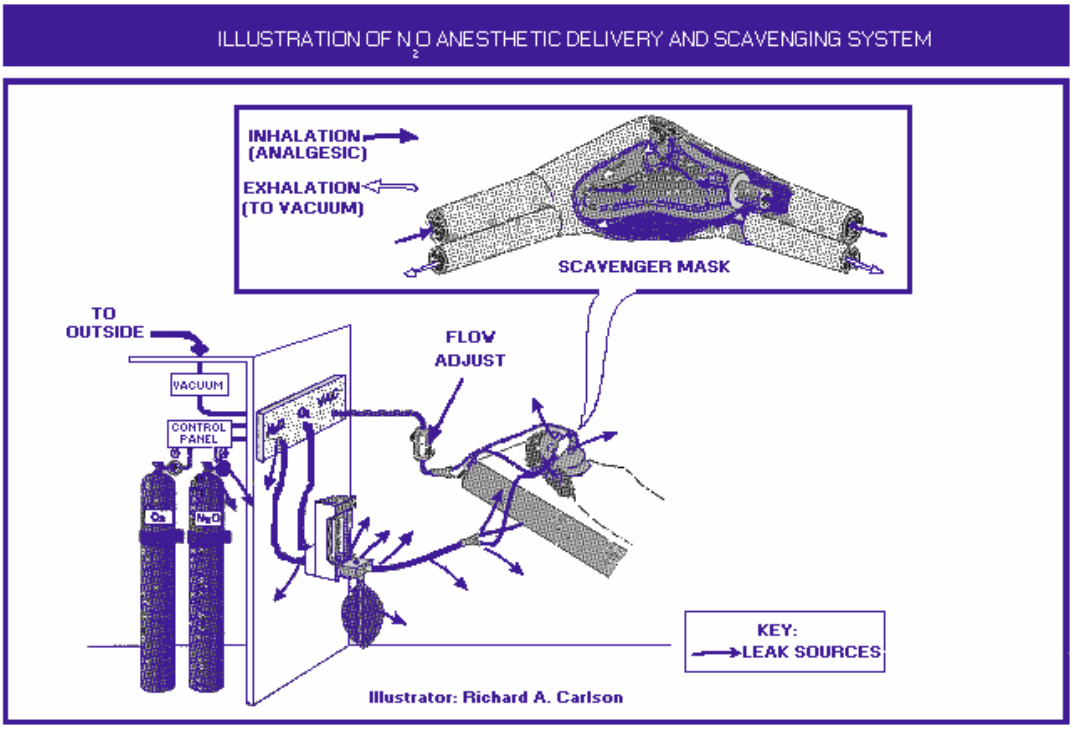

Figure 1.2 Illustration of a nitrous oxide anesthetic delivery and scavenging system with potential leak sources (40).

Nevertheless, ventilation and scavenging systems for nitrous oxide may not be effective due to leaks, poor maintenance, improper use, or lack of training for those administering nitrous oxide. Several studies in the 1990's concluded that scavenging systems were not reducing nitrous oxide levels below the recommended limits (8; 23; 38). However, Borganelli et al. (1993) and Henderson & Matthews (2000) reported that scavenging systems are able to keep nitrous oxide below recommended limits if properly used and maintained (7; 22). Yet, a study in 2017 by Boiano et al. showed that adherence to standard procedures for administering nitrous oxide can be lacking (6), raising questions as to how well these systems are maintained and used to prevent occupational exposures.

More recent studies suggest that exposure to levels of nitrous oxide that exceed exposure limits recommended by ACGIH and NIOSH is common. A British study of

pediatric dental units showed that only 38% of procedures kept nitrous oxide exposures below 100 ppm, which is the 8 hour Time-Weighted Average (TWA) exposure limit in the United Kingdom (19). In 2008, Westberg et al. studying Swedish midwives and midwife assistants showed that 25% were overexposed to nitrous oxide based on the ACGIH 8 hour TWA of 50 ppm (62). Furthermore, even when 8 hour TWA standards are met, it is possible to see short duration excursions of up to 1,000 ppm or higher (20; 22; 62).

1.4 Exposure Assessment Strategies

1.4.1 Current Sampling Methods

Assessments of nitrous oxide exposure published by NIOSH and OSHA utilize direct reading instruments and conventional passive sampling monitors (passive badges). NIOSH has validated two methods, NIOSH 6600 and NIOSH 3800, where air is analyzed using either a portable infrared spectrophotometer, or a Fourier Transform Infrared (FTIR) spectrometer, respectively (39; 41). Currently, OSHA's passive sampling method (Method ID-166) utilizes passive dosimeter badges that are thermally desorbed and analyzed by infrared spectroscopy (46). Ideally, a singular method should be able to accurately quantify nitrous oxide exposure, be easy to use and analyze, be inexpensive, and is able to be utilized under a variety of conditions such as variable sampling times, concentrations, temperatures, humidities and pressures. The following sections will discuss each of these techniques, and their advantages and disadvantages.

1.4.1.1 Direct Reading Instruments

Common direct reading instruments for measuring nitrous oxide use either non-dispersive infrared radiation (NDIR) or FTIR technologies. Both instruments pass

infrared light through a sample to determine how much of the infrared light is absorbed (64). Different compounds absorb infrared light in a unique way, essentially "fingerprinting" the compound (27). Once the IR light has passed through the sample, the remaining IR that was not absorbed by the sample is detected. The result is an IR absorbance spectra that can be used to both identify compounds and quantify them.

NDIR devices utilize a bandpass filter to narrow the IR band to a single, or potentially a few, wavelength(s) (64). Different bandpass filters are used based on the IR spectra produced by certain compounds in order to match the preferred wavelength with the individual compound. This allows the device to measure a particular compound due to the compound absorbing the wavelength. FTIR devices utilize an interferometer, which splits the IR beam and allows for analysis of the IR light at multiple wavelengths simultaneously (45). The interferometer produces an interferogram, which is then Fourier transformed into an IR spectra. Quantification of IR spectra is possible via the Beer-Lambert Law, which states that the concentration is directly proportional to the absorbance (27).

Infrared direct reading instruments provide an effective mechanism for nitrous oxide data collection, including in situations where short sampling periods are necessary. However, most of these direct reading instruments are expensive, require high levels of training, and may require periodic calibration. Furthermore, direct reading instruments must either be present at the sampling site or the technician can take bag samples to be analyzed later. This makes it difficult for organizations to manage comprehensive compliance programs if they have many clinics in a large geographic area, since a technician will either need to bring the instrument to the sampling location or take bag

samples (as seen in NIOSH method 6600). Bag samples are limited by the size of the sample bag and can be cumbersome to transport. It is also difficult to collect bag samples in numerous areas in a single sampling session. Since both NIOSH methods require the use of direct reading instruments, a passive sampling method with TD tubes adopted by NIOSH would provide a viable alternative when direct reading instruments are not available or feasible.

1.4.1.2 Passive Dosimetry

Passive dosimetry is a common method for healthcare workers to monitor their nitrous oxide exposure. Passive dosimetry is based on the use of an adsorbent material (sorbent) capable of capturing and retaining nitrous oxide. The most common adsorbent material used for sampling of nitrous oxide is zeolite molecular sieve 5 angstrom (3; 33; 55; 56). Passive dosimetry utilizes Fick's laws of diffusion, which describe how solutes flow from areas of high concentration to areas of low concentration. The adsorbent material acts as a concentration sink, allowing for continuous, albeit potentially fluctuating, flow of the compound onto the sorbent (15). Once the nitrous oxide has adsorbed onto the sorbent, the sorbent can then be analyzed.

There are a variety of commercial passive monitors available for nitrous oxide. OSHA method 166 describes a specific commercial badge, Landauer (46), which, according to the manufacturer, is no longer available (30). The technique uses 5Å molecular sieve sorbent, which thermally desorbs the nitrous oxide and analyzes the gas with IR spectroscopy. A commercial example that asserts to use this method is Sensors Safety Products' nitrous oxide passive dosimeter (55). Passive badges produced by other manufacturers may use alternate means to extract or analyze the 5Å molecular sieve. For

example, Assay Technology Inc., uses water to extract the nitrous oxide, and then analyzes the headspace of the vial using gas chromatography and an electron capture detector (3). SKC Inc. recommends thermal desorption of their sampler sorbent followed by analysis with gas chromatography and electron capture detector (56).

Conventional passive monitors are single use and often require proprietary analytic techniques. The Landauer badges validated for OSHA Method 166 were proprietary and required analysis by the manufacturer, which limited other laboratories from conducting their own analysis. With appropriate means, an organization responsible for an exposure monitoring program can conduct sampling and analysis on their own, which can be economically beneficial in the long term.

1.4.1.3 Thermal Desorption Tubes

Thermal desorption tubes containing 5Å molecular sieve sorbent have been proven as effective sampling devices for N_2O since the mid-1980's, with analysis performed using a variety of detectors, including electron capture, infrared absorption, and a mass selective detector, but with sampling times all greater than 1 to 2 hours (15; 58; 62). The major difference between thermal desorption tubes and passive badge dosimetry with regard to nitrous oxide sampling are the sampler geometry and the number of times the sorbent can be analyzed. TD tubes used in nitrous oxide sampling are stainless steel tubes 89 mm in length with a 5 mm inner diameter, are pre-packed, and have precisely 15 mm of space between the opening and the edge of the packed sorbent (path length). By comparison, badge samplers generally have a relatively large cross-sectional area about the size of a quarter, a short path length, and are either pre-packed with sorbent or the user is required to load the dosimeter. Analysis of thermal desorption

tubes does not require that the sorbent be removed from the sampler, which allows for multiple reuses of each tube (typically up to 100 times or more depending on the sorbent (65)), before the sorbent must be replenished. Badge-type samplers are typically one-time use products.

Despite studies showing their effectiveness in sampling and advances in techniques for their analysis, validated methods within NIOSH and OSHA remain dependent on expensive and relatively complicated direct reading instruments and conventional passive badges that often require proprietary laboratory analysis. Passive sampling using thermal desorption tubes addresses these shortcomings, as they are relatively inexpensive, can be reused 100 (or more) times, require no pumps, no toxic/flammable solvents, require no sample preparation, and can reach desorption efficiencies of up to virtually 100% (65).

1.4.2 Current Analytical Methods

1.4.2.1 Gas Chromatography (GC)

A gas chromatograph is an analytical technique used to separate various analytes within a sample (57). A gas or volatilized compound is introduced to a column and is swept through it by a carrier gas, or mobile phase. The lining within various columns, or stationary phase, interacts with the various analytes in the samples and determines how quickly each compound moves through the column. The rear portion of the column is connected to a detector, such as a mass selective detector/spectrometer (MSD) that can help the user identify and quantify the compounds within the sample.

Gas chromatography was used to analyze nitrous oxide as early as the late 1960s (31). In 1984, Cox & Brown used GC connected to an electron capture detector (ECD) to

analyze thermal desorption tubes containing nitrous oxide. In 1997, Tschickardt further defined the methodology using GC/ECD for nitrous oxide (58). In 2008, a GC utilizing a mass spectrometer as a detector was used as method to analyze thermal desorption tubes containing nitrous oxide (62). Either detector can be used, but detectors other than IR, ECD, or MS may not be able to detect nitrous oxide due to its low molecular weight and chemical nature.

1.4.2.2 Mass Spectrometry (MS)

A mass spectrometer is a powerful tool that allows for the identification of various compounds. When coupled with a GC, the mass spectrometer produces a mass chromatogram, which identifies each compound based on the combination of how long it takes the compound to travel through the GC column (retention time), and a mass spectrum identification "fingerprint" (57). In order to generate a mass chromatogram, the compound is first subjected to a beam of electrons that ionize and fragment the compounds (Figure 1.3). Then the ionized compounds and fragments collide with a detector plate. A mass to charge (m/z) ratio is recorded for each collision, which is then recorded to calculate the abundance of each m/z (57). The mass chromatogram generated is the instrument response vs. time, and consists of "peaks" that quantify the m/z ratio when integrated.

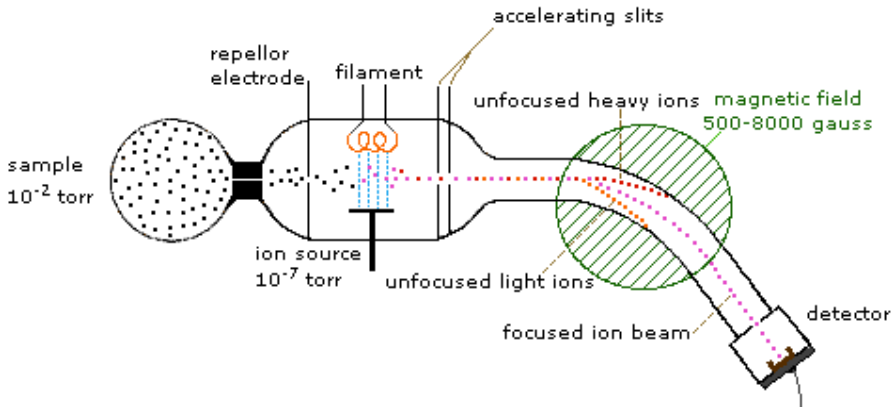

Figure 1.3 Diagram for mass spectrometry (48).

1.4.2.3 Other Detection Methods

An electron capture detector (ECD) is a commonly used tool for quantifying atmospheric nitrous oxide (67) and is also used for occupational exposure programs (3). ECDs utilize β-radiation to create high-energy electrons from high purity carrier gas. These high-energy electrons create a high standing current on a collector electrode. When analyte is introduced to this steady flow, the analyte reacts with the β-radiation and produces low energy electrons. These low energy electrons quantifiably reduce the current on the collector electrode (24). When combined with a GC, an ECD can quantify many compounds, but the appropriate GC column must be utilized if unknown analytes are present in the sample.

1.5 Specific Aims

The purpose of this study is to validate thermal desorption tubes for passive sampling of nitrous oxide. Shortcomings in nitrous oxide exposure assessment techniques have shown the need for an easy-to-use sampling method that is able to characterize

short-term exposures to high concentrations of nitrous oxide. In order to validate TD tubes for this purpose, several specific tests are required, including:

- Determine the analytical precision and accuracy of 5Å molecular sieve stainless steel thermal desorption tubes when nitrous oxide is passively sampled and the sorbent is analyzed using the Agilent 7890B / 5977B gas chromatograph/mass spectrometer.
- Experimentally determine the diffusive uptake rate of nitrous oxide collected on 5Å molecular sieve sorbent. Short sampling time periods (30 minutes) and high concentrations (10 – 400 ppm) will be a particular focus during this validation in order to allow quantification at the maximum exposure peaks and excursions likely to be observed.
- Determine desorption efficiency, reverse diffusion, sampler capacity, and the effects of humidity and storage.
- Develop a protocol to utilize the Gasmet DX4040 FTIR as a tool to validate dynamic flow chamber nitrous oxide concentrations.

CHAPTER 2: Methods

2.1 VALIDATION SCHEME

The validation of thermal desorption tubes containing 5Å molecular sieve sorbent followed the protocol set forth by the Occupational Safety and Health Administration (OSHA) for the development of industrial hygiene sampling and analytical methods (see Figure 2.1) (18). The protocol sets forth the requirements for review, devising and validating the analytical portion and sampling portion of the sampling method. A variety of tests are required to ensure completeness of the method as seen in Figure 2.1. While the guidelines do have specific standards, the needs of each validation is different, and therefore any deviation from the protocol or any solutions devised as a result of ambiguity are presented in detail.

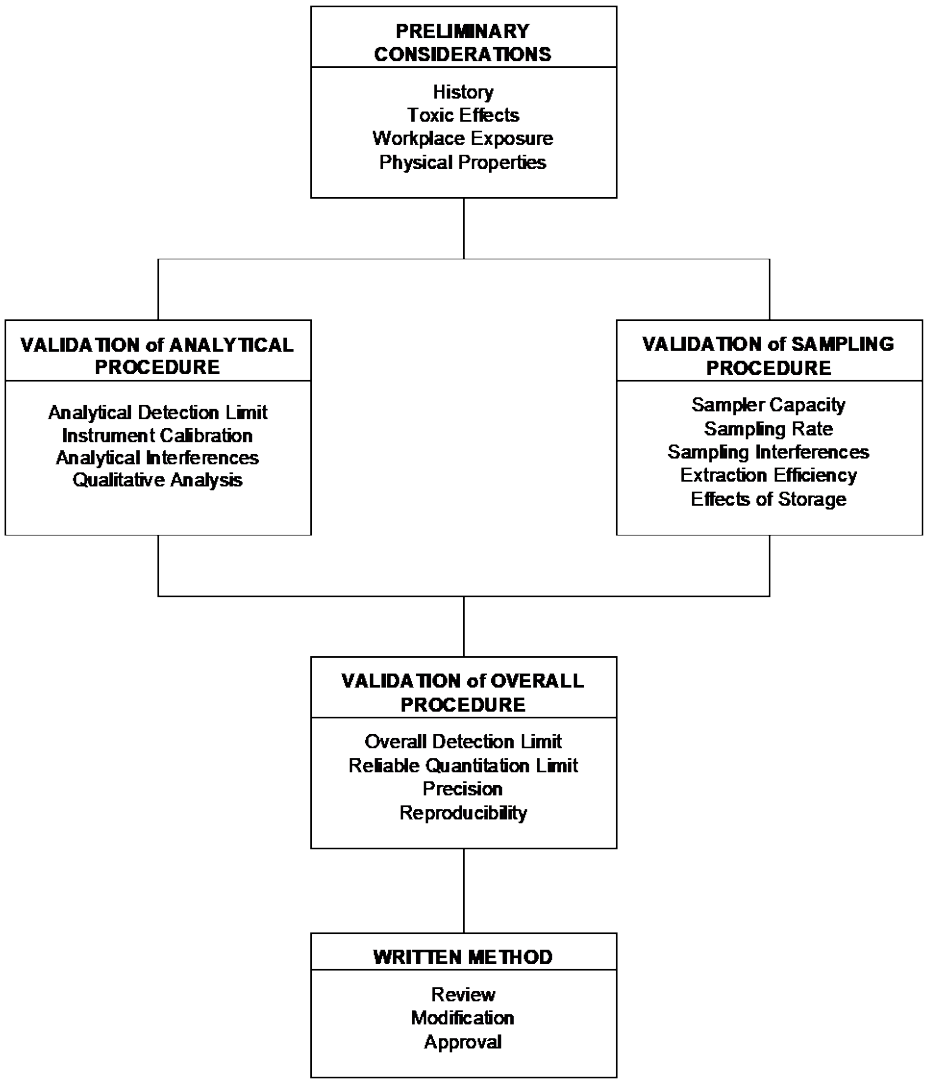

Figure 2.1 Guideline flow chart as provided by Validation of OSHA methods utilizing chromatographic analysis (18).

Thermal desorption tubes used in this study were 6 mm Outer Diameter (OD), 5mm Inner Diameter (ID) stainless steel tubes 89 mm in length purchased from Markes International (Llantrisant, UK). The adsorptive material contained within the tubes was 5Å molecular sieve, which has been shown to be effective at capturing nitrous oxide (15). Thermal desorption tubes were conditioned according to the manufacturer's recommendations prior to each use using a TC-20 thermal desorption tube conditioner

(Markes International, Llantristant, UK) in order to remove compounds from previous exposures or artifacts generated by the sorbent itself. Analysis of the thermal desorption tubes was performed using a 7890B / 5977B Gas Chromatagraph/Mass Spectrometer (Agilent Technologies, Santa Clara, CA), coupled with a Unity 2 / series 2 ULTRA thermal desorption unit / multi-tube autosampler (Markes International, Llantrisant, UK).

Spiked samples were used to test for storage effects and desorption efficiency. For all other tests, tubes were exposed using a dynamic flow exposure chamber coupled with an HCS-501 flow, temperature, and humidity control system (Miller-Nelson Instruments, Livermore, CA). The HCS-501 was used to generate concentrations of nitrous oxide in the dynamic flow chamber that ranged from 1 to 400 ppm.

For the range of concentrations, the lower concentration limit was selected based on an analytical method utilizing Electron Capture Detector (ECD), which found a limit of detection of 1 mL/m^3 for active sampling (58). Furthermore, the NIOSH and ACGIH limits of 25 ppm TWA and 50 ppm TLV (8 hour TWA) are included with a lower limit of 1 ppm. The upper concentration limit was selected based on a desire to validate beyond 500 ppm, which was reported by Hansen et al. (2017) as causing saturation of conventional passive dosimeter badges (20). However, the upper limit could not be achieved and was subsequently reduced to 400 ppm based on instrumentation limitations.

Validation of all dynamic flow chamber concentrations was performed with a Gasmet DX4040 portable gas analyzer (Gasmet, Vantaa, Finland) that has been user-calibrated specifically to the concentration range of nitrous oxide described above. Relative humidity (RH%) and temperature of the dynamic flow chamber atmosphere was constantly monitored using a TSI Velocicalc multi-function ventilation meter 9565-P

(TSI, Shoreview, MN). Absolute Humidity (AH) was recorded for each test due to variation in pressure and temperature within the laboratory.

2.2 CREATING STATIC ATMOSPHERES

Static atmospheres are known concentrations within a known volume that are fixed, and are used within this study to spike samples. Static atmospheres were created using Tedlar bags, Hamilton Gastight syringes, and 99.9% nitrous oxide from Roberts Oxygen Inc (Rockville, MD). In order to achieve atmospheres at various concentrations, dilutions were used to reduce the concentration from a 999,000 ppm stock bag taken from the nitrous oxide cylinder. The calculation used for dilutions was:

Equation 1: Dilution

$$C_1V_1 = C_2V_2 \qquad (1)$$

Where:

$C_1 = \text{Initial Concentration (ppm)}$
$V_1 = \text{Initial Volume (L)}$
$C_2 = \text{Final Concentration (ppm)}$
$V_2 = \text{Final Volume (L)}$

2.3 CALIBRATION OF THE AGILENT 7890B / 5977B GC/MS

A 7890B / 5977B GC/MS (Agilent Technologies, Santa Clara, CA), coupled with a Unity 2 / series 2 ULTRA thermal desorption unit / multi-tube autosampler (Markes International, Llantrisant, UK) were used to analyze the samples. The 7890B GC used a PoraPlot Q analytical column (25 m length, 0.25 mm inner diameter, 8 μm film) (Agilent Technologies, Santa Clara, CA) to separate nitrous oxide from carbon dioxide (CO_2) and other potential analytical interferences (1). The Markes Unity 2 thermal desorption unit required a U-T16GHG-2S Greenhouse Gases cold trap to ensure efficient

capture/concentration of the desorbed nitrous oxide prior to its introduction into the analytical column (35).

Since the analyte of interest was known, a Selected Ion Monitoring (SIM) method was used to maximize analytical sensitivity and accurate quantification of nitrous oxide. SIM methods isolate one m/z value in order to remove potential interferences by other ions. Nitrous oxide and carbon dioxide both have a molecular weight of about 44 grams/mole, which precluded the molecular ion m/z = 44 from being used to accurately quantify nitrous oxide. A review of the respective mass spectra for nitrous oxide (Figure 2.2) and carbon dioxide (Figure 2.3) showed that m/z = 30 is present in the nitrous oxide mass spectrum, but is not present in the carbon dioxide mass spectrum (43; 44). As a result, m/z = 30 was used as the target ion in the SIM method.

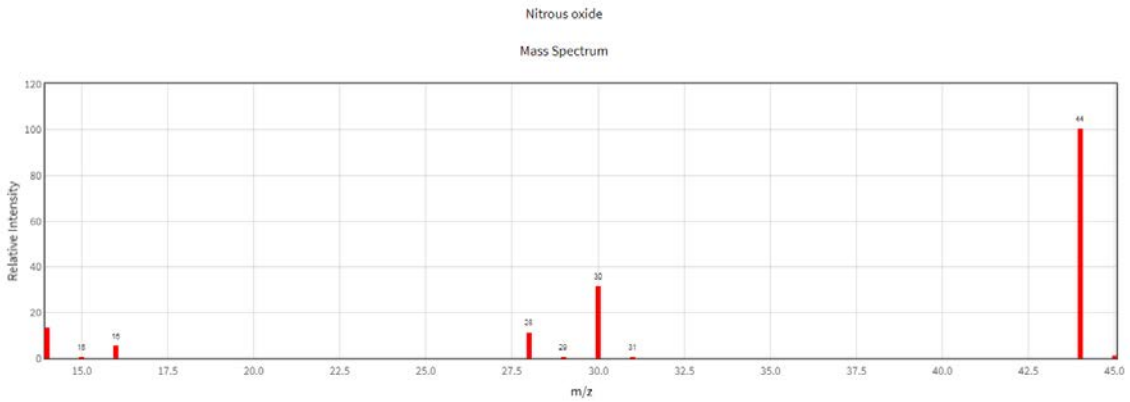

Figure 2.2 Nitrous oxide mass spectrum (44).

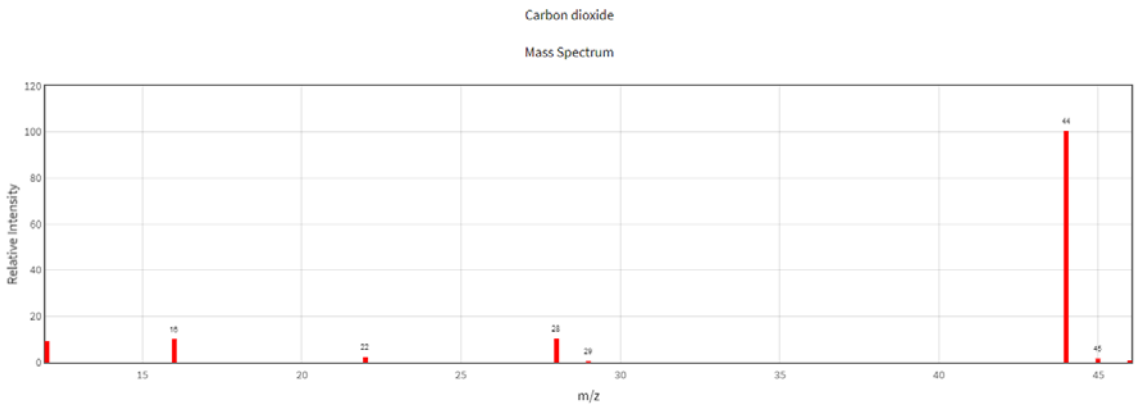

Figure 2.3 Carbon dioxide mass spectrum (43).

A total of 9 points were utilized to create a calibration curve for the GC/MS. The maximum and minimum concentrations of the curve were designed to be approximately 10% higher and lower, respectively, than the values targeted as necessary for the study. This method was utilized to provide higher confidence in any concentrations near the limits of the curve.

In order to estimate the masses required for the calibration curve, the following equation for Fick's first law of diffusion from Markes Application Note 025 (32) was used:

Equation 2: Fick's law

$$M = C * UR * t \quad (2)$$

Where:

$M =$ Mass (ng)
$C =$ Concentration (ppm)
$UR =$ Uptake Rate (ng $\text{ppm}^{-1}\text{min}^{-1}$)
$t =$ Time (min)

Mass was estimated using utilizing an uptake rate of 1.25 $ng\ ppm^{-1}min^{-1}$, as described in Markes International Application note 001 (34). The concentration was the target concentration (1-425 ppm) and the time was for a 30-minute exposure.

Once the estimated mass collected over a 30 minute sampling time was calculated, then the 5Å molecular sieve sorbent thermal desorption tubes were spiked with known masses of nitrous oxide using a Model LP-1200 Hand Pump (Rae Systems, Sunnyvale, CA) as seen in Figure 2.4. This was performed by drawing 100 mL from 3L SKC Tedlar® bags (SKC Inc, Eighty Four, PA) containing a known concentration of nitrous oxide. Static atmospheres in the Tedlar® bags were created with the method described in section 2.2. The concentrations required were calculated using equation 7. Equation 7 is determined from equations 3 through 6. The first step is converting ppm to ng/mL, followed by multiplying the result by the volume drawn with the RAE Systems hand pump as seen below:

Equation 3: ppm to ng/mL conversion

$$\frac{ng}{mL} = \frac{PPM * MW}{Molar\ Volume} \tag{3}$$

Where:

$PPM =$ target concentration (ppm) for cal curve point
$MW =$ Molecular Weight of nitrous oxide (g)

Equation 4: Calculating molar volume

$$Molar\ Volume = \frac{(R)(T_{lab}+273)}{(760/P_{lab})}\ (\frac{L}{mol}) \tag{4}$$

Where:

$T_{lab} =$ laboratory Temperature (°C)
$P_{lab} =$ laboratory Pressure (mmHg)
$R =$ Ideal gas constant $= 0.0821\ \frac{\mathrm{L} * \mathrm{atm}}{\mathrm{mol} * \mathrm{K}}$

Then:

Equation 5: Calculating mass loaded

$$Mass\ Loaded = \left(\frac{ng}{mL}\right) * V \quad (5)$$

Where:

$V =$ Volume drawn by the RAE Systems hand pump (mL)

Equation 6: Combining equations 3 and 5

$$Mass\ Loaded = \frac{C*MW*V}{Molar\ Volume} \quad (6)$$

Equation 7: Rearranging equation 6 to solve for the concentrations of the bags:

$$C = \frac{Mass\ Loaded*Molar\ Volume}{MW*V} \quad (7)$$

Figure 2.4 Draw setup from known concentration with Rae Systems Pump.

Starting on the left, a static atmosphere in a Tedlar bag is connected to a thermal desorption tube. The tube is then connected to the Rae Systems Pump. The box elevates the pump to prevent undesired pressure on the bag from the pump.

Before analyzing the loaded tubes for the calibration curve, the generation of the appropriate split ratio was completed using the Markes Unity 2 software. A split ratio reduces the amount of analyte that ultimately enters the GC/MS. It is important to have a split ratio that removes enough analyte to prevent instrument saturation. However, if the split ratio is too high, then too little analyte will enter the detector, potentially resulting in censored data (masses below the Limit of Quantification). The Markes Unity 2 has an inlet split before the cold trap, and an outlet split after the cold trap. Using the lowest mass outcome from equation 2, which came from a target concentration of 1 ppm, several tubes were analyzed at various split ratios to determine the optimal split flow. The split flow rates selected for this experiment were 100 mL/min at the inlet split and 100 mL/min at the outlet split. Split ratios were calculated to be 7.7:1 at the inlet split, 101:1 at the outlet split, and a total split ratio of 774.3:1.

Conditions of the lab including temperature and pressure were documented at the time of the static atmosphere creation. These conditions were taken into account when calculating the molar volume used to calculate loaded mass.

Each point was analyzed in triplicate to determine precision. Linearity (R^2) above 0.99 was required for the calibration curve to be deemed "acceptable". Calibration curve accuracy (% error) was determined by comparing the theoretical nitrous oxide mass with the predicted nitrous oxide mass estimated by applying the instrument response to the calibration curve trendline.

Equation 8: % error

$$\%Error = \left|\frac{Theoretical\ Value - Observed\ Value}{Theoretical\ Value}\right| \quad (8)$$

2.3.1 Desorption and Cold Trap Parameters

The Markes Unity 2 desorption parameters are based on the recommendations by the manufacturer (33). Prior to desorption, the TD tubes were purged with 99.999% pure helium carrier gas (Airgas, Radnor, PA). For desorption, the tubes were oriented in the Markes ULTRA autosampler in the direction of air flow.

The following table lists the various parameters used in the desorption method:

Purge time	6 minutes at 20 mL/min
Desorption temperature	170 °C for 6 minutes
Cold trap capture temperature	-25 °C
Cold trap flash heat temperature	300 °C

Table 2.1 Markes Unity 2 desorption and cold trap parameters.

2.3.2 GC/MS Parameters

The following table lists the various parameters used in the analytical method:

Column Flow	1 mL/min
Column Pressure	8 psi
Initial Oven Temperature	40 °C for 3 min
Ramp Temperature	20 °C/min until 180 °C
Final Temperature	180 °C for 10 min
MS Source	230 °C
MS Quad	150 °C
Acquisition Type	SIM for m/z = 30

Table 2.2 GC/MS Analytical Parameters

2.4 Storage Stability

A storage stability test was performed to investigate the stability of nitrous oxide adsorbed on the 5Å molecular sieve sorbent during storage at refrigerated and typical room temperatures (2°C and 23°C, respectively). For each test, all TD tubes were loaded from the same bag on the same day. Three tubes were immediately analyzed and averaged to determine the mean recovery prior to storage. Subsequent analyses were performed in triplicate every 3 days for a total of 28 days.

2.5 Desorption Efficiency

Eighteen TD tubes were loaded with the same mass of nitrous oxide. Each tube was then analyzed twice, consecutively. Nitrous oxide found on the TD tube during the second desorption was considered to be nitrous oxide that failed to be desorbed during the first desorption. Assuming that the nitrous oxide was completely desorbed after the second desorption, the two values equal the sum of nitrous oxide on the tube. Dividing each initial nitrous oxide value with the secondary nitrous oxide value provides the amount (%) left on the tube after the first desorption. The average percent is then calculated for all 18 tubes, resulting in an overall desorption efficiency for the analytical method.

2.6 The Dynamic Flow Chamber

The dynamic flow chamber was comprised of a sealed rigid container that was connected to a diluent air feed (compressed house air) and an air exhaust. The chamber was used for the creation of dynamic atmospheres, which allowed for TD tubes to be exposed at a constant concentration over time. The feed system consists of a diluent air inlet and an HCS-501-200 Flow, Temperature, and Humidity control system (Miller-

Nelson Instruments, Livermore, CA). The tubing material connecting the HCS-501 to the dynamic flow chamber was Teflon tubing material.

The HCS-501-200 module controls the flow of air into the train between 20 LPM to 200 LPM and controls humidity with a pressurized water vessel using deionized water. RH% inside the dynamic flow chamber was constantly monitored. The RH% used for validation tests was 80%. The RH% used for the low humidity test was 20%.

After the diluent air was set to the appropriate flow, temperature, and humidity, it flowed through a tube into the dynamic flow chamber. In the connection tube between the HCS-501 and the dynamic flow chamber, there was a direct connection to a source of 99.9% pure nitrous oxide tank (Airgas, Radnor, PA).

There were three instruments used for validation of parameters within the dynamic flow chamber. The first was an Aalborg GFM57 mass flow meter (Aalborg, Orangeburg, NY) that verified the diluent air flow into the dynamic flow chamber. It was factory calibrated before any experiments took place. The second was a TSI Velocicalc multi-function ventilation meter 9565-P (TSI, Shoreview, MN) that verified temperature and pressure. The third was a Gasmet DX4040 portable gas analyzer (Gasmet, Vantaa, Finland) that verified the concentration of nitrous oxide within the dynamic flow chamber prior to and for the duration of each validation test.

2.6.1 Calibration of the Gasmet DX4040

The Gasmet DX4040 was calibrated using a closed loop system. The inlet and outlet were both connected to Nalgene tubing that created the closed loop system. The closed loop system consisted of the DX4040 sample cell with a volume of 0.454 L provided by the manufacturer, and the Nalgene tubing with a volume of 0.01 L.

The calibration curve had 11 points corresponding to the nitrous oxide concentrations generated in the dynamic flow chamber. Each point was generated by injecting the appropriate volume of 99.9% pure nitrous oxide into the closed loop system. The specific volume of nitrous oxide injected was calculated using equation 1. Following the injection of the known amount of nitrous oxide, the output (instrument response) of the DX4040 for each nitrous oxide concentration was recorded. The loop system was purged until the DX4040 stabilized at the background nitrous oxide concentration after the instrument response at each calibration point was recorded.

The resulting pressure in the Gasmet sample cell was investigated to ensure that the injections of nitrous oxide into the closed loop system did not result in a significant rise in sample cell pressure (which could affect the accuracy of the readings). Pressure change was calculated using Avagadro's Law and the molecular volume of air at the lab temperature. Since the container (the loop system within the Gasmet) is rigid, the equation is:

Equation 9: Avagadro's law

$$\frac{P_1}{n_1} = \frac{P_2}{n_2} \tag{9}$$

Where:

$P_1 = \text{initial Pressure of container (mmHg)}$
$P_2 = \text{Pressure of container including injection (mmHg)}$
$n_1 = \text{initial moles of air in container (moles)}$
$n_2 = \text{moles of air in container including injection (moles)}$

Once all points of the calibration curve had been generated, accuracy was determined using percent error comparing the theoretical nitrous oxide concentration with the nitrous oxide concentration determined with the calibration curve.

2.6.2 Sampling Rate and Capacity

Sextuplicate samples were exposed in the dynamic flow chamber to concentrations of nitrous oxide ranging from 10 to 400 ppm for 30 minutes. As per guidance in the OSHA validation protocol, the temperature of the chamber was set at 22 °C and the RH% was 80% (AH 15.7 g/m^3). A sampling rate was calculated at each concentration for 30 minutes using the following equation from OSHA's validation protocol:

Equation 10: Sampling rate calculation

$$R_{SS} = \frac{1000M}{CtE_E} \quad (10)$$

Where:

$R_{SS} = \text{sampling rate at the sampling site } (\frac{\text{mL}}{\text{minute}})$
$M = \text{Mass collected (µg)}$
$C = \text{Concentration of the test atmosphere } \left(\frac{\text{µg}}{\text{L}}\right)$
$t = \text{sampling time (minutes)}$
$E_E = \text{Extraction Efficiency}$
$1000 = \text{unit conversion from L to mL}$

The result of this formula provides an uptake rate in mL min^{-1}. Molar volume and molar mass were used to convert the sampling rate to ng ppm^{-1} min^{-1}. The following equation was used, slightly modified for ease of use from Markes International Application Note 025 (32):

Equation 11: Converting mL/min to ng ppm^{-1} min^{-1}

$$UR = \frac{MM}{MV} * R_{SS} \quad (11)$$

Where:

$UR = \text{Uptake Rate (ng ppm}^{-1}\text{ min}^{-1}\text{)}$
$MM = \text{Molar Mass (g mol}^{-1}\text{)} = 44.01$
$MV = \text{Molar Volume (L mol}^{-1}\text{) (see eq. 4)}$
$R_{SS} = \text{Sampling Rate at the sampling site (mL min}^{-1}\text{)}$

In order to define sampler capacity, a 10% change in the preliminary sampling rate (PSR) must be seen. OSHA defines a preliminary sampling rate as the average sampling rate for samples exposed at twice the desired concentration for 0.5, 1, and 2 hours (18). Since this experiment is at various concentrations for one time interval (30 minutes), the PSR will be defined from the sampling rates at 50, 100, and 150 ppm.

2.6.3 Sampling Interferences

Sampling interferences refer to conditions or processes that have a potential effect on the sampling rate and capacity of the sorbent and any other functional aspect of the sorbent such as its ability to retain nitrous oxide. The two tests for sampling interferences completed in this study are for reverse diffusion and the effects of low relative humidity on the sampler. Reverse diffusion refers to the analyte unbinding from the sorbent before analytical desorption.

2.6.3.1 Reverse Diffusion

Six TD tubes were initially placed into the dynamic flow chamber set to a 150 ppm atmosphere at 22 °C and a relative humidity of 80% (Test 1 AH 15.7 g/m^3, Test 2 & 3 AH 17.6 g/m^3). After 10 minutes, 3 tubes were removed and capped, and the chamber was flushed with laboratory air. The 3 remaining TD tubes continued sampling in uncontaminated air for 20 minutes and were capped when complete. All 6 TD tubes were then analyzed. This test was completed three times.

2.6.3.2 Low Humidity

Six TD tubes were placed into the dynamic flow chamber set to a 200 ppm atmosphere at 22 °C and a humidity of 20% for 30 minutes (AH 3.9 g/m^3). All 6 TD tubes were then analyzed.

2.7 Overall Detection Limit and Reliable Quantitation Limit

Overall detection limit or detection limit of the overall procedure (DLOP) was calculated using the guidance in OSHA's validation protocol. The equations to calculate DLOP use information from the GC/MS calibration curve and are:

Equation 12: Detection Limit

$$L_D = \frac{3 * S_{YX(DLOP)}}{A} \tag{12}$$

Where:

$L_D =$ Detection Limit of the overall procedure
$S_{YX(DLOP)} =$ standard error of estimate
$A =$ analytical sensitivity (slope)

And:

Equation 13: Standard error of estimate

$$S_{YX(DLOP)} = \sqrt{\frac{\Sigma(Y_{OBS} - Y_{EST})^2}{n-k}} \tag{13}$$

Where:

$Y_{OBS} =$ observed response
$Y_{EST} =$ estimated response
$n =$ total number of data points
$k = 3$ (for polynomial regression)

Reliable Quantitation Limit (RQL) was also calculated using OSHA's validation protocol.

Equation 14: Reliable quantitation limit

$$L_{RQL} = 10\frac{S_{YX(DLOP)}}{A} \quad (14)$$

Where:

L_{RQL} = Reliable Quantitation Limit

2.8 STATISTICAL METHODS

Performance of the TD tubes was conveyed using descriptive statistics through Microsoft Excel. Accuracy and sampling uptake rate was described using arithmetic means. Precision was described using standard deviation, mean variation, and coefficient of variation. Linearity of response was determined using coefficient of determination (R^2).

Chapter 3: Results

3.1 Calibration of the Agilent 7890B / 5977B GC/MS

Four calibration curves were generated from the data. The first two curves were fit based on linear and polynomial models. The remaining curves were linear and polynomial models of logarithmically transformed data. The calibration curve with the lowest overall percent error (error between the known mass and the mass predicted by the regression equation) was the model generated by the logarithmic transformation of the data and a polynomial trendline as seen in Figure 3.1 and Table 3.1. The overall percent error of the trendline was 3.24%.

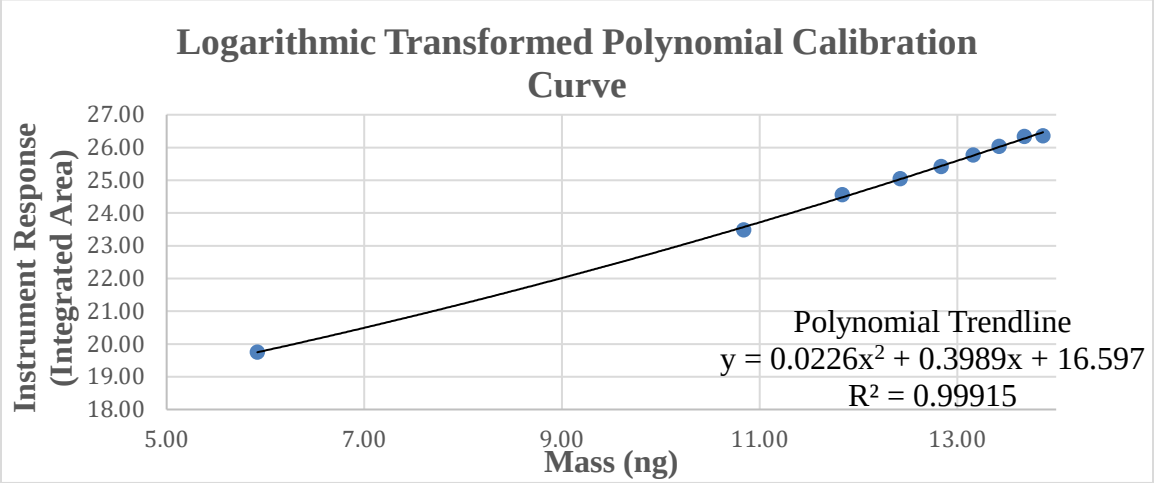

Figure 3.1 Calibration curve for the Agilent 7890B/5977B GCMS

Theoretical Mass (ng)	Observed Mass (ng)	Percent Error
60.	60.	0.68%
1,830	1,713	6.37%
3,660	3,879	6.03%
5,490	5,559	1.29%
7,320	7,247	0.97%
9,150	9,243	1.05%
11,000	11,116	1.27%
13,100	13,721	4.63%
14,900	13,915	6.88%
	Average Percent Error	3.24%

Table 3.1 Percent error calculations for the Agilent 7890B/5977B GCMS

3.2 STORAGE TEST

The room temperature and refrigerated storage tests were not begun on the same day due to time constraints. Following day 1, tubes from both experiments were analyzed on the same day. The data files for Days 10 and 9 of the room temperature test and refrigerated test, respectively, were lost.

Both storage temperatures saw a decrease in nitrous oxide desorbed to below 90% of the original loaded amount within the first week of storage (Table 3.2). A decrease of over 10% in nitrous oxide mass was observed after 3 days of storage at room temperature (Figure 3.2). Tubes that were refrigerated saw a slight increase in nitrous oxide desorbed (13% difference between day 4 room temperature and day 3 refrigerated), but fell below 90% after 6 days of storage (Figure 3.3 & Table 3.3). After 27 & 28 days, both tests had 55% nitrous oxide remaining.

Room Temperature (22 °C) Storage Test		
Day	Observed Avg Mass (ng)	% Recovery
1	7,982	100%
4	6,831	85%
7	6,287	78%
10	Data file lost	
13	5,576	69%
16	5,606	70%
19	5,283	66%
22	4,961	62%
25	4,536	56%
28	4,391	55%

Table 3.2 Room temperature storage test recovered mass values

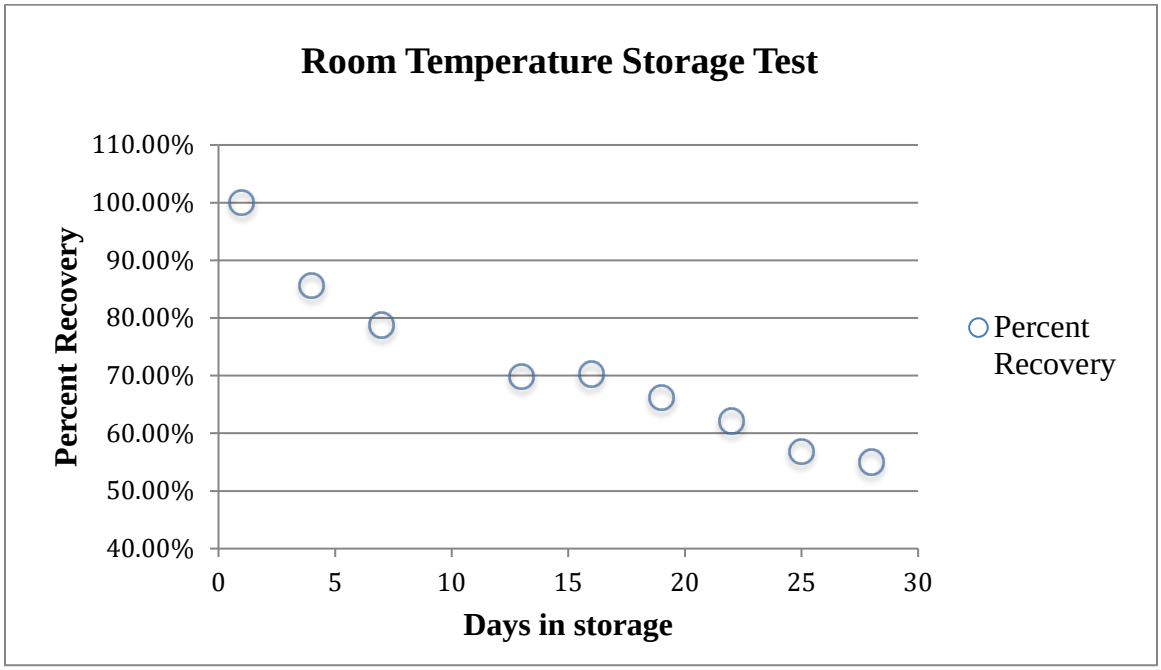

Figure 3.2 Plot of room temperature storage test percent recovery vs number of days in storage.

Refrigerated Storage Test		
Day	Observed Avg Mass (ng)	% Recovery
1	7,475	100%
3	7,343	98%
6	6,207	83%
9	Data file lost	
12	6,133	82%
15	6,011	80%
18	5,214	69%
21	5,084	68%
24	4,778	63%
27	4,111	54%

Table 3.3 Refrigerated storage test recovered mass values

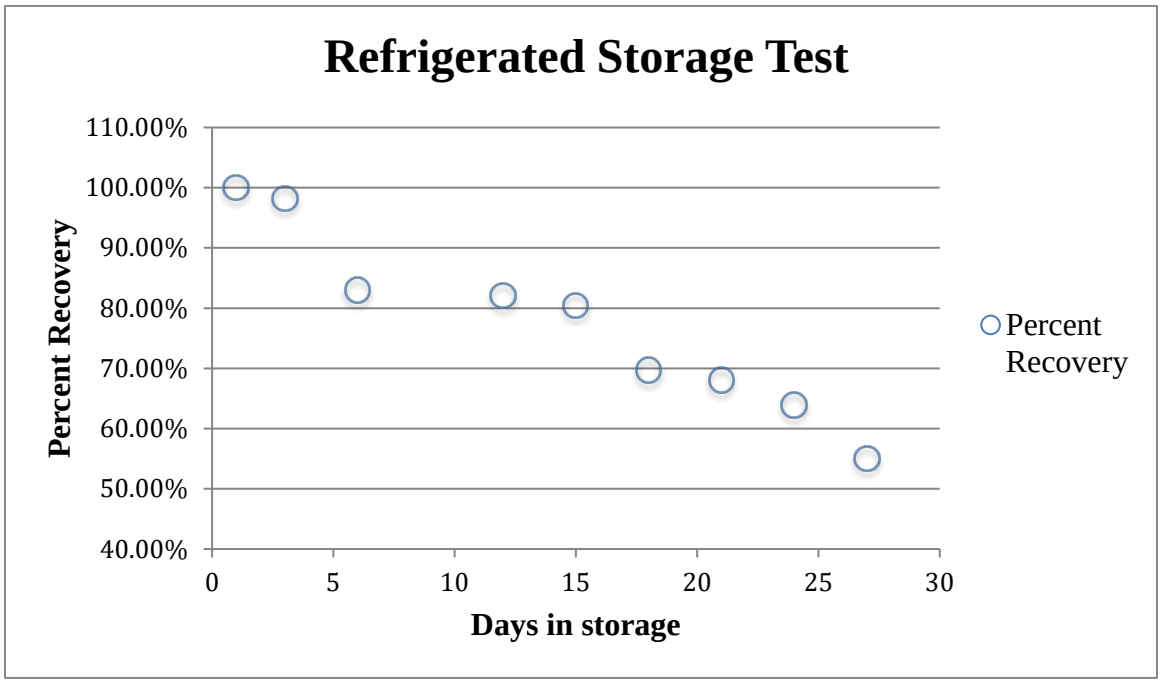

Figure 3.3 Plot of refrigerated storage test percent recovery vs. number of days in storage.

3.3 DESORPTION EFFICIENCY

The desorption efficiency test was completed twice, once at a low loaded mass and once at a high loaded mass within the analytical calibration curve, to test if the desorption efficiency may be dependent on mass adsorbed. In both tests, the secondary desorption identified no remaining nitrous oxide on the sorbent (Table 3.4). The coefficient of variation of the desorption efficiency tests were 14.4 and 10.4.

Desorption Efficiency Test			
Low Concentration		High Concentration	
Primary Desorption Mass Recovered (ng)	Secondary Desorption Mass Recovered (ng)	Primary Desorption Mass Recovered (ng)	Secondary Desorption Mass Recovered (ng)
82	<1.6	9,911	<1.6
126	<1.6	10,801	<1.6
88	<1.6	10,915	<1.6
80	<1.6	9,403	<1.6
85	<1.6	11,852	<1.6
85	<1.6	10,856	<1.6
84	<1.6	9,606	<1.6
84	<1.6	10,058	<1.6
75	<1.6	8,856	<1.6
77	<1.6	9,777	<1.6
80	<1.6	9,881	<1.6
70	<1.6	8,959	<1.6
77	<1.6	8,897	<1.6
72	<1.6	11,262	<1.6
75	<1.6	8,731	<1.6
71	<1.6	10,931	<1.6
82	<1.6	8,046	<1.6
77	<1.6	8,753	<1.6

Table 3.4 Desorption efficiency test results for low and high loaded masses.

3.4 GASMET DX4040 CALIBRATION

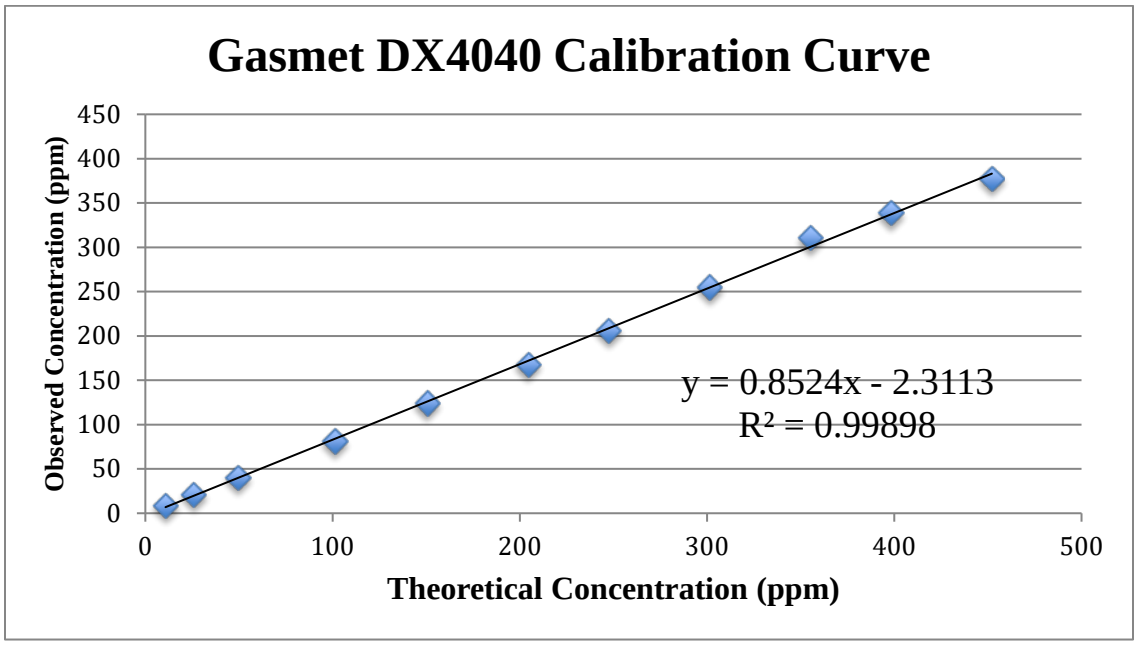

Figure 3.5 Calibration curve for the Gasmet DX4040.

The Gasmet DX4040 has a factory calibration of 1 – 100 ppm for nitrous oxide . Beyond the factory calibration of 100 ppm, the Gasmet DX4040 showed a linear response of $R^2 > 0.99$ up to 450 ppm of nitrous oxide (Figure 3.5).

The pressure increase in the rigid sample cell resulting during calibration was estimated. Table 3.5 shows the added pressures for each point along the calibration curve. At most, the additional pressure added to the cell was less than 0.4 mmHg, which was deemed negligible. While the significant figures within table are not correct, they are provided to clarify the minimal additional pressure.

Pressure Calculations for DX4040 Sample Cell			
Desired Concentration (ppm)	Injection Volume (μL)	Post-Injection Pressure (mmHg)	Pressure Change (mmHg)
10	4.6	752.008	0.008
25	11.6	752.019	0.019
50	23.2	752.038	0.038
100	46.4	752.075	0.075
150	69.7	752.113	0.113
200	92.9	752.151	0.151
250	116.1	752.188	0.188
300	139.3	752.226	0.226
350	162.6	752.263	0.263
400	185.8	752.301	0.301
450	209.0	752.339	0.339

Table 3.5 Theoretical changes in pressure within the DX4040 sample cell with various injection volumes.

3.5 SAMPLING RATE & CAPACITY

Table 3.6 shows that the average sampling rate for 5 angstrom molecular sieve TD tubes exposed to nitrous oxide concentrations between 50-150 ppm was observed to be 1.27 ng ppm^{-1} min^{-1}. The average sampling rate observed when exposed between 10-400 ppm was 1.08 ng ppm^{-1} min^{-1}. When exposed to concentrations less than 50 ppm or greater than 200 ppm, the sampling rate of the tube did not remain within 10% of the preliminary sampling rate (PSR) (Figure 3.6). The PSR was determined using the sampling rates at 50 ppm, 100 ppm, and 150 ppm for 30 minute exposures. The sampling rate at low concentration (10 ppm) was considerably less than any other sampling rate determined between 50 – 400 ppm for 30-minute exposures.

Sampling rates at various concentrations when 5 angstrom molecular sieve is exposed to nitrous oxide for 30 minutes			
Concentration (ppm)	Sampling Rate (ng ppm^{-1} min^{-1})	Preliminary Sampling Rate (PSR) Average (50 ppm - 150 ppm) (ng/(ppm*min))	1.270
9.69	0.78		
49.40	1.40		
97.60	1.23		
149.25	1.19	10% above PSR	1.397
202.38	1.15		
253.33	1.11		
295.12	1.07	10% below PSR	1.143
352.42	0.96		
399.47	0.84		

Table 3.6 Sampling rates at various concentrations when 5 angstrom molecular sieve thermal desorption tubes are exposed to nitrous oxide for 30 minutes.

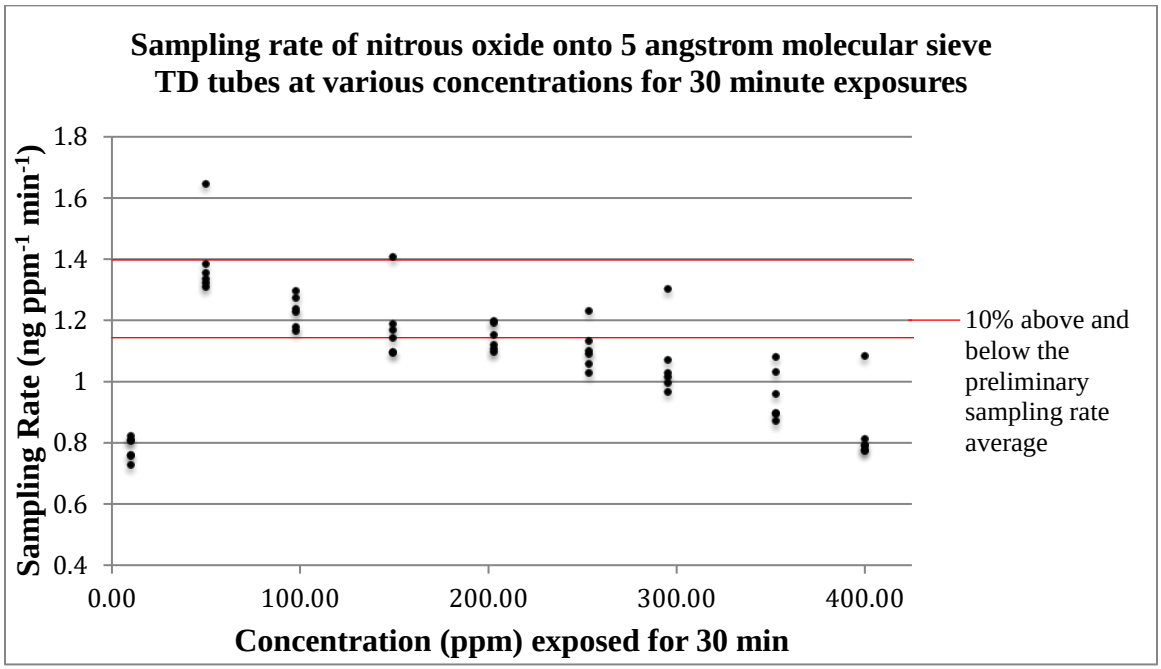

Figure 3.6 Sampling rates at various concentrations when 5 angstrom molecular sieve thermal desorption tubes are exposed to nitrous oxide for 30 minutes.

3.6 REVERSE DIFFUSION

Only one of three reverse diffusion tests showed that the sorbent effectively retained nitrous oxide after nitrous oxide had been removed from the air. The average recovery was 85% after a 10 minute exposure to 200 ppm of nitrous oxide (Figure 3.7).

Test 1				
Exposure	Sample Tube	Mass (ng)	Average Mass (ng)	Percent Recovery
10 min at 200 ppm	1	3,136	3,034	89%
	2	3,066		
	3	2,901		
10 min at 200 ppm, then 20 min to air	4	2,750	2,703	
	5	2,697		
	6	2,661		
Test 2				
Exposure	Sample Tube	Mass	Average	Percent Recovery
10 min at 200 ppm	1	2,753	2,828	91%
	2	2,656		
	3	3,077		
10 min at 200 ppm, then 20 min to air	4	2,811	2,562	
	5	2,660		
	6	2,214		
Test 3				
Exposure	Sample Tube	Mass (ng)	Average Mass (ng)	Percent Recovery
10 min at 200 ppm	1	2,684	3,149	76%
	2	4,030		
	3	2,732		
10 min at 200 ppm, then 20 min to air	4	2,761	2,381	
	5	2,168		
	6	2,224		

Table 3.7 Reverse diffusion test data.

3.7 Effect of Low Relative Humidity

The average percent error observed between known mass and predicted mass on the thermal desorption tubes was 14%. The air temperature and relative humidity during this test were 22.2 degrees C and 20% relative humidity, respectively. The absolute humidity was 3.9 g/m^3.

Sample Tube	Mass (ng)	Expected Mass (ng)	Percent Error	Average Percent Error
1	6,482	6,955	7%	14%
2	6,063	6,955	13%	
3	5,556	6,955	20%	
4	5,436	6,955	22%	
5	6,050	6,955	13%	
6	6,196	6,955	11%	

Table 3.8 Effects of low relative humidity.

Average error between observed mass and expected mass when tubes are exposed in air to a concentration of 200 ppm for 30 minutes at 20% RH.

3.8 Overall Detection Limit and Reliable Quantitation Limit

In order to calculate the overall detection limit (DLOP) and the reliable quantitation limit (RQL), a slope is required. While a linear slope can be estimated for a point along a non-linear line, a more accurate estimate of the DLOP and RQL can be acquired utilizing a linear model of the calibration data. Of the two linear models for the calibration data, a logarithmically transformed linear model is more accurate than a linear model. Therefore, in order to calculate the DLOP and RQL, the slope of the logarithmically transformed linear model was used. In order to provide a relative value in ng, the anti-log of the calculation was necessary. The logarithmic linear model had a slope of 0.843 and a standard deviation of 0.185. Equations 12 through 14 were utilized. The DLOP, after conversion, was 1.6 ng and the RQL was 4.6 ng.

Chapter 4: Discussion

4.1 Analytical Method

For the primary (first) desorption in the Markes Unity 2, the tube is oriented in the direction of carrier gas flow. Normally, the tube is desorbed opposite to the direction of carrier gas flow so that the compound travels through the minimum amount of sorbent. However, 5 angstrom molecular sieve is very hydrophilic, which means that it will effectively collect water vapor during sampling. As a result, special precautions must be taken during analysis of this sorbent. If heated too quickly, water will flash to steam and quickly expand, which can cause damage to the sorbent's micropores. Furthermore, it is important to prevent water from entering the analytical instrumentation. Therefore, it is recommended that desorption take place at a relatively low temperature, with the tube oriented in the direction of carrier gas flow. This orientation, as explained by the tube manufacturer, allows for the nitrous oxide to be desorbed from the sorbent while minimizing the likelihood of captured water entering the analytical system or damaging the sorbent (33).

Another compound that is effectively captured by 5 angstrom molecular sieve is carbon dioxide. During analysis, carbon dioxide was not well resolved (separated) from nitrous oxide in the analytical column. Therefore, a selected ion method (SIM) at m/z = 30 was utilized to minimize interference by carbon dioxide. An m/z of 30 was selected since it is the second most abundant ion in the nitrous oxide mass spectrum (Figure 2.2) and does not appear in the carbon dioxide mass spectrum. While this method would prevent interference by carbon dioxide, it should be noted that potential interference by

other compounds in the dental office or surgical suite may interfere with nitrous oxide analysis if their fragmentation also produces ions of m/z = 30.

4.2 Calibration of the Agilent 7890B / 5977B GC/MS

The Agilent 7980B/5977B GC/MS was calibrated based on mass estimates using equation 2. The corresponding concentration range for the mass estimates was 1 ppm – 400 ppm. Above 400 ppm, the calibration curve became non-linear, which indicates that the Upper Limit of Linearity was approximately 400 ppm at the specific split ratio used for analysis. Above this limit, the MS would begin to become saturated and the instrument response would become less sensitive. This event was similar to Tschickardt (2007), where it was stated that the upper range of concentrations of nitrous oxide would create a non-linear response with an ECD (58).

There are a few methods that can potentially overcome this obstacle. The first would be to use a non-linear calibration curve. However, when using a non-linear calibration curve (R^2<0.99), responses at the high end of the calibration curve are subject to a less sensitive line, and therefore accuracy may decrease. The second method is adjusting the split flows. However, if the split flows were adjusted, the low end of the calibration curve would then be raised, which would decrease the ability of the instrument to detect low concentrations of nitrous oxide. The third method would be to utilize the Markes Unity 2 recapture capability. With this capability activated, the Markes Unity 2 can capture any nitrous oxide that is split off before entering the GC. If the mass then corresponds to a concentration outside the limits of the calibration curve, the split flow can be adjusted, and the tube can be desorbed again. The nuances of this method could be explored in future studies.

4.3 Storage Test

A deviation of 10 percent from the pre-storage recovery baseline indicates that tubes have reached the maximum storage time as stated in OSHA's protocol: Validation of OSHA Methods Utilizing Chromatographic Analysis. Five angstrom molecular sieve TD tubes deviated from the pre-storage baseline in less than 3 days if not refrigerated, and less than 5 days if refrigerated. This is similar to the Cox & Brown (1984) finding of an average loss of 27% after one week of storage. The recommendation then remains the same, and that is to analyze 5 angstrom molecular sieve TD tubes as quickly as possible after sampling.

4.4 Desorption Efficiency

In OSHA's validation protocol, extraction efficiency is used to denote how much analyte can be successfully removed from a sorbent and introduced into the analytical system. In order to do this, a known amount of analyte is loaded onto a sorbent, which is then analyzed. The resulting instrument response is then applied to a calibration curve and extraction efficiency is determined. However, extraction efficiency cannot be determined in this experiment for two reasons. The first is that extraction efficiency within the OSHA validation protocol is designed for solvent extraction, and therefore cannot be applied word for word to thermal desorption. However, extraction efficiency could still be measured if a loaded tube could be compared to a standard that has not been exposed to the sorbent. A method to accomplish this would be to compare a loaded tube to an analytical standard injected directly onto the GC column. A difference in the two masses could then be compared in order to determine if nitrous oxide remained on the sorbent. However, within the specific GC system utilized, there was no capability to

directly inject analytical standards onto the GC column. Therefore, a loaded tube could not be compared to an analytical standard directly injected onto the column. Instead, desorption efficiency was calculated for the analytical method.

The desorption efficiency test is limited. This test specifically identifies if the desorption parameters remove all the nitrous oxide that is possible to be removed, not if all the loaded nitrous oxide is removed. Since the desorption efficiency test showed below the limit of detection for all tubes on the second desorption, then desorption efficiency is 100%. If there was any remaining nitrous oxide on the tubes, it will not desorb under the current desorption parameters. For future experiments, obtaining the capability to inject analytical standards onto the GC column and then utilizing the analytical standard calibration curve for the extraction efficiency test would better characterize if all of the loaded nitrous oxide is desorbing off of the TD tubes.

4.5 Sampling Rate and Capacity

In OSHA's validation protocol, sampling is done at a specific concentration over many different exposure times (from 5 minutes to 10 hours). Averaging the sampling rates for 0.5 hrs, 1 hr, and 2 hrs identifies a preliminary sampling rate (PSR). This PSR is used as a benchmark to determine when the other sampling rates deviate by 10%, indicating that the capacity of the tube has been reached. For this experiment, the time remained the same while the concentration varied. In order to find sampling capacity, the PSR was determined using 50 ppm, 100 ppm, and 150 ppm.

The sampling rate for 5 angstrom molecular sieve TD tubes varied significantly between 10 ppm and 400 ppm of nitrous oxide. When sampling 10 ppm of nitrous oxide for 30 minutes, the sampling rate average was 0.782 ng ppm^{-1} min^{-1}, which is 48%

different from the PSR of 1.27 ng ppm^{-1} min^{-1} and 32% different from the overall average sampling rate of 1.079 ng ppm^{-1} min^{-1}. After initially having a low sampling rate (relatively) at 10 ppm for 30 minutes, the sampling rate increased when sampling at 50 ppm and then steadily declined as it moved up to 400 ppm. From 50 ppm to 400 ppm, the sampling rate acted as expected based on results from Cox & Brown (1984) i.e. there was a dose dependent response as the sorbent becomes saturated. Within a TD tube, the sorbent closest to the opening will become saturated first, forcing future nitrous oxide to move further into the sorbent to find a binding site. This, in effect, increases the path length (L) that the nitrous oxide must travel, which decreases the sampling rate according to equation 15. Path length can be seen in Fick's law (Equation 2) when uptake rate (UR) is broken down as seen in equation 15.

Equation 15: Uptake Rate from Fick's Law

$$UR = \frac{DA}{L} \qquad (15)$$

Where:

UR = Uptake Rate (cm^3/s)
D = Diffusion coefficient (cm^2/s)
A = Cross-sectional area (cm^2)
L = Path length (cm)

For the 10 ppm sampling rate, a possible explanation of the low sampling rate at 10 ppm could be due to concentration starvation. While the diffusion coefficient of gases is not effected by concentration, the amount of mass that loads onto the tube can be limited if there are not enough analyte molecules in the mixture. Interference of other molecules that are in much higher concentration and that have a very high affinity for 5Å molecular sieve sorbent, such as water and/or carbon dioxide, could play a significant role. These high concentration/high affinity molecules could be binding preferentially to

the sorbent, which would reduce the number of binding sites available for nitrous oxide. This would effectively force the nitrous oxide molecules deeper into the sorbent, which would increase the path length (decreasing the observed uptake rate). This means that at some point between 10 ppm of nitrous oxide and 50 ppm of nitrous oxide, a point is reached where there are enough nitrous oxide molecules in the mixture to reduce the effect of water and/or carbon dioxide on the uptake rate. To determine this point, further experimentation could be done with small increments between 10 ppm and 50 ppm within the dynamic flow chamber, or potentially calculating sampling rates using a carrier gas instead of air.

The overall average sampling rate from 10-400 ppm was 1.08 ng ppm^{-1} min^{-1}, which is 14% different from the uptake rate utilized by Markes International Inc. of 1.25 ng ppm^{-1} min^{-1} (34). The PSR of 1.27 ng ppm^{-1} min^{-1} was much closer to the Markes International Inc. uptake rate, with a 1.6% difference. A possible explanation of the difference between this experiment's sampling rate and Markes International Inc. uptake rate could be if the Markes International Inc. uptake rate does not include high doses of nitrous oxide (i.e. greater than doses of 200 ppm for 30 minutes as seen in Table 3.6). While the difference could be due to error, there was a also a difference in uptake rates when compared to the study conducted by Cox & Brown (1984).

Cox & Brown (1984) reported an uptake rate of 1.03 ng ppm^{-1} min^{-1} when sampling at 50 ppm and an uptake rate of 0.96 ng ppm^{-1} min^{-1} when sampling at 100 ppm. A 30% difference was seen when sampling at 50 ppm for 30 minutes, with a sampling rate average of 1.40 ng ppm^{-1} min^{-1}, and a 25% difference was seen when sampling at 100 ppm for 30 minutes, with a sampling rate average of 1.23 ng ppm^{-1}

min^{-1}. A possible explanation of the difference could be that Cox & Brown (1984) averaged their uptake rates over many different time periods: from 30 minutes up to 8 hours. Averaging their uptake rates over a long period of exposure would decrease the overall average because the higher exposure times would lead to greater doses, bringing down the overall average uptake rate (since uptake rate is dose dependent).

4.6 Reverse Diffusion

The reverse diffusion test showed an average retention of 85%, indicating that the tubes were not able to effectively hold nitrous oxide when exposed to uncontaminated air for 20 minutes. This finding contradicts Cox & Brown (1984), which stated that reverse diffusion of nitrous oxide was not an issue with 5 angstrom molecular sieve. However, it is possible that this finding may be affected by the age of the sorbent. The tubes used in the experiment had undergone several desorption cycles (estimated 20-30) for other experiments within this and other studies. While the number of desorption cycles were far below the approximate 100 cycle lifespan as suggested by Woolfenden (2013), it is possible that they may have been affected by use and therefore less able to hold nitrous oxide.

4.7 Effect of Low Humidity

The sampling rate for 5 angstrom molecular sieve TD tubes was tested using 80% relative humidity (AH 3.9 g/m^3) at 22 °C. When the dynamic flow chamber was set to 20% relative humidity (AH 3.9 g/m^3), the sampling rate decreased, as shown by a 14% reduction from expected mass to observed mass. This is the opposite of what was expected with reduced humidity, as binding sites within the sorbent would have less

interaction with water. Further exploration of sampling rates at low relative humidity may illuminate the cause of this finding.

4.8 Overall Detection Limit and Reliable Quantitation Limit

The DLOP and RQL of 1.6 ng and 4.6 ng, respectively, allow for concentrations of nitrous oxide to be analyzed with GC/MS instrumentation. The analytical limitations do not seem to be a major factor in the sampling of nitrous oxide, as a 30-minute sample of 1 ppm nitrous oxide is estimated to yield around 60 ng. It is not practical to develop analytical capabilities that go beyond the limits presented here for the purposes of industrial hygiene practice.

Chapter 5: Conclusion

The 5 angstrom molecular sieve TD tubes used in this study have shown a number of inconsistencies with existing literature. The most concerning inconsistencies are with regard to 5 angstrom molecular sieve's tendency for significant reverse diffusion of nitrous oxide and the variation in sampling rate with low humidity and low nitrous oxide concentration. Furthermore, after confirmation of the inability of 5 angstrom molecular sieve TD tubes to retain nitrous oxide during storage for greater than only few days, it is not likely that they will provide an ideal nitrous oxide sampling alternative to direct reading instruments or passive dosimeter badges.

However, past studies have shown that 5 angstrom molecular sieve TD tubes are a viable option for nitrous oxide sampling. If these tubes are chosen for sampling use, it is important to ensure that the tubes are analyzed as quickly as possible after sampling (preferably within 3 days of sampling). It is also important to keep a thorough log of all environmental conditions within the sampling environment, such as temperature, pressure, and humidity in order to assess for any variables that may affect sampling rate and potentially provide inaccurate data. When utilizing 5 angstrom molecular sieve TD tubes, it is recommended to use the 50 ppm to 150 ppm sampling rate of 1.27 ng ppm^{-1} min^{-1}, which may lead to a skewed result towards higher exposure, but it will be a more conservative estimate of exposure. If the limitations of 5 angstrom molecular sieve thermal desorption tubes are well understood and accommodated by the user, then they may be utilized successfully for the passive sampling of nitrous oxide.

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