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Occupational exposure to isoflurane anesthetic gas in the research environment

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OCCUPATIONAL EXPOSURE TO ISOFLURANE ANESTHETIC GAS IN THE RESEARCH ENVIRONMENT

by

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This dissertation is a compilation of studies related to the halogenated anesthetic gas isoflurane. Historically, halogenated anesthetic gases have been used in the health care industry. In 1977 the National Institute for Occupational Safety and Health (NIOSH) issued a recommended exposure limit (REL) of two parts per million (ppm) averaged over one hour of exposure for halogenated anesthetic gases (NIOSH 1977). The purpose of the standard was to protect healthcare workers from exposure to halothane, methoxyflurane, and chloroform. However, isoflurane only became available after the NIOSH REL was adopted. Therefore, the NIOSH REL is not directly applicable to isoflurane. Moreover, use of isoflurane in healthcare has diminished over the years, and it is now more widely used in medical research laboratories and veterinary clinics. The purpose of this dissertation is to demonstrate the need for an updated occupational exposure limit for isoflurane. Four studies were conducted toward the completion of this goal; a systematic review of the literature to investigate human health effects associated with occupational exposure to isoflurane, a case study of a high exposure to isoflurane and its control, an assessment of occupational exposure of isoflurane to researchers, and a comparison of the effectiveness of control methods in reducing isoflurane waste anesthetic gas (WAG).

In the first study, we searched the PubMed and Embase databases were searched for articles with data on health effects associated with occupational isoflurane exposure. Thirteen studies were found during the search that fit the review criteria. Five of the studies reported no adverse human health effects. Eight of the studies reported

human health effects ranging from genetic mutations, changes in cellular function, symptoms of acute exposure, and congenital anomalies in the offspring of exposed women.

In the second study, we found that researchers working with isoflurane in a small unventilated space had exposures close to 30 ppm over a short-time period (0.48 and 1.15 hours) for the main researcher. Other members of the group had exposures above 2 parts per million (ppm). An active scavenging ventilation control which reduced isoflurane exposure by an average of 86%.

In the third study, we showed that isoflurane exposure to researchers at a medical research institution was significantly associated with scavenging technique and role of the investigator ($p = 0.02$ and 0.04, respectively). Researchers using passive scavenging canisters were exposed to a mean concentration of 3.18 ppm (%CV = 123) and researchers using active scavenging were exposed to a mean isoflurane concentration of 0.83 ppm (% CV = 89). Researchers who performed the greater part of the procedures were exposed to a mean of 2.71 ppm (%CV = 108) and researchers who assisted were exposed to a mean of 1.18 ppm (%CV = 97).

In the final study, we evaluated isoflurane exposures when using active scavengers, passive canister scavengers, and combinations of both scavenging techniques. We also evaluated isoflurane exposures with no scavenging control. Isoflurane concentration was significantly associated with control method ($p < 0.0001$). Post hoc Tukey's comparison showed the significant difference ($p = 0.05$) in isoflurane concentration between no scavenging and active scavenging conditions, no scavenging and combination active and passive scavenging conditions, and passive and active scavenging conditions. There was no difference between no scavenging and passive scavenging conditions or active scavenging and combination scavenging conditions. The mean isoflurane concentration while using no scavenging controls was 10.23 ppm (%CV

= 12), and was 10.35 ppm (%CV = 58) while using passive scavenging. Isoflurane concentration using active scavenging was 1.43 ppm (%CV = 15) and 0.59 ppm (%CV = 46) while using the combination scavenging method.

Researchers who use passive scavenging methods are more likely to be at risk for isoflurane exposure above 2 ppm. Researchers should use active scavenging to control isoflurane WAG.

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LIST OF ABBREVIATIONS

ACGIH	American College of Governmental Industrial Hygienists
ACVAA	American College of Veterinary Anesthesia and Analgesia
ANOVA	Analysis of Variance
BSC	BioSafety Cabinet
CDC	Centers for Disease Control and Prevention
CNS	Central Nervous System
DOT	Department of Transportation
Embase	Excerpta Medica dataBASE
EPA	Environmental Protection Agency
HEPA	High-Efficiency Particulate Absorption
HVAC	Heating, Ventilating and Air Conditioning
IARC	International Agency for Research on Cancer
kWh	Kilowatt-hour
LOD	Limit of Detection
LOQ	Limit of Quantification
L/min	Liters per minute
MeSH	Medical Subject Headings
NAICS	North American Industry Classification System
NIOSH	National Institute for Occupational Safety and Health
OSHA	Occupational Safety and Health Administration
PAPR	Powered Air Purifying Respirator
PEL	Permissible Exposure Limit
PI	Primary Investigator
ppm	parts per million
REL	Recommended Exposure Limit
ROS	Reactive Oxygen Species
U.S.	United States of America
WAG	Waste Anesthetic Gas
WEL	Workplace Exposure Limit
WHO	World Health Organization

CHAPTER 1

ISOFLURANE EXPOSURE IN THE RESEARCH SETTING: OVERVIEW OF THE PROBLEM

Anesthetic gases are used to induce a state of unconsciousness in humans and animals during healthcare procedures. Anesthetic gases are also used to conduct research procedures. There are approximately 100,000 medical scientists in the U.S. (U.S. Department of Labor, 2015) who are potentially exposed to anesthetic gas in the workplace. There are also approximately 143,000 individuals in the veterinary field who are also likely to be exposed (U.S. Department of Labor, 2015). Cubizolles et al. (1992) reported that workers exposed to anesthetic gases report symptoms of nausea, headache, slower reaction time, neuropsychological syndrome, difficulties with memory, peripheral neurological disorders and fatigue. The National Institute for Occupational Safety and Health (NIOSH), an institute within the U.S. Centers for Disease Control (CDC) also cites irritability, difficulties with judgement and coordination as well as liver and kidney disease (NIOSH, 2007).

Occupational exposure to nitrous oxide and other anesthetic gases have been associated with adverse birth outcomes such as spontaneous abortion, low birth weight, premature birth, and infertility (Cohen et al., 1971, Knill-Jones et al., 1972, Rosenberg et al., 1973). Occupational exposure to anesthetic gas is mainly due to leakage from the induction, maintenance, or recovery process. Anesthetic gas that is leaked to the surrounding environment is referred to as waste anesthetic gas (WAG). Best practice guidelines state that WAGs should be controlled using scavenging techniques to protect employees (NIOSH, 2007). Scavenging is the collection and subsequent containment or evacuation of WAGs. Two commons methods of scavenging WAGs are active scavenging using vacuum exhaust lines and passive scavenging with canisters.

Isoflurane is a halogenated anesthetic gas that was introduced in the 1980s, after NIOSH had published a recommended exposure limit (REL) for halogenated anesthetic gases. At the time of the REL publication there was limited health data available for these gases. However, with the data available for halothane, a two parts per million (ppm) limit was recommended for all halogenated ethers (NIOSH, 1977). The rationale behind this limit was to recommend the lowest possible exposure, which was determined by the lowest level that the analytical method was able to detect at the time. However, in the absence of a standard that specifically took into account isoflurane, the NIOSH REL for halogenated gases has been applied to isoflurane and the newer halogenated ethers by researchers (Barberio et al., 2006, Barker et al., 1997, Franco et al., 1992, Friembichler et al., 2011, Gardner, 1989, Hobbhahan et al., 1998, Hoerauf et al., 1996, Säre et al., 2011, Smith et al., 2002, Taylor et al., 2009)

Acute exposure to isoflurane has been associated with central nervous system toxicity. Symptoms include headache, nausea, dizziness, loss of consciousness, asphyxia. Severe occupational overexposures to isoflurane may result in death (OSHA, 2006). Newer publications have documented possible pathways of central nervous system (CNS) toxicity (Acharya et al., 2015, Dittmar et al., 2015, Joksovic et al., 2015, Kim et al., 2014, Sun et al., 2014, Uchimoto et al., 2014, Xie et al., 2007).

Isoflurane exposure in humans has been linked to an increase of chromosomal abnormalities and DNA mutations (Bilban et al., 2005, Costa Paes et al., 2014, Hoerauf et al., 1999, Hoerauf et al., 1999). These changes could result in negative health outcomes including cancer and adverse birth outcomes. The International Agency for Research on Cancer (IARC) has not designated isoflurane as carcinogenic because of. Inadequate evidence of carcinogenicity to humans and animals (IARC, 1998).

Recent studies in animal and in vitro models have described biological mechanisms that could potentially cause negative health effects in humans. Isoflurane is metabolized in the liver by cytochrome P450 2E1 into trifluoroacetic acid and inorganic fluoride (Kharash et al., 1999). Several studies have described neurological effects of isoflurane in animals (Archaraya et al., 2015, Dittmar et al., 2015, Joksovic et al., 2015). In a recent study, isoflurane exposure has been shown to cause reactive oxygen species accumulation in cells that results in apoptosis (Sun et al., 2014).

There has been limited epidemiological data concerning the health effects of isoflurane exposure to humans. Most of these studies have been concerned with exposure to anesthetic gases as a class of chemicals (Bilban et al., 2005, Costa Paes et al., 2014, Hoerauf et al., 1999, Hoerauf et al., 1999). As a result, there are only a few studies that focus on isoflurane exposure. Furthermore, anesthetic gases do not have the same mechanisms, so it is not appropriate to generalize. As an example, nitrous oxide exposure has been better studied than the other anesthetic gases. Health effects associated with nitrous oxide do not necessarily reflect those of isoflurane. Nitrous oxide is structurally different from isoflurane and has different pathways in the body. Therefore, studies with a mixed exposure of isoflurane and nitrous oxide may not give an accurate picture of the health effects of isoflurane. Isoflurane is similar in structure to the other halogenated anesthetic gases desflurane, enflurane, halothane, and sevoflurane (Corbett, 1976, Baden et al., 1990, Barker et al., 1997).

As isoflurane is now primarily used by researchers and veterinarians working with animals, occupational exposures may be higher among these workers than among health care workers. Operating theatres are designed with ventilation controls specifically to reduce WAGs and other possible air pollutants. Research and veterinary setting may have less rigorous institutional policies to govern how isoflurane is used and

controls in place to reduce WAGs. Researchers may have to work in rooms that have not been designed for the use of anesthetic gases. This may result in high exposures to isoflurane that could be potentially hazardous to the health of the researchers.

The purpose of this dissertation is to demonstrate the need for an updated occupational exposure limit for isoflurane. This study has been designed to achieve this objective and focusses primarily workers in the scientific research and development services North American Industry Classification System (NAICS), 541700 (US Department of Labor, 2014). This research may also benefit individuals working in the veterinary services field, NAICS 541940 (Veterinary Services) (US Department of Labor, 2014). Four studies were conducted to achieve this objective. These studies are presented in the following order: A systematic review of available literature was conducted to determine what health effects are associated with occupational isoflurane exposure (Chapter 2). The objective of the systematic review was to find health effects and exposure data that could be used to suggest a safe exposure limit for isoflurane. It was hypothesized that occupational isoflurane exposure is associated with adverse human health outcomes.

We conducted an intervention study in research dentists using isoflurane for a research protocol. We evaluated exposures before and after the installation of a ventilation control. (Chapter 3). The purpose of the study was to reduce isoflurane exposures through a ventilation control. Researchers were relying on passive scavenging canisters, as opposed to active methods such as ventilation, to evacuate air, vacuum scavenging lines or working in a biosafety cabinet. It was hypothesized that employees were exposed to high concentrations of isoflurane and that an active scavenging vacuum line could be used to reduce isoflurane exposure to the researchers.

Due to the high exposure of isoflurane found in the study of dental researchers (Chapter 3), we determined that more information was needed about how isoflurane was used in the institution. An exposure assessment study of several researchers, conducting a variety of procedures, in different locations in the institution was conducted to determine how isoflurane is used at the medical research institution (Chapter 4). The purpose of this study was to identify working conditions and work practices that were associated with high levels of isoflurane exposure. It was hypothesized that scavenging controls, role of the researcher during the procedures, species of animals used for the procedure, and number of animals would be associated with isoflurane exposure to researchers.

More data was needed to be able to justify isoflurane WAG controls methods to researchers. A comparison of current, commonly used isoflurane controls were compared to determine which controls most effectively reduce isoflurane WAG exposure to workers. Isoflurane concentration in an experimental setting was measured using three common isoflurane control techniques (Chapter 5). The purpose of the study was to determine how well each technique controlled isoflurane WAG. These techniques were activated charcoal canisters for the passive scavenging of isoflurane, a vacuum exhaust line for active scavenging, and a combination of passive and active scavenging. It was hypothesized that isoflurane concentration would be different while using different WAG control methods. The secondary hypothesis was that isoflurane concentration would be different while using different brands of the passive scavenging canisters.

The combined studies present a full picture of isoflurane use from known health effects to current occupational exposures and exposure control methods. The systematic review revealed that occupational exposure to isoflurane may be associated with changes in cell function and genetic damage. The exposure assessments of researchers showed that researchers are at risk for high exposures to isoflurane WAG when performing research procedures. Active scavenging methods were shown to more effectively control WAG than passive scavenging methods in three of the studies (Chapters 3—5).

CHAPTER 2

HEALTH EFFECTS RELATED TO OCCUPATIONAL ISOFLURANE EXPOSURE: A SYSTEMATIC REVIEW

ABSTRACT

Isoflurane is the most commonly used anesthetic gas in the veterinary and research fields. Few studies concerning health outcomes associated with isoflurane have been published, since its introduction in the 1980s. The objective of this review is to determine what health outcomes are associated with occupational exposure to isoflurane. PubMed and Embase databases were searched for all studies concerning occupational exposure to isoflurane anesthetic gas. There were thirteen studies that met all of the eligibility criteria. Eligibility requirements were studies conducted on human subjects, in which the population had documented occupational exposure to isoflurane, health effects information related to the exposure, and published in English.

Eight of the studies reported some kind of adverse health outcome associated with occupational exposure to isoflurane. Five of the studies reported that isoflurane exposure was not associated with the adverse health outcomes of interest. Health outcomes varied from the cellular level to the individual level. Four of the studies found differences in rates of genetic mutations. One study found an increase in congenital abnormalities and one found an association with symptoms such as headache, nausea, and dizziness. Isoflurane is associated with some adverse health outcomes, although more data is needed to determine the mechanism behind these outcomes.

INTRODUCTION

Isoflurane is the most commonly used anesthetic gas in the veterinary and animal research fields. There have been no regulations or exposure recommendations published by either the Occupational Safety and Health Administration (OSHA) or the National Institute for Occupational Safety and Health (NIOSH) specifically for isoflurane. This may in part be due to the lack of health effects data available for isoflurane exposure. Acute exposure effects of isoflurane exposure are known to be headache, nausea, loss of consciousness, and other symptoms of central nervous system (CNS) effects (Occupational Safety and Health Administration, 2006). However, health effects associated with chronic exposures likely to be observed in occupational studies have not been well documented. These data are necessary to develop occupational exposure limits for isoflurane.

Most studies completed on isoflurane focus on exposures and control methods. These studies have not included health symptom data of any kind. The studies with isoflurane exposure data often use healthcare workers as the sole study population. Isoflurane is commonly used in the veterinary and research fields, so the healthcare population does not truly represent the current exposed population. Isoflurane exposure due to use in humans is also likely to be considerably different from exposures due to use in animals. This is attributable to many factors, including induction and maintenance techniques, and equipment as well has differences in ventilation between hospital operating theaters and veterinary and research procedure rooms.

NIOSH published a recommended exposure limit (REL) of two parts per million (ppm) for a one hour time period for halogenated anesthetic gases in 1977 (NIOSH, 1977). The standard was developed with data from the anesthetic gases chloroform, trichloroethylene, halothane, methoxyflurane, enflurane, and fluroxene. Most of these

anesthetics are no longer in use today. Notably, the newer generation halogenated anesthetic gases sevoflurane, desflurane, and isoflurane are not mentioned in the standard. At the time the standard was written these drugs were not in use.

The NIOSH standard of two ppm should not be interpreted as a safe exposure level. At the time the standard was developed, little health data was available for these anesthetic agents. Therefore, NIOSH set the REL as low as possible, which was defined by the reliability of the sampling methods available. Two ppm was the lowest reliable concentration of the anesthetic gases that could be sampled using charcoal adsorption and analyzed using gas chromatography. There have been many improvements in both sampling and analysis techniques. The limit of detection (LOD) of the MIRAN sapphire direct reading instrument is 0.03 ppm and the LOD of passive monitoring badges is 0.04 ppm. Therefore, if the spirit of the NIOSH REL were to be applied to isoflurane today the standard would be 0.04 ppm or lower.

The NIOSH standard was also specifically determined with healthcare workers in mind. It was not anticipated that halogenated gases would be used outside of the strictly controlled healthcare environment. Today, halogenated gases are more likely to be used in veterinary practices and research environments than in the healthcare setting. Veterinarians and researchers may not have the same training for safe anesthetic gas use that healthcare workers are provided.

The NIOSH standard is not only outdated because it lacks the inclusion of newer halogenated anesthetic gases; it is also outdated in respect to the population using and exposed to the gases and the exposure assessment tools to evaluate exposure. The NIOSH REL needs to be updated. Health effects data concerning isoflurane and the newer halogenated anesthetic gases are needed to develop a reasonable and justifiable exposure limit.

A systematic review of the literature was conducted to determine what health effects are associated with occupational exposure to isoflurane. The search was limited to studies with an occupationally exposed study population and a non-exposed control group. All health effects and occupational settings were included.

METHODS

We conducted a computerized search of PubMed and Embase for all studies on occupational environmental exposure to isoflurane or other anesthetic gases. A combination of the following MeSH heading and keywords were used: “occupational exposure” or “environmental exposure” and “isoflurane” or “anesthetic gases”. The search was conducted in December of 2014 and again in June of 2015. A limit of human studies was applied to the search. No language filters were used. The search strategy was translated for the Embase search interface. The full PubMed search strategy can be found in Appendix A and the Embase strategy in Appendix B.

Studies without a comparison to a control group, without isoflurane exposure data or did not include isoflurane exposure data during data analysis were excluded. Papers that were non-English publications were excluded. The remaining studies were thoroughly reviewed and assigned a grade of low, medium, or high quality using the Newcastle-Ottawa Scale for Observational studies. A point-scale was used to determine quality. Categories were based on traditional grading scales. Studies that received less than one half of the available points were graded as poor. Studies that received 50—70% of the points were graded as medium quality, and studies that received 80—100% of the points were graded as high quality. All studies were graded by two reviewers (A.M., C.A.). Studies were reviewed and graded separately by each reviewer. Differences in grades were discussed until the reviewers reached a consensus.

RESULTS

A total of 453 unique records were found in PubMed and Embase. Of these, 361 were studies on anesthetic gases other than isoflurane and were excluded from the study. Non-English publications concerning isoflurane exposure were also excluded (n=24). Studies with isoflurane exposure data, but no health outcomes data were also excluded from the study (n=52). This left 16 studies that had both isoflurane exposure data and health effects data. Two of these studies had no comparison group and were excluded. One study had exposure information on isoflurane but did not include these data in the analysis of health effects. This study was excluded (Figure 2.1).

Of the remaining 13 studies, five reported no adverse health effects associated with occupational exposure to isoflurane. These five studies included one cohort study design (Shuhaiber et al., 2002) and four studies of cross-sectional design (Franco et al., 1993, Krenzischek et al., 2002, Lucchini et al., 1997, Scapellato et al., 2011). The cohort study was rated of low quality and one cross-sectional study was rated high quality and three were rated as medium (Table 2.1).

Four studies reported a significant difference in genetic damage associated with exposure to isoflurane. These studies reported changes in chromosomes and DNA damage. All studies reporting genetic differences were cross-sectional. Two of the studies were rated as medium (Bilban et al., 2005, Costa Paes et al., 2014) and two were rated as high (Hoerauf et al., 1999, Hoerauf et al., 1999) (Table 2.1).

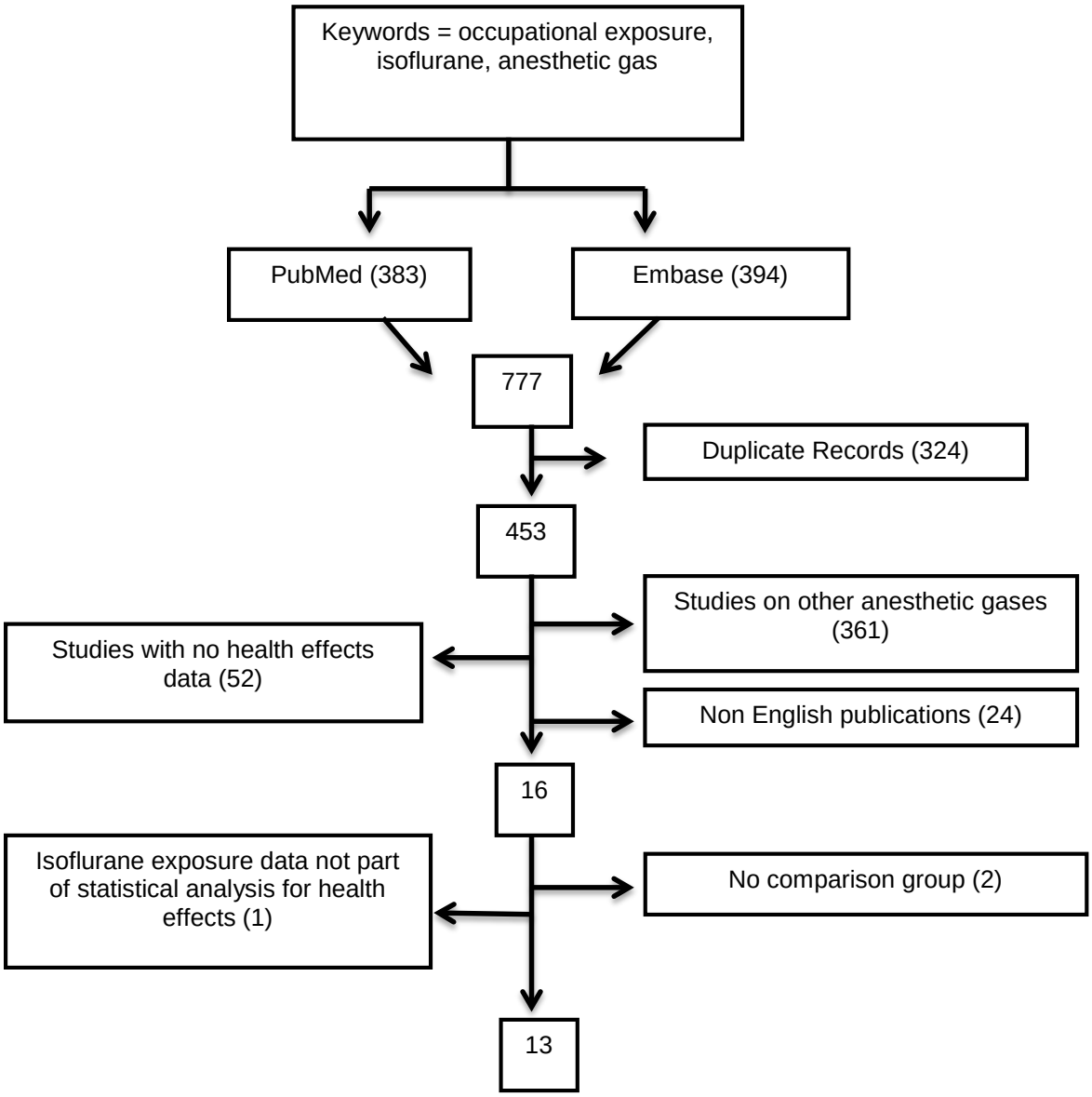

Figure 2.1: Study selection process for systematic review of health effects associated with occupational exposure to isoflurane. Number of studies is indicated in parentheses.

Four studies reported other adverse health effects. These included a range of health outcomes from symptoms associated with acute isoflurane exposure (Hill et al., 1998) to changes in biomarkers (Franco et al., 1992) and cell functions (Goto et al., 2000). One study found that isoflurane exposure was associated with an increase in congenital anomalies in offspring of female veterinarians who were occupationally exposed to isoflurane (Teschke et al., 2011). One of these studies was a cohort study rated as medium in quality; the other three studies were cross-sectional and ranged in quality from low to high (Table 2.1).

Table 2.1: Summary of Studies Included in Review

Author Year	Title	Study Design	Findings	Grade (points)
Bilban, M., et al., 2005	Cytogenetic tests performed on operating room personnel (the use of anesthetic gases)	Cross-sectional Study population: Health care workers, women (Subjects 153 Controls 197 and 153) Statistical analysis: Pearson correlation coefficient	Occupational exposure to a mixture of nitrous oxide, isoflurane, and halothane anesthetic gases was associated with and increased frequency of structural chromosomal aberrations ($p<0.001$), sister chromatid exchanges ($p<0.001$), and increased number of micronuclei ($p<0.001$).	Medium (7)
Costa Paes, R., et al., 2014	DNA damage and antioxidant status in medical residents occupationally exposed to waste anesthetic gases	Cross-sectional Study population: Health care workers (Subjects 15, Controls 15) Statistical analysis: Student's t test	Occupational exposure to isoflurane and other anesthetic gases was associated with an increase in lymphocyte DNA damage ($p=0.001$), and altered antioxidant protection ($p>0.001$).	Medium (7)
Franco G., et al., 1992	Occupational exposure of operating-theater personnel to isoflurane and nitrous oxide	Cross-sectional Study population: Health care workers (Subjects 24, Controls 24) Statistical analysis: Student's t test, Chi square	Occupational exposure to isoflurane and nitrous oxide was associated with an increase of urinary D-glucaric acid ($p<0.001$).	High (8)
Franco, G., et al., 1993	Drinking habits and occupational exposure to inhalation anesthetics at low doses	Cross-sectional Study population: Health care workers (Subjects 80, Controls 92) Statistical analysis: ANOVA	Occupational exposure to isoflurane and nitrous oxide does not cause changes in serum aminotransferases ALT and AST or mean corpuscular volume (MCV) of red blood cells ($p>0.05$).	Medium (7)
Goto, Y., et al., 2000	Does chronic occupational exposure to volatile anesthetic agents influence the rate of neutrophil apoptosis?	Cross-sectional Study population: Health care workers (Subjects 20, Controls 10) Statistical analysis: Student's t test	Neutrophil apoptosis was inhibited by occupational exposure to nitrous oxide, sevoflurane, and isoflurane ($p=0.008$).	Low (4)
Hill, D., et al., 1998	Occupational injuries and illnesses reported by zoo veterinarians in the United States	Cross-sectional Study population: Zoo veterinarians (Subjects 277) Statistical analysis: Chi square	Ten percent US zoo veterinarians reported adverse health effects related to anesthetic gas exposure; isoflurane was the anesthetic gas for 78.6% of the adverse reactions). Female veterinarians were more likely to report an adverse exposure to anesthetic gas ($p=0.04$).	Medium (5)

Hoerauf, K., et al., 1999	Genetic damage in operating room personnel exposed to isoflurane and nitrous oxide	Cross-sectional Study population: Health care workers (Subjects 10, Controls 10) Statistical analysis: Paired Student's t test	Exposure to nitrous oxide and isoflurane leads to an increased frequency of sister chromatid exchanges (p=0.036).	High (8)
Hoerauf, K., et al., 1999	Waste anaesthetic gases induce sister chromatid exchanges in lymphocytes of operating room personnel	Cross-sectional Study population: Health care workers (Subjects 27, Controls 27) Statistical analysis: Student's t test	Simultaneous exposure to isoflurane and nitrous oxide is associated with increased frequency of sister chromatid exchanges (p<0.05).	High (8)
Krenzischek, D., et al., 2002	Phase I collaborative pilot study: Waste anesthetic gas levels in the PACU	Cross-sectional Study population: Health care workers (Subjects 6, Controls 3) Statistical analysis: Student's t test	Halogenated anesthetic gases are not associated with adverse health symptoms (p=0.48).	Medium (7)
Lucchini, R., et al., 1997	Neurobehavioral functions in operating theatre personnel: a multicenter study	Cross-sectional Study population: Health care workers (Subjects 112, Controls 135) Statistical analysis: Student's t test	Trace levels of nitrous oxide and isoflurane exposures are not associated with neurobehavioral functions such as stress and arousal. Exposure to anesthetic gases was not associated with decrease or self-reported quality of life.	High (8)
Scapellato, M., et al., 2001	Occupational exposure to anaesthetic gases and urinary excretion of D-glucaric acid	Cross-sectional Study population: Health care workers (Subjects 229, Controls 229) Statistical analysis: stepwise multiple regression	Exposure to the anesthetic gases isoflurane and nitrous oxide is not associated with urinary D-glucaric acid (OR 0.68).	Medium (7)
Shuhaiber, S., et al., 2002	A prospective-controlled study of pregnant veterinary staff exposed to inhaled anesthetics and x-rays	Cohort Study population: Veterinary staff, women (Subjects 95, Controls 95) Statistical analysis: Fisher's exact or Chi square	Occupational exposure to isoflurane and halothane anesthetic gases is not associated with low birth weight (p=0.06) or major malformations (p=0.96).	Low (4)
Teschke, K., et al., 2011	Exposure to anesthetic gases and congenital anomalies in offspring of female registered nurses	Cohort Study population: Registered nurses, women (Subjects 56,213) Statistical analysis: binary unconditional logistic regression model	Congenital anomalies are associated with "ever" and "probable" exposure to halogenated anesthetic gases (OR 1.49, OR=2.61) and nitrous oxide (OR=1.42, OR=1.82).	Medium (6)

Table 2.1: An overview of articles included in the systematic review. Studies were assigned points based on the Newcastle-Ottawa Scale for observational studies. The listed scores reflect quality of the study and were based on the following point system: Low (1—4 points), Medium (5—7 points), and High (8—10 points).

DISCUSSION

Most of the studies included in this review were cross-sectional studies, which would have allowed easy comparison between studies if the outcomes of the studies had been similar. However, a variety of health outcomes were reported. The most frequently studied health outcomes of isoflurane exposure in humans were related to genetic damage. This includes studies focusing on singular aspects of genetic damage including chromosome abnormalities, DNA damage, and sister chromatid exchanges. Changes in cell function and other biomarkers were also studied. Most of these studies were not associated with symptoms that employees could report as the outcomes are on the cellular or sub-cellular level. These outcomes were also associated with low levels of isoflurane exposure as demonstrated by the exposure assessment data in many of the studies.

Four of the included studies contained self-reported health outcomes by employees and with occupational exposure to isoflurane. Of these, only one study focused on symptoms known to be associated with isoflurane anesthetic gas use such as headache, nausea, and dizziness. This study relied on the participants to disclose workplace exposures and did not estimate the exposures (Hill et al., 1998). As such, we cannot determine if isoflurane is associated with these symptoms in a dose-dependent manner. This information would be most helpful in establishing exposure limit recommendations for isoflurane.

A limitation of this review is that most of the studies included had a mixed exposure of isoflurane with other anesthetic gases. The most common mixture of gases was nitrous oxide with isoflurane. A few studies also reported exposures to other halogenated anesthetic gases. Health outcomes reported in these studies may result from exposure from the other anesthetic gases, isoflurane, or the combination of

exposure to isoflurane with other anesthetic gases. These studies were mostly conducted with healthcare workers as the participants. Isoflurane is no longer commonly used in this setting. However, veterinarians and researchers almost exclusively use isoflurane for anesthesia. More epidemiological studies focused on veterinarians and researchers could be beneficial in determining what health effects are associated with isoflurane and at what concentrations. A strength of this review is that only observational studies with control comparison groups were included.

A case study of three healthcare workers showed that in these cases exposure to isoflurane and sevoflurane was associated with asthmatic symptoms and itchy rash (Vellore et al., 2006). Symptoms in the three healthcare workers abated once they were transferred to duties not associated with isoflurane and sevoflurane exposure. Cope reported that exposure to isoflurane depressed central neurorespiratory activity in healthcare workers (Cope et al., 2002). Long term effects of chronic exposure to isoflurane have not been well documented or investigated. Studies in animal and cell models have reported a number of health effects. Corbett noted that isoflurane and other halogenated anesthetic ethers are similar in structure to human carcinogens bis (chloromethyl) ether and chloromethyl methyl ether (Corbett, 1976). He went on to investigate the possible mutagenic properties in mice and found that male offspring of pregnant mice exposed to isoflurane at certain times during pregnancy went on to develop hepatic neoplasms. The offspring were exposed to isoflurane during gestation and after birth (Corbett, 1976). Four studies in this review reported genetic and DNA damage was associated with isoflurane exposure. It is not outside the realm of possibility that isoflurane could be a potential carcinogen.

A study in rats found that isoflurane negatively impacts short term cognitive function. Uchimoto et al. found that isoflurane exposure impaired hippocampal learning

in the exposed rats (Uchimoto et al., 2014). Cellular studies have found that isoflurane exposure can induce apoptosis in cells (Xie et al., 2007) and accumulation of reactive oxygen species (ROS) in cells (Sun et al., 2014). These studies indicate that isoflurane is toxic at the cellular level which may translate to human toxicity.

Nitrous oxide and other anesthetic gases have been associated with adverse birth outcomes such as spontaneous abortion, low birth weight, premature birth, and infertility (Cohen et al., 1971, Knill-Jones et al., 1972, Rosenberg et al., 1973). One study included in this review found an association between halogenated anesthetic gases and nitrous oxide and congenital abnormalities in the offspring of exposed female veterinarians (Tescheke et al., 2011). Again, the genetic and DNA damage reported in other studies could be linked to this outcome. More studies with isoflurane as the only exposure are needed to determine if isoflurane is associated with adverse birth outcomes in humans.

This systematic review was unable to find the necessary health effects data to recommend a safe exposure limit for isoflurane. Many of the studies were conducted with study populations with a mixed exposure of anesthetic gases and populations in the healthcare field. If researchers turned to study populations working in the veterinary and research fields, a more homogenous picture of health effects associated with isoflurane may be obtained. However, until those studies become available, no exposure limits can be determined based on health effects. In the meantime, following the NIOSH REL of a cumulative maximum of two ppm for one hour is the best plan of action.

CONCLUSION

Isoflurane in combination with other anesthetic gases is associated with a variety of potential health outcomes. These are related to more obvious symptoms such as headache, nausea, and dizziness as well as to less noticeable outcomes such as increased rates of DNA and genetic damage. No dose-dependent data was available for these outcomes. We can only recommend that isoflurane exposure is kept to a minimum. The NIOSH REL of two ppm cannot be determined as protective or non-protective at this time.

CHAPTER 3

EVALUATION AND CONTROL OF ISOFLURANE DURING EXPERIMENTAL DENTAL PROCEDURES ON RODENTS

ABSTRACT

The purpose of this study was to assess exposures to isoflurane to employees performing an experimental dental procedure on rodents. Isoflurane was used as an anesthetic for rodents during injections and placement of dental apparatus into the mouth. We collected eight full-shift samples using passive badges over a 3-week period. Results showed that employees were being exposed to isoflurane above the National Institute for Occupational Safety and Health recommended exposure level for halogenated anesthetic gases of two parts per million. The procedure was performed once a week for approximately 45 minutes each day. The employer installed a ventilation system in the procedure room after the initial sampling and results. We collected twelve full-shift samples after the ventilation system was in place. Results showed an approximate 86% decrease in isoflurane concentration.

INTRODUCTION

Exposure to waste anesthetic gas (WAG) in occupational settings has been found to significantly increase the risk of miscarriage and infertility (Cohen et al., 1971, Cohen et al., 1974, Knill-Jones et al., 1972, Rosenberg et al., 1973). The approximate increased relative risk of miscarriage is 1.3—1.9 (Sessler et al., 1998). Several epidemiological studies have found an increased risk of spontaneous abortion in occupationally exposed women and the female partners of occupationally exposed men (Knill-Jones et al., 1975, Tomlin, 1979, Cohen et al., 1980, Guirguis et al., 1990). Chronic exposure to halogenated gases is also significantly correlated with an increased risk of infertility in both men and women. Metabolites of halogenated gases accumulate in the body and can then be exhaled in substantial amounts resulting in a secondary exposure of others (Smith et al., 2005). Aside from reproductive toxicity, volatile anesthetic gases are also associated with increased risks of hepatic and renal diseases as well as psychological impairments among workers (NIOSH, 1977). The National Institute for Occupational Safety and Health (NIOSH) recommended exposure level (REL) for halogenated anesthetics states that exposure levels should not exceed two parts per million (ppm) for any given task or time (NIOSH, 1977).

Isoflurane is a halogenated anesthetic gas and today it is one of the most commonly used inhalation anesthetics for experimental and veterinary animal procedures (Säre et al., 2011). Isoflurane has not been consistently linked with reproductive toxicity in human studies. However, cellular studies have found that isoflurane exposure inhibits mitochondrial function that may lead to increased reactive oxygen species (ROS) levels. ROS have been found to be toxic to many cellular functions. Uchimoto et al. showed that isoflurane induced anesthesia was associated with learning deficits and young adult rats (Uchimoto et al., 2014). There is no

Occupational Safety and Health Administration (OSHA) permissible exposure level (PEL) for isoflurane; in addition, the NIOSH REL does not directly apply to isoflurane as it was developed before isoflurane became available. Use of inhalation anesthetics can result in the pollution of anesthetic gas in the workplace and resulting exposure to employees. In animal procedures, an anesthetic state is often induced and maintained using a face mask or nose cone. Several studies have already documented that using face masks to induce anesthesia in humans results in a significant increase of WAG concentrations that may exceed occupational exposure limits (Weber, 1994, Schuyt et al., 1996, Hoerauf et al., 1997, Hoerauf et al., 1999). Using a scavenging mask or double masking have been shown to reduce occupational exposure during human procedures. Scavenging masks for rodents are also commercially available, but the efficacy of these masks has not been sufficiently proven.

Appropriate engineering controls such as ventilation can be used to effectively control WAGs. Surgical procedure rooms used for human procedures are often designed and regulated to protect employees from high concentrations of WAGs. In contrast, animal researchers must often utilize spaces for surgical procedures that have not been designed for anesthetic gas use. The use of these spaces may lead to high exposures of WAG to researchers. The purpose of this study was to investigate an isoflurane exposure to animal researchers and the effectiveness of an engineered ventilation control.

METHODS

In this intervention study, we measured study participants' exposure to isoflurane before and after the installation of an engineering control. Samples were collected using passive badges (Advanced Chemical Sensors Inc., Boca Raton, FL). The badges were placed in the breathing zone of the study participants. We collected three full-shift

samples from a single researcher over a three-week period. We then collected eight full-shift personal samples from five dental researchers (the entire research group) over a two-week period. Samples were collected once a week identical to how often the researchers performed the procedure. This study was conducted as part of institutional safety requirements and with approval of the University of Nebraska Medical Center institutional review board.

Room ventilation conditions were 12.21 air changes per hour. Intake air was filtered with a high-efficiency particulate absorption (HEPA) filter, and the room was kept under positive pressure. The procedure room was small (area =106 ft^2 or 32.31 m^2, volume = 848 ft^3 or 24.0 m^3).

An engineering control was designed and installed in the procedure room after the initial sampling periods. After the installation, eleven full-shift personal samples for isoflurane were collected from four employees over a three-week period. Samples were collected once a week while researchers performed the research procedure. Job titles and duties of the employees are listed in Table 3.1.

TABLE 3.1: Description of Study Participants and Duties

Study Participant	Job Title	Gender	Duties
Employee 1	Technician	Female	Anesthetize and monitor animals
Employee 2	Dental Resident	Female	Conduct procedure
Employee 3	Visiting Surgeon	Female	Observe
Employee 4	Advising Professor	Male	Assist
Employee 5	Dental Surgeon	Male	Assist

Process Description

The technician placed the animal in an induction chamber to induce anesthesia. Isoflurane was introduced into the chamber at a concentration of 3% isoflurane and flow rate of 1 liter per minutes (L/min) using an isoflurane vaporizer. The technician then placed the animal on the surgical field so that the animal's nose was inside of a nose-cone. The technician then manipulated the vaporizer, so that 1.5% isoflurane flowed to

the nose cone at a rate of 1 L/min. The technician held the animal in place for the duration of the procedure. F/Air activated charcoal canisters were used to scavenge waste isoflurane gas. One canister was connected to each anesthesia station, the induction chamber and the nose-cone.

Employee two, the lead researcher, injected the animal's gums on each side of the mouth. Employees four and five assisted with the correct placement of the injections by manipulating the animal's head, mouth, and tongue. Employee three observed the procedure. After the injections had been completed, the researchers placed an experimental dental apparatus in the mouths of the animals in the treatment group. The animals recovered in a separate room. Control animals did not receive the dental apparatus and recovered immediately after the injections.

Installation of Engineering Control

The institution had an engineering control designed and installed to remove isoflurane WAG from the procedure room. This engineering control consisted of two vacuum scavenging lines attached from the building exhaust to the procedure room. The exhaust vented outside of the building away from any windows and air intake units. The engineered control included a flow meter/ ball valve and 1 ½ inch modular hose for both the induction chamber and the procedure table. The modular hose for the induction chamber was fitted with a tapered oval anti-static nozzle. The modular hose for the procedure table could me maneuvered into position by the researchers for optimal scavenging and ease of use (Figure 3.3). Modular hoses were designed to be stored attached to exhaust line and on the wall for easy access. The total cost of the system was approximately $2,000.

RESULTS AND DISCUSSION

Isoflurane Exposure Levels Pre-Installation of Engineering Control

We hypothesized that the technician would be at the highest risk for isoflurane exposure due to the responsibility of handling the rodents and the isoflurane. The technician's exposure to isoflurane was monitored during three sampling periods prior to monitoring the entire research team. Isoflurane concentration was at or above the two ppm REL for each of these three sampling periods. Exposure time ranged from 0.75 to 3.27 hours (Table 3.2).

TABLE 3.2: Isoflurane Exposure to Employee 1 (Technician).

Day	Isoflurane Concentration (ppm)	Time (hrs.)
1	11.00	3.27
2	11.30	1.50
3	2.00	0.75

The entire research team was monitored for isoflurane exposure during two subsequent experimental procedures. Interestingly, the exposure levels of the other researchers exceeded that of the technician. Employee two, the dental resident and lead researcher, had the highest isoflurane exposure at 28.40 ppm over 0.48 hours and 29.30 ppm over 1.15 hours. The exposure most likely resulted from isoflurane leaking from the nose-cone while the researcher leaned over the rodent while conducting the procedure (Figure 3.1). The technician wore a powered air purifying respirator (PAPR) due to previously identified allergy to animal dander. It is possible that the measurement of the technician’s isoflurane exposure was impacted by air turbulence caused by air flow from the bottom of the PAPR mask (Figure 3.1).

Employees three, four, and five were each monitored for one sampling period. During the experimental procedures employees four and five assisted and also leaned

over the animal. The source of the isoflurane exposure was likely the nose-cone. Isoflurane exposure for employee four was 17.20 ppm over a 0.47 hours sampling period and exposure for employee five was 13.20 ppm over 1.03 hours, both were well above the NIOSH REL. Employee three observed the procedure, standing further from the surgical field than the other employees. Isoflurane exposure was 7.90 ppm over 0.47 hours (Figure 3.2). The isoflurane concentration was below the limit of detection for all field blank, and laboratory blank samples indicating the isoflurane exposures found for the study participants resulted from the use of isoflurane as an anesthetic during their investigations. The dental surgeons wore gloves and surgical masks. However, surgical masks do not protect workers from isoflurane and other WAGs. It should also be noted that the procedure did not take place in a fume hood or under any form of visible ventilation.

Figure 3.1: A) Researchers conducting procedure. Note that the researchers lean in over the animal. B) Procedure was conducting on the mouth.

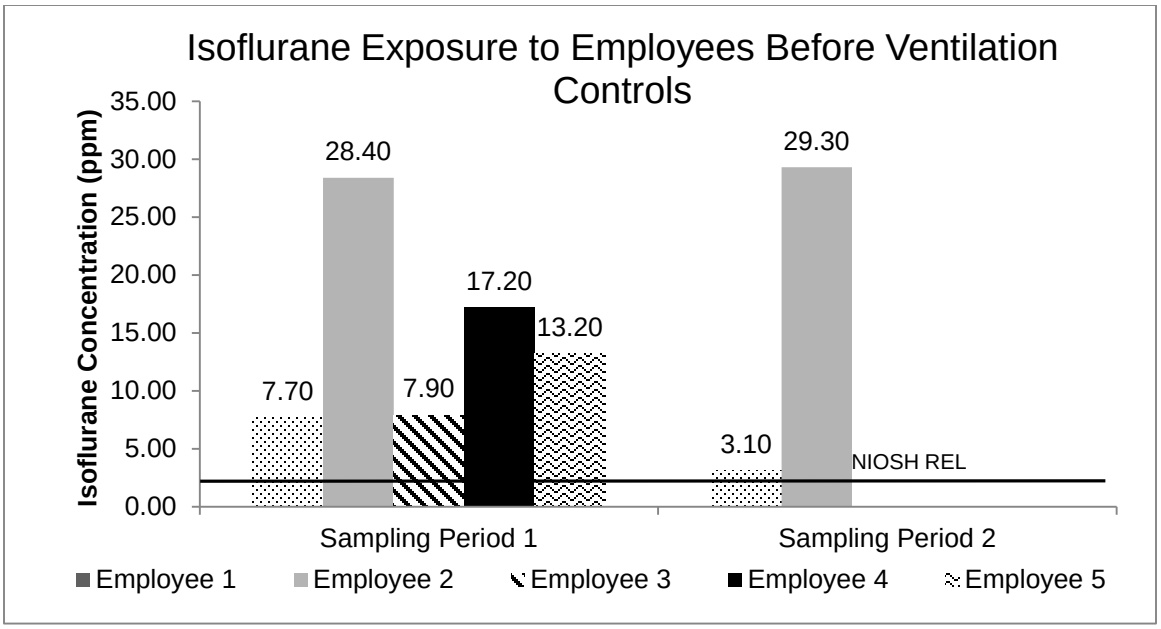

FIGURE 3.2: Isoflurane exposure for study participants before installation of ventilation control. Note that isoflurane concentration for employee two is well over ten times the NIOSH REL of 2 ppm.

Isoflurane Exposures Post-Installation of Engineering Control

After installation of the engineering control, the average isoflurane exposure decreased approximately 86% from 10.96 ppm in 1.17 hours to 1.57 ppm in 1.43 hours. The first sampling period after the scavenging unit was installed, the mean isoflurane exposure for employees one, two four, and five was 2.52 ppm in 2.04 hours. While the mean isoflurane exposure is above the two ppm NIOSH REL, employees one and five, are below the REL while employees two and four are at four ppm. It should be noted that during this sampling period, adjustments to the isoflurane apparatus had to be made which could have led to an increase in the exposure levels. These adjustments were due to the process of exchanging the oxygen tank, which was used to supply the carrier gas. These adjustments are common, but not part of a daily use routine. The adjustments increased the amount of time the researchers spent performing the procedure. The second sampling period after the installation of the engineering control, the mean isoflurane exposure was 0.46 ppm, which was a decrease of approximately 97% (Figure 3.4).

During the third sampling period after the installation of the engineering control, the researchers made minor adjustments to the procedure table scavenging unit and the nose-cone. A thinner hose was coupled to the larger hose of the scavenging unit to allow the hose to be nearer the procedure without being in the way of the researchers. During the prior experimental procedures, it was observed that the large hose of the scavenging unit was bulky and at times hard for the researchers to work around. The opening of the nose-cone was wrapped in clear tape, and then a smaller hole was cut to allow for the rodents nose to enter the cone. This adjustment was meant to decrease the amount of isoflurane gas that escaped from the nose cone by making a more snug fit around the animal's nose.

The researchers placed the thinner hose, used to modify the engineering control, directly next to the nose -cone. The researchers were able to work efficiently around the thinner hose while still remaining close to the nose-cone. The average isoflurane exposure during the procedure using these modifications was 2.13 ppm, slightly above the NIOSH REL. This was an approximate 81% decrease compared to the sampling periods before the engineering control was installed. Employees four and two had the highest isoflurane concentration at 2.70 ppm and 2.40 ppm, respectively (Table 3.3).

TABLE 3.3: Comparison of Isoflurane Exposure Before and After Installation of Ventilation Control

Date	Description	Mean (%CV), ppm	Range
10/5/2011	Pre-Engineering Control	15.3 (64.0)	7.7—28.4
10/12/2011	Pre-Engineering Control	15.2 (86.9)	3.1—29.3
1/25/2012	Implementation of Ventilation Control		
1/25/2012	Post-Engineering Control	2.1 (81.5)	0.6—4.0
2/1/2012	Post-Engineering Control	0.5 (57.6)	0.3—0.8
2/8/2012	Post-Engineering Control	2.1 (34.6)	1.3—2.7

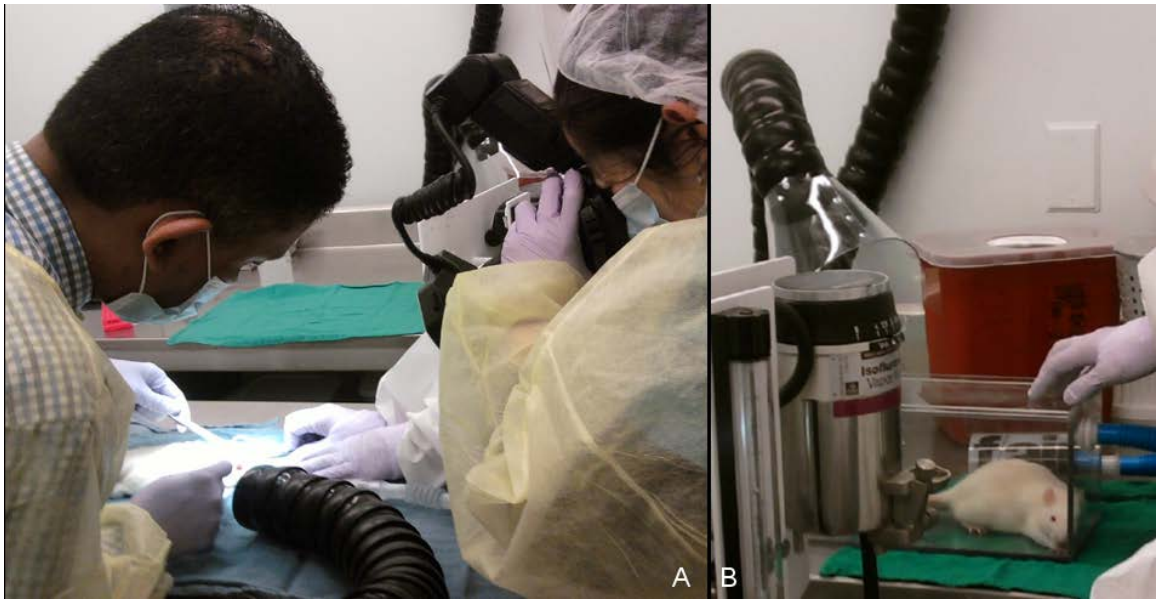

Figure 3.3: Researchers using ventilation control during procedure. A) Vacuum scavenging line placed on the procedure table to scavenging from the nose-cone. B) Vacuum scavenging line placed near the induction chamber.

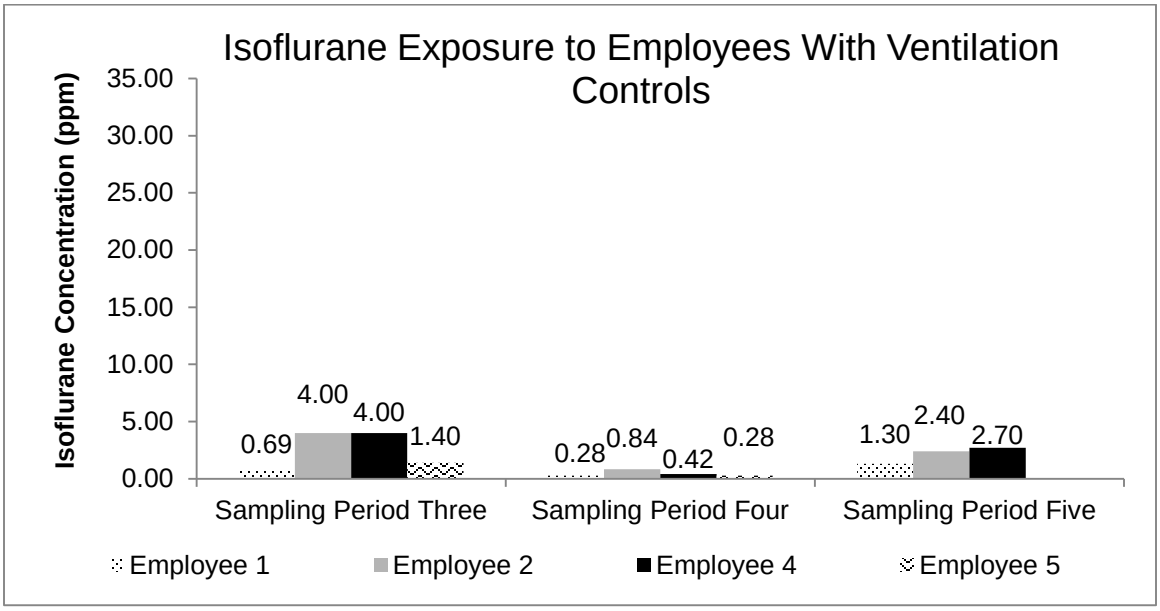

FIGURE 3.4: Isoflurane exposure to employees after installation of active ventilation controls. The ventilation controls dramatically reduced isoflurane exposure. Note that all employee exposures are under or near the NIOSH REL of 2 ppm.

Installation of the engineering control reduced isoflurane concentration a maximum of 97% (29.3 ppm to 0.84 ppm) close to the NIOSH REL of two ppm. The mean reduction in isoflurane exposure was approximately 85%. The engineering control removed isoflurane vapors from the procedure room most efficiently without the attachment of the smaller hose. Researchers continued to use F/Air canisters during procedures in conjunction with the engineering control.

A strength of this study was that the same procedure was conducted in the same room by the same researchers both before and after the use of a ventilation control. Researchers also had clear roles that did not change through the different sampling periods. A limitation of the study was that not all researchers participated in each of the procedures periods that were sampled.

CONCLUSIONS

In conclusion, employees were exposed to isoflurane concentrations well above the NIOSH REL for halogenated anesthetic gases while using passive scavenging techniques to control isoflurane vapors. Our initial hypothesis that the employee responsible for anesthetizing and handling the animals would have the highest isoflurane exposure was incorrect. The highest concentrations of isoflurane were experienced by researchers bending over the anesthetized animal to perform the research procedure. The lowest isoflurane exposure was experienced by the technician anesthetizing the animals. WAG leaking from the nose-cone was likely to have contributed the most to the exposure. Our results demonstrate the importance of considering all sources of isoflurane waste anesthetic gas. An engineering control of additional ventilation effectively reduced isoflurane concentration in the room. The isoflurane exposure was reduced a maximum of 97% and a mean of 86%.

CHAPTER 4

ASSESSMENT OF ISOFLURANE EXPOSURE TO RESEARCHERS DURING ANIMAL PROCEDURES

ABSTRACT

Isoflurane is a commonly used anesthetic gas in veterinary and animal research procedures. Symptoms of acute exposure to isoflurane include headache, nausea, dizziness, unconsciousness, and asphyxia. However, isoflurane exposure to medical researchers has not been well documented. We conducted a study of research procedures using isoflurane at a medical research institution. Full-shift isoflurane exposures to researchers were monitored using passive badges for halogenated anesthetic vapors. Exposures ranged from 0.04 parts per million (ppm) to 9.6 ppm for a 15-minute time period. Species of animal used during the procedure, number of animals, and procedure time were not found to be significant factors associated with isoflurane exposure. Waste anesthetic gas (WAG) control method was found to be a significant factor. Active scavenging methods such as biosafety cabinets and exhaust vacuum lines were significantly more effective in controlling isoflurane WAG than passive scavenging canisters. The role of the researcher was also a significant factor. Researchers who were involved in anesthesia induction and performed the majority of the animal procedure had higher isoflurane exposures than researchers who assisted during the procedure.

INTRODUCTION

Isoflurane is a core anesthetic on the World Health Organization's (WHO) list of essential medicines published in April 2013 (WHO, 2013). It is the preferred anesthetic technique for most animal research and veterinary procedures today (Säre et al., 2011). In the 1980s several studies showed that individuals using isoflurane on animals were at risk for high exposures. Studies also indicated associations between waste anesthetic gas exposure (WAG) and several adverse health effects including reproductive toxicity, organ toxicity, and central nervous system toxicity (Cohen et al., 1980, Guirguis et al., 1990, Spence et al., 1978). The lack of epidemiological data focused on isoflurane WAG exposure has led to confusion concerning the potential long-term health risks caused by occupational isoflurane exposure. The limited knowledge of health outcomes associated with chronic isoflurane exposure is particularly troubling due to the ubiquitous use of isoflurane in research and veterinary practice.

Although isoflurane is widely used, there has been little progress towards any occupational exposure guidelines in the United States. In 1977, the National Institute of Occupational Safety and Health (NIOSH) published a recommended exposure limit (REL) of two parts per million (ppm) for halogenated anesthetic gases (NIOSH, 1977). This standard was actually developed with the anesthetic gas halothane involved. Isoflurane was not included in the REL as it had not been developed for use at that time. NIOSH has not updated the halogenated gas REL or published an REL for isoflurane.

Exposure to isoflurane in animal research settings has not been well characterized. Possible reasons may be the large-scale use of isoflurane use and the unsupported belief that isoflurane is harmless. Inconsistency in research surgical spaces, procedures performed, and species involved in procedures may also contribute to the difficulty of characterizing isoflurane exposure to animal researchers. Many species of animals are utilized in research, and there are different anesthesia techniques

for induction and maintenance based on species of animal. Anesthesia methods may also differ between research protocols. However, isoflurane is widely used to maintain anesthesia during most animal surgical procedures including those performed in veterinary settings.

Use of inhalation anesthetics can result in the pollution of anesthetic gas in the workplace resulting in exposure to employees. In large animal procedures, an anesthetic state is often induced by using a face mask and maintained by intubation. Induction chambers and nose cones are used in small animal procedures. Several studies have already documented that using face masks to induce anesthesia in humans results in a significant increase of WAG concentrations that may exceed limit values (Hoerauf et al., 1997, Hoerauf et al., 1999, Schuyt et al., 1996, Weber, 1994).

The goal of this study was to measure researchers' isoflurane exposure during animal procedures and to observe and document work practices of researchers during isoflurane use. WAG control methods, species of animals used during the procedure, and roles of the investigators were compared to determine which variables were more likely to contribute to isoflurane exposure.

METHODS

Study Population

Participants were identified by the institution's Comparative Medicine department. All researchers at the institution using isoflurane during the time of the study were eligible to participate. A total of 33 laboratories were identified as owning isoflurane vaporizers, which are devices used to deliver isoflurane for anesthesia. An additional seven laboratories were identified as scheduling procedures using an isoflurane vaporizer with the Comparative Medicine department. Forty letters were sent to the principle investigator (PI) or contact person on record for the laboratories requesting researchers to participate in isoflurane monitoring by contacting the study investigators.

A total of thirty-six (90%) replies were received. Of these, twenty of the researchers replied that they were not using isoflurane at this time, the remaining respondents agreed to participate in the study and scheduled a time to be monitored for isoflurane exposure. Four researchers who did not reply were contacted a second time.

A total of 23 different research procedures and thirty-nine individual researchers from 16 different laboratories were included in the study. The study participants included five PIs, twenty-one research technicians, and thirteen junior researchers including graduate students and post-docs. All participants were sampled while using isoflurane to anesthetize animals during research procedures. No interventions were used during this study. Employees were asked to perform work tasks as usual, including all safety and controls methods normally used. The study was conducted between November 2014 and June 2015. This study was conducted as part of institutional safety requirements and with the approval of the University of Nebraska Medical Center institutional review board.

Isoflurane Sampling

Full-shift personal air samples were collected using halogenated anesthetic vapor passive badges purchased from Advanced Chemical Sensors (Boca Raton, FL). Sampling medium in the badges was activated carbon molecular sieve adsorbent. The limit of quantification for the badges was 0.04 ppm for an 8-hour period. The limit of detection for isoflurane was 0.01 ppm for 15 minutes (Advanced Chemical Sensors). One field blank was collected per procedure for quality control and comparison purposes.

The badges were clipped to the collars of lab coats of personnel to represent exposures in their personal breathing zone. Work practices of study participants, procedure descriptions, and scavenging techniques for waste anesthetic gas were

recorded during isoflurane use. The passive badges were analyzed per OSHA method 103 (OSHA, 1994) by Advanced Chemical Sensors.

Statistical Analysis

Differences in isoflurane exposure between researchers working on large or small animal species and scavenging type were analyzed using Mann-Whitney analysis for nonparametric analysis. Large animals were defined as animals that were intubated to maintain anesthesia (pig and rabbit). Small animals species were defined as animals that anesthesia was induced inside an induction chamber and maintained using a nose-cone. Scavenging techniques were categorized as active or passive. Active scavenging was defined as the use of a biological safety cabinet (BSC) or fume hood, and the use of an exhaust line to evacuate isoflurane WAG. Passive scavenging was defined as the use of an activated charcoal canister specifically marketed to control WAG. Only one researcher per procedure was included in the study. Procedures that were repeated by the same researchers were only included once in the study analysis. Samples were chosen based on representativeness of the personal exposure to the individual. A two-sided p-value < 0.05 was considered significant.

Isoflurane exposure was compared between primary and secondary researchers using the Wilcoxon signed rank test. Researchers were divided into two groups: primary or secondary. The primary researcher was defined as the individual who performed the bulk of the procedure including the induction and maintenance of anesthesia. Secondary researchers were defined as individuals who assisted in the procedure and were not responsible for the greater amount of work involved with the animal procedure. Only procedures in which at least two or more researchers were involved were included in the analysis. Two researchers per group were used in the analysis. Procedures performed by the same research group were only included once. A p-value < 0.05 was considered significant.

Differences in isoflurane concentration and the length of time of procedures and the number of animals used in the procedures were analyzed using Spearman correlation statistics. Only researchers who worked with mice were included in this analysis as this was the only species with enough data to conduct the analysis. One researcher per procedure was included in the analysis. There were no repeated procedures included in the analysis. A p-value < 0.05 was considered significant. Full data set is presented in Appendix C.

RESULTS

Procedure Descriptions

Researchers used isoflurane for several procedures. Table 4.1 summarizes the procedures observed for the study. Detailed descriptions of these procedures are provided in Appendix D. Studies included non-survival and survival procedures in pigs, rats, and mice. A single survival study was conducted using rabbits. Non-survival studies included studies that used isoflurane for anesthesia maintenance during a procedure followed by dissection and protocols that used isoflurane as a euthanasia technique. Survival studies included surgical implantation of monitoring devices, imaging, injection delivery, and outcomes of new treatments.

Anesthesia induction and maintenance varied by species of animals used in the procedure. Large animals such as pigs and rabbits were intubated to maintain anesthesia. Anesthesia was induced in pigs using a face-mask. Anesthesia was induced in the rabbit using an injectable anesthetic. Anesthesia was induced in small animals, such as rats and mice, using an induction chamber. Both the open-drop method of using an isoflurane soaked material in an air tight container and isoflurane vaporizer connected to an induction chamber were observed. Protocols that required anesthesia to be maintained for the entire procedure used a nose-cone to keep the animal under sedation.

Several locations were used to conduct the research procedures. The controls available varied by room. Most procedure rooms in the Comparative Medicine facility housed either a biosafety cabinet (BSC) or vacuum exhaust line or both. Researchers working outside of this facility did not always have access to these WAG scavenging controls. If no BSC or vacuum exhaust line was available the researchers relied on passive scavenging canisters to control isoflurane vapors.

Table 4.1: Overview of Monitored Procedures

Procedure Description	Number of Researchers Monitored	Scavenging Method	Animal Species	Number of Animals
Cardiac Arrest Treatment	2	Passive	Pig	1
Cardiac Arrest Treatment	2	Passive	Pig	1
Cardiac Arrest Treatment	2	Passive	Pig	1
Cardiac Arrest Treatment	3	Passive	Pig	2
Surgical Robot Test	5	Active	Pig	1
Acute Cardiac Disease Model	1	Passive	Rabbit	1
Cardiac Arrest Treatment	4	Active	Rat	1
Intraperitoneal Injections	2	Passive	Rat	12
*Liver Cancer Model	3	Passive	Rat	4
Telemeter Implantation	1	Passive	Rat	3
#Body Composition	1	Active	Mouse	23
#Body Composition	1	Active	Mouse	15
Dissection	1	Active	Mouse	10
Dissection	1	Active	Mouse	3
Dissection	4	Passive	Mouse	4
Drug Pump Implantation	1	Passive	Mouse	3
Embryo Transfer Surgery	2	Active	Mouse	6
Heart Ultrasound	2	Passive	Mouse	6
#Humanized Liver Model	2	Active	Mouse	4
Kidney Ultrasound	1	Passive	Mouse	8
MRI	2	Active	Mouse	1
Skin Disease Model	1	Active	Mouse	20
Time Course Model	2	Active	Mouse	5

*Researchers used active scavenging during anesthesia induction and passive scavenging during procedure.
#Researchers used passive scavenging during induction and active scavenging during procedure.

Active vs. Passive Scavenging Techniques

Four active scavenging techniques were observed during the study. They included active vacuum lines, vacuum lines used with passive scavenging canisters, BSC, and BSC used with canisters (Figure 4.1). Researchers who used BSCs with passive canisters were classified as using active scavenging (Table 4.2). Differences in mean isoflurane concentrations between the active scavenging techniques were not calculated due to the small sample size.

Table 4.2 : Active Scavenging Technique Used During Procedures

Scavenging Technique	n	Isoflurane Exposure (ppm)	Mean (% CV), ppm
BSC	2	0.28, 0.64	0.46 (55)
BSC + Canister	5	0.12, 0.25, 0.42, 0.53, 0.56	0.38 (50)
Vacuum Line	2	0.61, 3.6	2.1 (100)
Vacuum Line + Canister	1	1.3	
Total	10	0.12, 0.25, 0.28, 0.42, 0.53, 0.56, 0.61 0.64, 3.6, 1.3	0.83 (123)

Isoflurane exposure for researchers using active scavenging was statistically lower than for researchers who relied on passive scavenging canisters as the only way to control isoflurane WAG ($p = 0.02$) (Table 4.3). A typical surgical procedure area setup using passive scavenging and active scavenging are shown in Figure 4.2 A-B.

Table 4.3: Isoflurane Exposure by Scavenging Technique

Scavenging Technique	n	Mean (%CV), ppm	Range, ppm
Active	10	0.83 (123)	0.12—3.60
Passive	10	3.18 (89)	0.09—9.50

Isoflurane exposure was lower for researchers using active scavenging compared to researchers using passive scavenging, Mann-Whitney, $p = 0.02$.

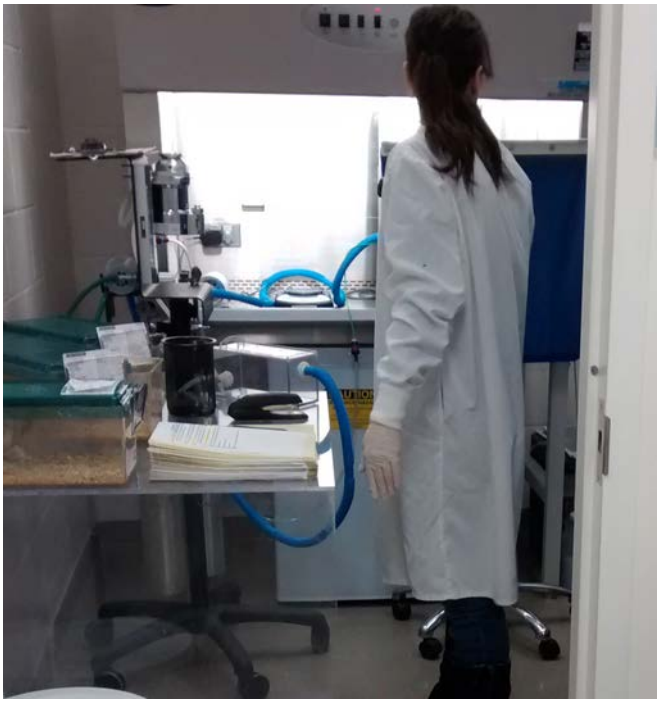

Figure 4.1: Combination of passive and active scavenging controls. Researcher used passive scavenging for induction and BSC for active scavenging during the procedure.

Figure 4.2: Typical setup for small animal surgery. A) Passive scavenging canister is used to control WAG. B) Vacuum lime is used to control WAG.

Large vs. Small Animals Species

Large animals such as the pigs and rabbits were intubated to maintain anesthesia while rats and mice were placed on nose cones to maintain anesthesia. Isoflurane exposure to researchers was not statistically different between researchers working with large animals or small animals (p = 0.08) (Table 4.4).

Table 4.4: Isoflurane Exposure by Species

Species	n	Mean (%CV), ppm	Range, ppm
Small	17	2.07 (122)	0.12—9.5
Large	3	1.66 (107)	0.09—3.6

Isoflurane exposure did not differ by animal species, p = 0.08.

Isoflurane Concentration by Researcher Role

The overall mean isoflurane exposure to researchers was 1.95 ppm. Researchers who had the largest roles during the procedures had higher mean exposure to isoflurane (2.71 ppm) compared to researchers who had secondary or assisting roles (1.18 ppm). This was a statistically significant difference in isoflurane exposure (p = 0.04). A total of 22 researchers (11 pairs) were included in the analysis (Table 4.5).

Table 4.5: Isoflurane Exposure by Role

Role	n	Mean (%CV), ppm	Range, ppm
Primary	11	2.71 (108)	0.25—9.50
Secondary	11	1.18 (97)	0.15—3.70

Isoflurane exposure was higher for researchers who had the primary role in the procedure versus researchers who were assisting, p = 0.04.

Isoflurane Concentration by Procedure Time and Number of Animals

Isoflurane concentration for researchers working with mice was not correlated with the length of the procedure ($p = 0.26$). The number of animals used during the procedure was also not correlated with isoflurane exposure to researchers ($p = 0.20$). A total of 13 samples were included in the analysis.

DISCUSSION

Approximately 31% (12/39) of the researchers included in the study had isoflurane exposures above the NIOSH REL of two ppm for halogenated anesthetic gases; so by current practice standards we can state that 31% of the participants were over-exposed to isoflurane vapors.

We used passive sampling badges that adsorb isoflurane to monitor for isoflurane exposure. Some research groups have monitored isoflurane exposure by using biomarkers to determine actual isoflurane concentration in the body (Accorsi et al., 2001, Al-Chanem et al., 2008, Imbriani et al., 1988). These studies found that ambient air levels of isoflurane were correlated to urinary isoflurane concentration. Periago et al., (1993), reported that isoflurane concentration in the exhaled breath of exposed individuals was also correlated to ambient isoflurane concentration.

While these sampling methods have the benefit of quantifying isoflurane load in the body, they require biological samples. Biological samples are generally more difficult to obtain and have higher costs associated with storage, shipping, and analysis. Due to these constraints, biological monitoring of isoflurane has not been used to monitor isoflurane exposure in the field.

Other sampling methods for isoflurane include active sampling methods. Personal pumps which draw air into a charcoal tube can be used to more accurately quantify exposure. However, the pumps are more susceptible to human error, as they can be accidently turned off during the monitoring period or develop mechanical

difficulties. Real-time exposure monitoring instruments have been developed that can be used to measure isoflurane concentration in the air. These units are associated with a high initial cost and are usually not justifiable in most occupational settings. We chose to use passive monitoring badges as they are the most cost efficient, easy to use, and the most used method to monitor for isoflurane concentration.

Procedures being conducted at a single research institution can vary widely. We sampled nineteen different procedures using four different species of animals. Research was conducted in several different rooms and four different building on the same campus. Air exchanges in the different rooms and buildings may impact isoflurane concentration. Older buildings may have less efficient exhaust which would increase isoflurane concentration in the room. However, due to the equipment required for the procedure and familiarity of laboratory spaces, researchers may be disinclined to work in newer rooms specifically designed with a high number of air exchanges. Scavenging capabilities may also be impacted by the procedure room. Some of the procedures took place in rooms that had a BSC or vacuum exhaust line available, while other rooms had no means of active scavenging.

As many research procedures as possible were included in the study. However, the sample size was too small to fully investigate all of the different variables. Isoflurane concentration and flow rate were too variable to be compared in the small sample size. However, these factors have the potential to influence individual isoflurane exposure. This study was also not able to evaluate the effectiveness of different active scavenging methods. It would be interesting to compare BSCs and vented fume hoods to vacuum exhaust lines to determine if they are equally efficient in removing isoflurane vapors. We were not able to compare researchers' work practices, such as latching an induction chamber closed vs. keeping the latch open, or concentration and flow rate of isoflurane used during the procedures. We would also be interested in conducting a study to

examine different nose-cones in rodents to determine how nose-cones may impact isoflurane exposure. Future studies with a larger number of participants could compare some of these factors.

There are several individual work practices that can be implemented to reduce exposure to isoflurane. An example of simple behavior is ensuring that induction chambers are closed and air-tight during use. Personnel can also decrease their exposure by performing tasks that do not require isoflurane before the procedure, thus reducing exposure time. These work practices can be taught but are difficult to enforce at an institutional level.

Environmental controls, however, are controls that institutions can regulate to reduce isoflurane exposure to many users. Rooms in which isoflurane are used should have an appropriate number of air changes and ventilation. NIOSH recommends 15 air changes per hour for clinical operating theatres (NIOSH, 2007). Recommended ventilation for laboratories is 4 to 12 air changes per hour (OSHA, 1990). Active scavenging methods should be used whenever possible. Active scavenging methods can be as simple as using a ventilated hood or biosafety cabinet. OSHA recommends that researchers use ventilated fume hoods whenever possible when working with hazardous or potentially hazardous chemicals (OSHA, 2011). Institutions may also provide vacuum lines attached to the building exhaust to remove isoflurane efficiently from procedure areas. Passive scavenging techniques using activated charcoal canisters are not enough to keep exposures below the two ppm NIOSH REL for halogenated anesthetic vapors.

CONCLUSION

Active scavenging techniques significantly reduce isoflurane exposure to animal researchers. Researchers who used only passive scavenging canisters had a mean isoflurane exposure twice as high as researchers who used active scavenging or active scavenging with passive scavenging canisters. Isoflurane exposure was also lower for researchers working with large animals compared to small animals. This difference is due to the difference in anesthesia induction and maintenance techniques. Researchers can induce anesthesia using injectable anesthetics and then intubate large animals, but must use nose-cones for small animals. Both induction chamber and nose cones can result in waste anesthetic gas due to leaks and opening of the induction chamber. The species of animal used during procedures did not have an effect on isoflurane exposure. Procedure time and number of animals did not have an effect of isoflurane exposure on researcher working with mice. Researchers who were designated as the primary individual conducting procedures had a higher exposure to isoflurane than researchers who assisted in procedures.

CHAPTER 5

COMPARISON OF PASSIVE AND ACTIVE SCAVENGING METHODS TO REDUCE EXPOSURE TO ISOFLURANE IN RESEARCH LABORATORIES

ABSTRACT

The two most common scavenging methods for isoflurane gas are passive scavenging canisters with activated charcoal and active scavenging. Passive scavenging is the use of a material to adsorb the air pollutant. Passive scavenging requires the pollutant to diffuse from the source into the scavenging material. In active scavenging, the polluted air is pulled out of the room by building exhaust through a vacuum line or ventilated hood. Refresh™, VaporGuard™, and F/Air™ are three brands of passive scavenging canisters that were included in the study. Isoflurane concentration was compared between four scavenging conditions (no scavenging, passive scavenging, active scavenging, and combined active and passive scavenging). The mean isoflurane concentration found without any controls was significantly higher compared to active scavenging (10.23 ppm, %CV = 11.53 and 1.43 ppm, %CV = 15.38, respectively). The mean isoflurane measured during the passive scavenging condition was 10.35 ppm (%CV = 57.97). Overall, there was a significant difference in isoflurane concentration between the conditions ($p > 0.001$). There was a slight difference in isoflurane concentration between canister brands; ReFresh canisters had a lower mean isoflurane concentration than the F/Air brand of canisters (6.48 ppm, %CV = 30 and 15.76 ppm, %CV = 50) ($p = 0.04$). However, mean isoflurane concentration while using all three brands of canisters exceeded the NIOSH REL of 2 ppm for halogenated anesthestic gases. Passive scavenging canisters are not equal to vacuum exhaust lines in scavenging capabilities. Researchers should use active scavenging methods to control isoflurane WAG exposures.

INTRODUCTION

Gas leakage from face masks or nose cones, anesthetic machinery, and malfunction of scavenging equipment are the primary sources of occupational exposure to isoflurane waste anesthetic gas (WAG) (Hoerauf et al., 1996, Kelly et al., 2011, Todd et al., 2013). As isoflurane is the primary anesthetic in the research setting, scientists and laboratory personnel are at risk for occupational exposures to isoflurane. Available literature has not provided an overall picture of health consequences associated with occupational exposure to isoflurane. A few studies have demonstrated adverse health effects, but the literature is not consistent. Low dose exposures typical of occupational exposures have been found to cause damage to sister chromatids similar to damage caused by cigarette smoking (Hoerauf et al., 1999). To reduce risk of health damage to workers, best practice guidelines state that all WAGs should be kept to the smallest amount possible (Barker et al., 1997).

Ways to control WAG include passive scavenging canisters, active vacuum scavenging devices, ventilation through a heating, ventilating and air conditioning (HVAC) system, as well as scavenging face masks and rebreathing circuits for larger animals. The use of passive scavenging canisters may not result in the intended result of lower isoflurane exposure. A study of three different brands of passive scavenging canisters found that there was large variability in scavenging capability both between brands and between canisters of the same brand (Smith et al., 2003). The study also found that several canisters actually emitted isoflurane vapor after use. Studies of isoflurane WAG in clinical settings found that passive scavenging canisters could effectively control isoflurane concentration (Coleman et al., 1994, Imberti et al., 1995). However, these studies overlook the effect of other WAG controls such as air exchanges in the spaces and the use of intubation to maintain anesthesia, which could actually be responsible for the low isoflurane concentration.

Studies of active scavenging techniques have found that these techniques efficiently control WAGs. Smith et al. (2002), found that fume hoods are able to efficiently reduce WAG exposure to personnel. Another study found that an exhaust vacuum line reduced isoflurane concentration during anesthesia induction and nose-cone maintenance for small animals (Nesbitt et al., 2013). A study on dogs found that active scavenging from the face-mask reduced isoflurane WAG (Friembichler et al., 2011). Appropriate use of vacuum scavenging lines has also been shown to reduce isoflurane concentration to close to the lower detection limit (Todd et al., 2013).

The literature on WAG exposure has reported several sources of isoflurane and other WAG exposure. Kelly et al. (2011), reported in a study of ten different isoflurane vaporizers that the vaporizers were highly inaccurate in delivering the isoflurane at the concentration and flow rate set by the users. Inaccurate vaporizers may not only result in an overexposure of isoflurane to users, it may also be dangerous for any patients or animals, as they are not receiving the intended level of medication.

Leakage from anesthesia maintenance devices has been found to significantly influence isoflurane exposure. Hoerauf et al. reported that use of laryngeal masks resulted in higher exposure than intubation (Hoerauf et al., 1996). A study of rodents found that isoflurane leakage from rodent nose-cones substantially contributed to isoflurane exposure to workers (Smith et al., 2006). Modified nose-cones for rodents were reported to help reduce this exposure. Nose-cones can be modified to be used with an active scavenging line (Nesbitt et al., 2013) or with a simple diaphragm (Smith et al., 2006, Todd et al., 2013).

Current guidelines published by professional veterinary societies to reduce WAG concentration, state that the use of scavenging devices is the most effective way to decrease waste anesthetic gases. The guideline goes on to say that both passive and

active scavenging systems are effective if used properly (American College of Veterinary Anesthesia and Analgesia, 2013). This statement seems to imply that passive and active scavenging devices are equally effective, which early studies negate (Gardner, 1989, Ward et al., 1982). We tested these recommendations by comparing isoflurane concentration in a procedure room when using passive scavenging canisters, active scavenging, and a combination of passive and active scavenging. We used these comparisons to test the hypothesis that passive scavenging canisters will reduce isoflurane WAG to two parts per million (ppm) or below during 30 minutes of exposure.

METHODS

Isoflurane concentration was measured during four environmental conditions in the same room. The room was part of the Comparative Medicine facility, where many animal procedures take place. Room dimensions were 10 feet 9 inches by 11 feet 5 inches with a ceiling height of 9 feet for a volume of 1026.54 feet^3 (312.89 meters^3). Ventilation for the Comparative Medicine facility was 27.96 air exchanges per hour as reported by the Comparative Medicine facility.

The four environmental conditions were “no scavenging” in which no effort was made to reduce isoflurane vapor in the room, “passive scavenging” in which we used activated charcoal canisters to control isoflurane vapor. The other two conditions involved the use of an exhaust vacuum line. The “active scavenging” condition was defined as the use of the vacuum line alone and the “combined scavenging” condition was the use of the vacuum line in tandem with passive scavenging canisters.

Isoflurane was introduced into the environment and controlled using a model vapor 19.1 isoflurane vaporizer (Drägerwerk, Lübeck, Germany) that had been serviced and calibrated within the past year. All hoses and anesthesia equipment were checked for leaks each day prior to use. Passive scavenging canisters were weighed daily for

four days prior to and after exposure to isoflurane gas to determine weight variability. The performance of the balance (Sartorius, Model L 2200 P, Gottingen, Germany), used to weigh the canisters was evaluated daily with a set of eight standard weights ranging in weight from 50 grams (g) to 500 g.

No Scavenging

Baseline isoflurane concentration was measured in the procedure room using a MIRAN Sapphire model 205 BXL (Thermo Fisher Scientific, Franklin, MA) infrared ambient air analyzer. An isoflurane vaporizer was connected to a rodent nose cone using flexible hosing. Isoflurane was introduced into the procedure room for thirty minutes at a concentration of 1.5% and flow rate of 1 liter per minute (L/min). Isoflurane concentration in the room was measured and logged at 90-second intervals until ambient isoflurane concentration returned to baseline. The 90-second interval was the shortest possible interval allowed by the instrument. The procedure was conducted a total of three times, consecutively, on one day.

Absorption of Isoflurane by Passive Scavenging Canisters

Three brands of passive scavenging canisters (F/Air™, VaporGuard™, ReFresh™) were weighed once daily for four days. A passive scavenging canister was connected to the exhaust line of a rodent nose cone with cotton plug representing a mouse, attached to the isoflurane vaporizer. Isoflurane was released for 30 minutes at a concentration of 1.5% and flow rate of 1 L/min. Isoflurane concentration in the room was logged at 90-second intervals until isoflurane concentration returned to baseline. Canisters were weighed immediately after exposure to isoflurane. Canisters were weighed once daily for three days after isoflurane exposure. The procedure was conducted with a total of five canisters per brand and each canister was tested once (n=15). This experiment was carried out in one day in triplicate. (Figure 5.1).

Figure 5.1: Control comparison setup. A) Isoflurane was measured using a MIRAN Saphhire direct reading instrument. B) Isoflurane was introduced into the room via a nose-cone with cotton plug representing a mouse. Isoflurane vaporizer line, passive canister, and vacuum line can be seen in the picture.

Effectiveness of Active Scavenging to Control Isoflurane Concentration

Flexible tubing was connected to the building exhaust and placed near the nose cone. Isoflurane was released for thirty minutes at a concentration 1.5% and flow rate of 1 L/min. Isoflurane concentration was recorded and logged at 90-second intervals until the isoflurane concentration returned to baseline. The active scavenging was discontinued after isoflurane was turned off. The procedure was conducted three times. The room was allowed to return to baseline isoflurane concentration between trials.

Effectiveness of a Combined Passive and Active Scavenging Technique

A passive scavenging canister was attached to the exhaust port of the nose cone. An active vacuum line as described above was placed near the nose cone. Isoflurane was released into the room for thirty minutes at a concentration of 1.5% and flow rate of 1 L/min. Isoflurane concentrations were recorded and logged at 90-second intervals until the room returned to baseline. Active scavenging was discontinued immediately after isoflurane was turned off. The passive canister was weighed immediately after isoflurane exposure. The procedure was conducted three times for each brand of canister (n=9).

Statistical Analysis

All statistical analysis was performed using SAS 9.3 software (Cary, NC). Linear regression and correlation statistics were used to determine whether daily measurements of known weight varied significantly over time. Linear regression analysis and correlation statistics were used to determine whether canister weight varied over time.

Repeated measures analysis of variance (ANOVA) for one factor was used to determine whether the isoflurane concentration was significantly different using scavenging methods compared to the no scavenging condition. Post hoc Tukey's

adjustment was used to compare differences between groups. A $p < 0.05$ was considered significant. Repeated measures (ANOVA) for one factor was used to determine whether individual trials of the same condition varied significantly. A $p < 0.05$ was considered significant. Repeated measures (ANOVA) for one factor was used to determine whether isoflurane concentration differed between each passive canister brand. Post hoc Tukey's adjustment was used to compare differences between groups. Only measurements from the passive scavenging condition were used in the analysis. A $p < 0.05$ was considered significant.

RESULTS

Canister Weight

The performance of the balance was evaluated with eight known weights over eighteen days. There was no change in the balance accuracy over time for any of the standard weights ($p > 0.05$). Linear regression analysis also showed no change in weight over time for the standard weights ($p > 0.05$) (Table 5.1).

Table 5.1: Balance Calibration

Known Weight	n	Mean (SD), g	Range, g	P - value
50	18	50.17 (0.01)	50.14—50.19	0.18
100	18	99.95 (0.01)	99.93—99.96	0.43
200	18	200.14 (0.05)	200.07—200.22	0.93
300	18	300.10 (0.05)	300.03—300.18	0.79
350	18	350.28 (0.05)	350.20—350.36	0.33
400	18	400.32 (0.02)	400.28—400.34	0.22
450	18	450.49 (0.03)	450.42—450.52	0.61
500	18	500.25 (0.12)	499.78—500.30	0.13

Canister weight for the ReFresh™ brand of canister was not correlated with time. Canisters did gain weight after exposure to isoflurane. However, the weight gain was small and the canisters did not lose weight after exposure. One of the VaporGuard™

and two of the F/Air™ canisters were marginally correlated with time ($p = 0.04$). All canisters gained a small amount of weight after exposure to isoflurane. Significant correlation over time may be related to weight gained due to isoflurane exposure. The weights of the other VaporGuard™ and F/Air™ canisters were not correlated with time (Table 5.2). Overall the weight of all of the canisters was stable over time.

Table 5.2: Canister Weight Before and After Isoflurane Exposure

		Before		After		
Canister	N	Mean (%CV), ppm	Range, ppm	Mean (%CV), ppm	Range, ppm	p-value
ReFresh 1	4	396.32 (0.01)	396.26—396.35	397.12 (0.07)	396.83—397.48	0.06
ReFresh 2	4	381.17 (0.01)	381.13—381.21	381.35 (0.09)	381.00—381.80	0.9
ReFresh 3	4	386.29 (0.01)	386.23—386.33	386.61 (0.08)	386.28—387.02	0.43
ReFresh 4	4	390.77 (0.01)	390.73—390.82	391.41 (0.07)	391.12—391.78	0.1
ReFresh 5	4	403.02 (0.01)	402.94—403.06	403.53 (0.06)	403.26—403.86	0.12
VaporGuard 1	4	369.11 (0.03)	368.93—369.23	370.14 (0.25)	369.14—371.31	0.45
VaporGuard 2*	4	364.35 (0.04)	364.13—364.48	363.50 (0.26)	362.45—364.74	0.01*
VaporGuard 3	4	367.71 (0.05)	367.46—367.85	367.30 (0.27)	366.21—368.58	0.08
VaporGuard 4	4	370.22 (0.05)	369.99—370.35	369.89 (0.24)	368.88—371.00	0.09
VaporGuard 5	4	369.08 (0.04)	368.87—369.19	369.00 (0.25)	367.95—370.13	0.26
F/Air 1	4	293.67 (0.03)	293.58—293.80	295.22 (0.07)	294.99—295.50	0.02*
F/Air 2	4	292.50 (0.03)	292.43—292.58	293.02 (0.06)	292.82—293.21	0.12
F/Air 3	4	311.18 (0.03)	311.10—311.27	311.21 (0.06)	311.00—311.45	0.41
F/Air 4	4	292.98 (0.04)	292.89—293.11	293.31 (0.09)	293.11—293.69	0.45
F/Air 5	4	294.38 (0.03)	294.30—294.47	295.41 (0.07)	295.18—295.68	0.04*

*Indicates significance at 0.05 level

Canister Comparison

The mean isoflurane concentration while using passive scavenging canisters was above 2 ppm for all brands of canisters. The highest isoflurane concentrations were recorded while using the F/Air™ canisters alone (Table 5.3). Comparison between the no scavenging condition and each of the passive scavenging canisters brands alone showed a slight difference in isoflurane concentration ($p = 0.03$). Post hoc Tukey's analysis showed a significant difference between ReFresh and F/Air canisters. No other comparisons were significant. Isoflurane concentration while using active scavenging and passive scavenging canisters was not included in the canister comparison.

Table 5.3: Isoflurane Exposure while using Passive Scavenging Canisters

Canister	n	Mean (%CV), ppm	Range, ppm
No Scavenging	3	10.23 (11.53)	9.11—11.47
*ReFresh	5	6.48 (29.94)	4.22—8.79
VaporGuard	5	8.80 (17.50)	6.92—10.56
*F/Air	5	15.76 (49.87)	5.58—23.88

*Indicates significance at the 0.05 level

Comparison of Scavenging Techniques

Repeated measures ANOVA model showed that type of scavenging was a significant factor in isoflurane concentration ($p < 0.0001$). Post hoc analysis of comparisons showed isoflurane concentration differed between no scavenging and combined scavenging, passive scavenging and active scavenging, and passive scavenging and combined scavenging at the 0.05 significance level. Comparisons of no scavenging and active scavenging alone showed that isoflurane concentration was also different between active scavenging and no scavenging ($p < 0.0001$) (Figure 5.2).

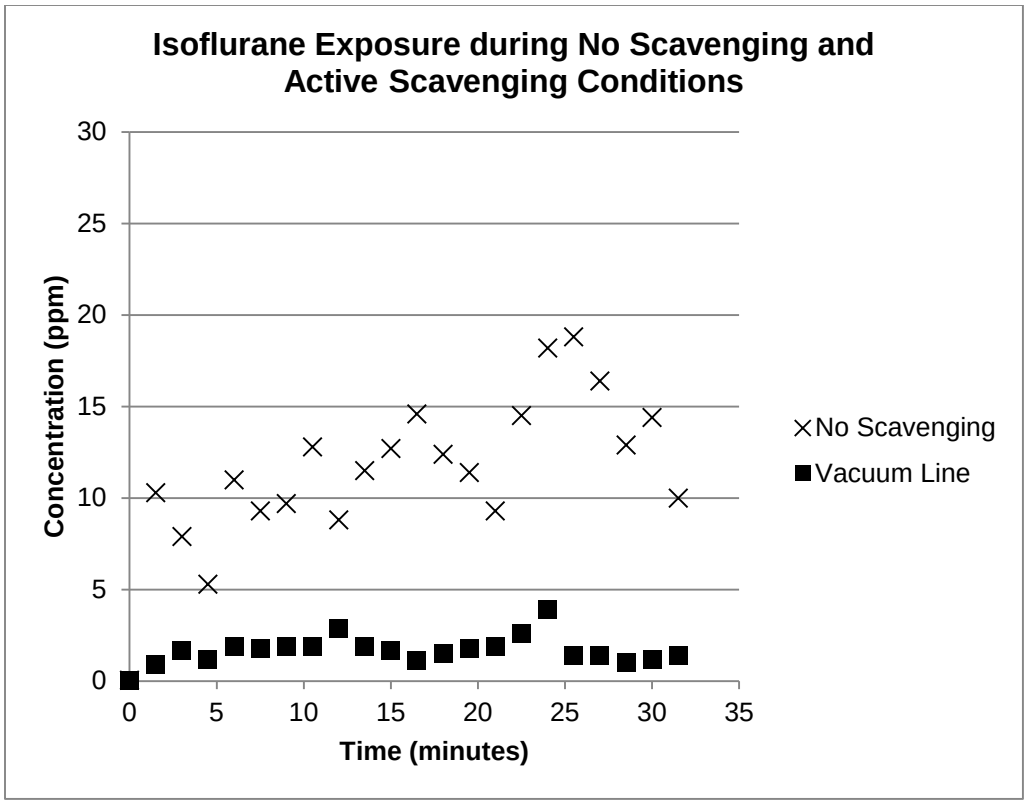

Figure 5.2: Isoflurane exposure without scavenging and while using an exhaust vacuum line. Isoflurane exposure was significantly lower while using active scavenging.

The mean isoflurane concentrations for the no scavenging and passive scavenging conditions were very similar (10.23 and 10.35, respectively) and higher than for the active and combined scavenging conditions (1.43 and 0.59, respectively) (Table 5.4). Both the “no scavenging” and passive scavenging conditions resulted in a mean isoflurane concentration well above the NIOSH REL of two ppm. Isoflurane concentration measured over time was similar between the “no scavenging” condition and while using ReFresh and VaporGuard canisters. Isoflurane concentration was higher while using F/Air canisters compared to the other passive canisters (Figure 5.3).

Table 5.4: Isoflurane Exposure during Experimental Scavenging Conditions

Condition	n	Mean (%CV), ppm	Range, ppm
No Scavenging	3	10.23 (11.53)	9.11—1.47
Passive Scavenging	15	10.35 (57.97)	4.22—23.88
Vacuum Line	3	1.43 (15.38)	1.28—1.68
Combined Scavenging	9	0.59 (45.76)	0.07—0.28

P-value < 0.0001

Active scavenging and combination scavenging resulted in a mean isoflurane concentration below two ppm. The maximum isoflurane concentration was also below two ppm while using these scavenging techniques.

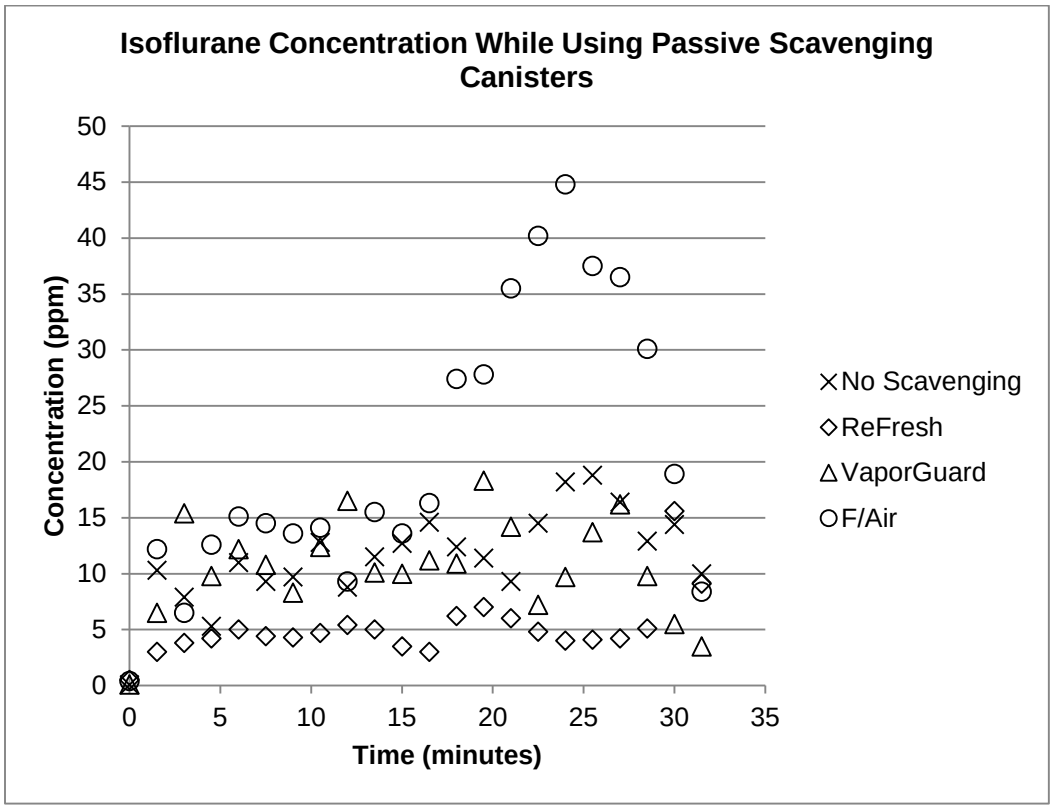

Figure 5.3: Isoflurane exposure during without scavenging and while using passive scavenging canisters. Isoflurane exposure was similar without scavenging and while using canisters.

Comparison of Active and Combined Scavenging Techniques

Combination scavenging technique combined a passive scavenging canister with an active vacuum scavenging line. Isoflurane concentration was reduced approximately 50% compared to active scavenging alone. Repeated measures ANOVA with post hoc Tukey's comparison analysis showed that the difference in isoflurane concentration was not significant. Repeated measures ANOVA also showed that there was no difference in isoflurane concentration between canister brands when used in combination with active scavenging (Figure 5.4).

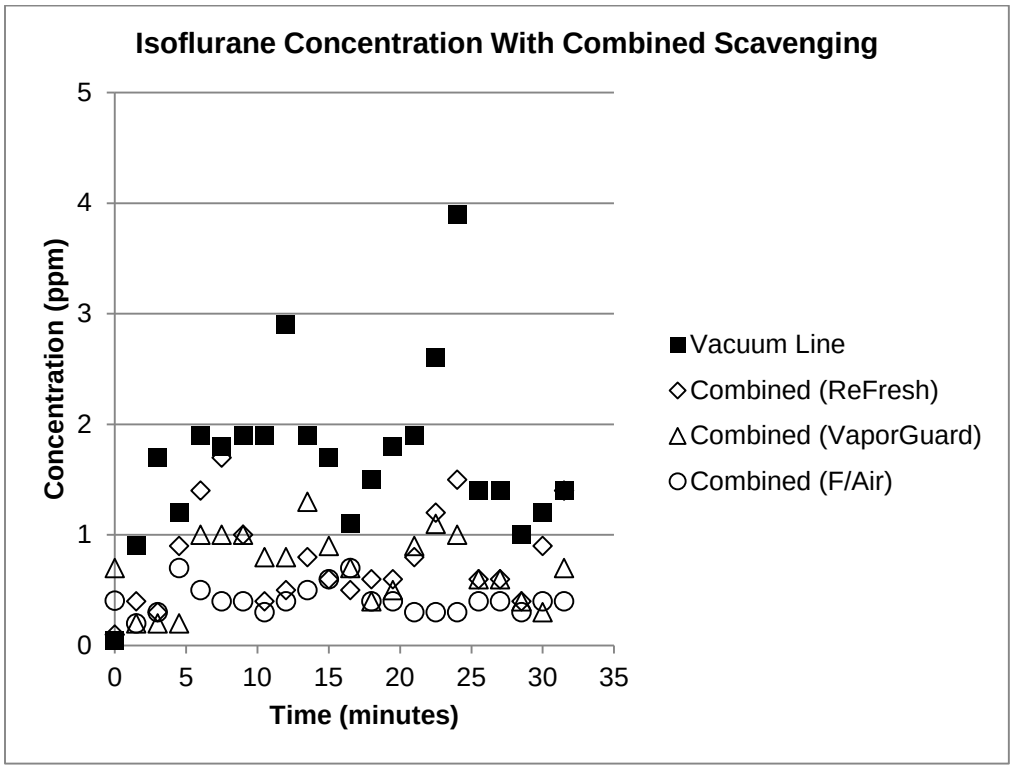

Figure 5.4: Isoflurane exposure was not statistically different between the vacuum line and the vacuum line combined with a passive scavenging canister.

DISCUSSION

Mean isoflurane exposure was below the NIOSH REL of two ppm for halogenated anesthetic gases when using an active scavenging source. The NIOSH REL is a 1-hour limit, meaning that exposure to halogenated gases should not be above two ppm, when used for 1-hour or greater. Although this standard was developed before the introduction of isoflurane as a halogenated anesthetic gas, it is the only guidance currently available that has been developed in the U.S. The American Conference of Governmental Industrial Hygienists (ACGIH) has listed isoflurane among chemical substances that are under study for the year 2015. This is the only U.S. body likely to publish an updated standard for isoflurane in the near future. A workplace exposure limit (WEL) of 50 ppm over an 8-hour work day has been adopted by member states of the European Union (United Kingdom Health and Safety Executive, 2013). However, we have shown that the NIOSH two ppm standard is achievable if active scavenging methods are employed. Examples of active scavenging methods are the use of a vacuum exhaust line similar to what was used in this study and exhausted chemical safety hoods such as class II BSCs.

Passive scavenging canisters, when used alone, did not reduce isoflurane concentration to an acceptable level. The passive scavenging canisters as a whole were not different from the no scavenging condition. Little manufacturer information is available concerning the charcoal used in the canisters and the construction of the canisters themselves, as this is proprietary information. It is difficult to determine what differences between canisters brands may be related to scavenging capability. All of the passive scavenging canisters contained activated charcoal, which is supposed to control isoflurane WAG by adsorbing the isoflurane to the charcoal. The problem with this strategy is that there is no air flow being directed into the canisters. We also observed

that isoflurane concentration was actually higher when using the F/Air canisters than when not using any scavenging. It is possible that any isoflurane-contaminated air that entered the canister actually exited the canister before the charcoal could adsorb any isoflurane. This is an effect known as channeling (Smith et al., 2003).

Flow rate may impact how well a passive canister adsorbs isoflurane because of this channeling effect. Higher flow rates are more likely to overload the canisters, thus reducing their ability to retain air long enough for the isoflurane to be exposed to the activated charcoal long enough to adsorb to it. We used a lower flow rate of 1.5%, to recreate what was used by a majority of the researchers to maintain anesthesia in rodents. We used a concentration of 1% isoflurane as this was the concentration most often used to maintain anesthesia.

Surgical wrap was used to mimic the effect that an animal would have in blocking some air flow from the nose-cone. The middle of the surgical wrap was shaped to be similar to the nose of a mouse and secured in the nose-cone using surgical tape. We did not use live animals because this could have resulted in random error due to the variability in the shapes of the animals' noses and respiration. The main objective of the study was to determine the effectiveness of WAG controls. As such, we kept to a minimum variables that could influence isoflurane exposure. This is also why only one procedure room was used during the course of the study. The procedure room was located in a secure, environmentally controlled underground facility. This allowed for the equipment to be housed in the room to reduce any variability that could be caused by exposure to the outside air or transport. The environmental controls ensured that temperature and relative humidity were stable throughout the study. Air exchanges were controlled by the facility.

Passive scavenging canisters are not a cost effective way to control isoflurane WAG. The purchasing cost of the canisters is between $8 and $10 each. The cost increases when disposal of the used canisters is considered. Disposal of the canisters is regulated by the Environmental Protection Agency (EPA) and the Department of Transportation (DOT). Canisters must be handled as EPA regulated D001 ignitable waste and shipped outside of facilities for disposal following DOT NA1361 regulations (Department of Transportation, 2010). The cost for canister disposal at our institution is $180 for approximately 60 canisters. If one canister is used per day the total cost of passive scavenging is approximately $4,750 per year. The active scavenging vacuum line greatly reduced isoflurane concentration. Costs associated with vacuum line scavenging will be based on individual exhaust systems and are difficult to quantify. However, active scavenging with a fume hood is likely to be as or more efficient than a vacuum line. Costs associated with a fume hood are initial purchase and installation and energy consumption. Conventional bypass fume hoods use approximately 35,000 kilowatt-hours (kWh) of electricity per unit and costs approximately $6,000 in energy use each year (U.S. Department of Energy, 2015). This cost is based on 24-hour per day use with a fully open sash (29 inches). Energy costs dramatically decline as the sash opening is reduced; energy associated with a sash opening of 5 inches is approximately 5.500 kWh and $1000 (U.S. Department of Energy, 2015). Energy costs will differ by city and state; however energy use will remain approximately the same.

It is possible that researchers may have a false sense of security when using passive scavenging canisters to control WAG. As we have shown, passive canisters do little to reduce isoflurane exposure to researchers. However, several researchers at the institution relied solely upon passive canisters to control WAG. Researchers may overestimate the protection offered by passive canisters. Researchers, who have access

to BSCs, fume hoods, or vacuum exhaust lines should be encouraged to utilize these tools responsibly by keeping the sash as low and possible, especially when not in use. If used responsibly, the energy costs associated with a fume hood will be considerably less than the cost associated with passive scavenging.

We have observed several researchers at our institution using active scavenging in combination with passive scavenging canisters. The results of this study show that there is no real benefit to using passive scavenging canisters with an active scavenging vacuum line. For the purpose of this study, it was assumed that ventilated hoods would reduce isoflurane exposure either as much as or greater than an active scavenging vacuum line. Therefore, the ability of ventilated hoods to reduce isoflurane exposure has been inferred based on the active scavenging vacuum lines.

A strength of this study is that we measured isoflurane concentration using a direct reading instrument. The instrument was set to measure isoflurane in an operator's breathing space. This allowed us to determine the effectiveness of different controls in protecting an employee from isoflurane vapors. Smith and Bolon (2003) compared the scavenging capabilities of passive scavenging canisters by measuring isoflurane concentration at the canister. This approach is sufficient in determining how well the passive canisters retain isoflurane vapor, but is not sufficient to determine if they are protective of the operator.

Several portable active vacuum scavenging instruments are now available to control WAGs. These devices use a pump to draw air into a reservoir of activated charcoal. These devices could be useful in rooms that do not have a source of active scavenging available. The use of these devices has not been reviewed in the scientific literature. A logical next step for this study would be to evaluate several of these devices to determine how effectively they control isoflurane WAG.

CONCLUSION

Isoflurane concentration was lower when using an active scavenging source compared to using no scavenging or passive scavenging canisters. Isoflurane concentration was only slightly lower when using passive scavenging canisters. However, using passive scavenging canisters resulted in a mean isoflurane concentration above the NIOSH REL of two ppm. The use of active scavenging and passive scavenging canisters, reduced isoflurane concentration by approximately 50% compared to active scavenging alone. However, there was no statistical difference in active scavenging and combined scavenging when compared to all experimental conditions.

The most cost effective control method to reduce isoflurane exposures in laboratory researchers is active scavenging. Active scavenging includes fume hoods (including biosafety cabinets) and vacuum lines. Active scavenging techniques reduced isoflurane exposures to laboratory researchers below the NIOSH REL for anesthetic gases of 2 ppm. Passive canisters are not effective in reducing exposures below the NIOSH REL. Combining the active and passive scavenging techniques further reduced exposures, though this reduction was not statistically significant from the active scavenging data alone. Given the cost of purchasing new canisters and discarding used ones, this approach may not be cost effective.

CHAPTER 6
CONCLUSION

This dissertation identified possible overexposures to medical researchers who use isoflurane. However, because an isoflurane-specific standard is not currently available, we are not able to conclude if these researchers are at a risk of developing health hazards associated with isoflurane. Nevertheless, the data underscores the need for an occupational exposure limit that includes isoflurane. The NIOSH REL for halogenated anesthetic gases is the closest standard applicable to isoflurane. The NIOSH REL needs to be revised to include isoflurane. At the time the standard was developed isoflurane and other newer generation halogenated anesthetic gases were not available.

In addition, this standard was written to protect health care professionals. The use of isoflurane has shifted to the veterinary and research fields. These populations need to be considered when revising the standard. When the NIOSH standard was developed there was insufficient health data available to determine what exposure concentrations of halogenated gases could be determined as reasonable safe. The result was a standard which was based mostly upon the sampling and detection techniques at the time. Isoflurane has been in use for decades.

Unfortunately, while research and technology have moved forward in sampling and analysis, epidemiological research on human health effects has remained stagnant. The systematic review of the literature in chapter two revealed that most studies with health effects data were focused on the healthcare population and a mixed anesthetic gas exposure. Future studies need to be conducted with study populations in the veterinary or research fields. These are the fields in which isoflurane is used the most

often. Studies looking for long-term health outcomes also need to be conducted. There is evidence that isoflurane may cause genetic mutations.

Employees in the research field are at risk for over exposure to isoflurane. In the study of dental researchers in chapter three, the most important source of isoflurane was the nose-cone. This was an unanticipated result, as we assumed that the induction chamber would be the greatest source. It is important to consider all possible sources of isoflurane WAG to accurately assess exposure and when designing controls. A ventilation control that could be manipulated for use with the induction chamber and the nose cone greatly reduced isoflurane exposure to the researchers. The isoflurane exposure was reduced a maximum of 97% and a mean of 86% after the installation and use of this control.

A study of several researchers and procedures in chapter four revealed scavenging technique and researcher roles during a procedure greatly affect isoflurane exposure. Researchers who used an active scavenging technique such as a BSC or exhaust vacuum line had significantly lower isoflurane exposures than researchers who relied solely on passive scavenging canisters. This indicates that passive scavenging in conjunction with normal room ventilation is not sufficient to protect researchers from over exposure. Almost one third of the researchers monitored for the study had an isoflurane exposure above the NIOSH REL of two ppm for halogenated anesthetic gases.

Species of animals on which the procedures were being performed was not associated with isoflurane exposure. This was a surprise because we would expect larger amounts of isoflurane to be used on larger animals, thus increasing the possibility of overexposure. However, large animals such as pigs are intubated to maintain anesthesia. This should have reduced the most likely source of the isoflurane, which is

the nose-cone or face-mask. This result may have been impacted by the small sample size available for large animal procedures.

In the control comparison study in chapter five, passive scavenging canisters did not reduce isoflurane exposure. Passive scavenging canisters also had a high variance between brands and between canisters of the same brand. Canister weight was stable before isoflurane exposure, which indicated that environmental conditions should not have had an impact on canister performance. Canister weight after exposure was also fairly stable which indicated that canisters were not likely to off-gas isoflurane. The lack of off-gassing, combined, with the high isoflurane concentrations measures in the room indicated that the canisters were not able to adsorb isoflurane efficiently. Active scavenging using an exhaust vacuum line greatly reduced isoflurane concentration. There was no significant difference in isoflurane concentration between the vacuum lines alone compared to using passive canisters with the vacuum line. It is recommended that researchers use of active scavenging methods to control isoflurane WAG.

Isoflurane in combination with other anesthetic gases, is likely to be associated with adverse human health effects such as genetic damage and changes in cellular functions. These changes could be related to other negative health outcomes such as CNS toxicity, reproductive toxicity, and organ toxicity. Researchers using isoflurane to anesthetize animals are at risk for high exposures to isoflurane. Researchers should use active scavenging methods to control isoflurane WAG. Active scavenging may not be reasonable in all research situations due to cost or limited space. Portable active scavenging systems are now available, which may be beneficial in these instances. These systems combine the portability of passive scavenging canisters with pump to pull polluted air into the activated charcoal. The portable active scavenging systems have yet to be evaluated in the literature. These devices should be investigated to determine if they are an efficient control for isoflurane

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APPENDIX A: FULL PUBMED SEARCH STRATEGY FOR SYSTEMATIC REVIEW

((((((((occupation[tiab] OR occupational[tiab]))) AND ((expose[tiab] OR exposed[tiab] OR exposure[tiab]))))) OR "occupational exposure") OR ((("Occupational Exposure"[Mesh]) OR "Occupational Diseases"[Mesh]) OR "Environmental Exposure"[Mesh:NoExp]))) AND (((((("Isoflurane"[Mesh]) OR isoflurane[tiab])) OR ("anaesthetic gas" OR "anaesthetic gases")) OR ("anesthetic gas" OR "anesthetic gases")) Filters: Humans.

APPENDIX B: FULL Embase SEARCH STRATEGY FOR SYSTEMATIC REVIEW

EMBASE®

Search Queries

No.	Query	Results	Date
#15	((('occupational exposure'/exp OR 'occupational disease'/exp) AND ('isoflurane'/exp OR (isoflurane:ti OR isoflurane:ab) OR ('anesthetic gas':ti OR 'anaesthetic gas':ti) OR ('anesthetic gases':ti OR 'anaesthetic gases':ti) OR ('anesthetic gases':ab OR 'anaesthetic gases':ab) OR ('anesthetic gas':ab OR 'anaesthetic gas':ab) OR ('anesthetic gas':ab OR 'anaesthetic gas':ab))) AND 'human'/de) AND ([embase]/lim NOT [medline]/lim)	70	19 Dec 2014
#14	[embase]/lim NOT [medline]/lim	6564799	19 Dec 2014
#13	(('occupational exposure'/exp OR 'occupational disease'/exp) AND ('isoflurane'/exp OR (isoflurane:ti OR isoflurane:ab) OR ('anesthetic gas':ti OR 'anaesthetic gas':ti) OR ('anesthetic gases':ti OR 'anaesthetic gases':ti) OR ('anesthetic gases':ab OR 'anaesthetic gases':ab) OR ('anesthetic gas':ab OR 'anaesthetic gas':ab) OR ('anesthetic gas':ab OR 'anaesthetic gas':ab))) AND 'human'/de	378	19 Dec 2014
#12	('occupational exposure'/exp OR 'occupational disease'/exp) AND ('isoflurane'/exp OR (isoflurane:ti OR isoflurane:ab) OR ('anesthetic gas':ti OR 'anaesthetic gas':ti) OR ('anesthetic gases':ti OR 'anaesthetic gases':ti) OR ('anesthetic gases':ab OR 'anaesthetic gases':ab) OR ('anesthetic gas':ab OR 'anaesthetic gas':ab) OR ('anesthetic gas':ab OR 'anaesthetic gas':ab))	483	19 Dec 2014
#11	'isoflurane'/exp OR (isoflurane:ti OR isoflurane:ab) OR ('anesthetic gas':ti OR 'anaesthetic gas':ti) OR	23477	19 Dec 2014

	('anesthetic gases':ti OR 'anaesthetic gases':ti) OR ('anesthetic gases':ab OR 'anaesthetic gases':ab) OR ('anesthetic gas':ab OR 'anaesthetic gas':ab) OR ('anesthetic gas':ab OR 'anaesthetic gas':ab)		
#10	'occupational exposure'/exp OR 'occupational disease'/exp	183140	19 Dec 2014
#9	'anesthetic gas':ab OR 'anaesthetic gas':ab	458	19 Dec 2014
#8	'anesthetic gas':ab OR 'anaesthetic gas':ab	458	19 Dec 2014
#7	'anesthetic gases':ab OR 'anaesthetic gases':ab	667	19 Dec 2014
#6	'anesthetic gases':ti OR 'anaesthetic gases':ti	409	19 Dec 2014
#5	'anesthetic gas':ti OR 'anaesthetic gas':ti	164	19 Dec 2014
#4	'occupational disease'/exp	132114	19 Dec 2014
#3	'occupational exposure'/exp	65409	19 Dec 2014
#2	isoflurane:ti OR isoflurane:ab	13200	19 Dec 2014
#1	'isoflurane'/exp	20403	19 Dec 2014

APPENDIX C: ASSESSMENT OF ISOFLURANE EXPOSURE TO RESEARCHERS DURING ANIMAL PROCEDURES (CHAPTER 4)

Procedure Description	ID	Isoflurane Exposure (ppm)	Role	Scavenging	Species
Acute Cardiac Disease Model	5679	0.09	Secondary	Passive	Large
Cardiac Arrest Treatment 1	2402	1.90	Secondary	Passive	Large
Cardiac Arrest Treatment 1	7894	0.69	Secondary	Passive	Large
Cardiac Arrest Treatment 2	2402	1.70	Secondary	Passive	Large
Cardiac Arrest Treatment 2	7894	0.24	Secondary	Passive	Large
Cardiac Arrest Treatment 3	2402	2.50	Secondary	Passive	Large
Cardiac Arrest Treatment 3	4608	0.22	Secondary	Passive	Large
Cardiac Arrest Treatment 4	2402	1.30	Secondary	Passive	Large
Cardiac Arrest Treatment 4	4608	0.16	Secondary	Passive	Large
Cardiac Arrest Treatment 4	4979	0.72	Primary	Passive	Large
Surgical Robot Test	5162	2.50	Primary	Active	Large
Surgical Robot Test	3443	3.60	Secondary	Active	Large
Surgical Robot Test	6295	0.63	Primary	Active	Large
Surgical Robot Test	5729	0.42	Secondary	Active	Large
Surgical Robot Test	6017	0.68	Secondary	Active	Large
Body Composition	1517	0.56	Primary	Active	Small
Body Composition	1235	0.53	Primary	Active	Small
Cardiac Arrest Treatment	7304	1.30	Primary	Active	Small
Cardiac Arrest Treatment	4608	0.77	Primary	Active	Small
Cardiac Arrest Treatment	2402	0.63	Secondary	Active	Small
Cardiac Arrest Treatment	4399	0.53	Secondary	Active	Small
Dissection (1)	5015	0.28	Primary	Active	Small
Dissection (2)	7776	0.64	Primary	Active	Small
Dissection (3)	1809	1.6	Secondary	Passive	Small
Dissection (3)	9131	1.5	Secondary	Passive	Small
Dissection (3)	4904	2.5	Primary	Passive	Small
Dissection (3)	2102	6.6	Primary	Passive	Small
Drug Pump Implantation	7584	3.6	Primary	Passive	Small
Embryonic Transfer Surgery	2437	1.6	Primary	Passive	Small
Embryonic Transfer Surgery	8747	0.26	Secondary	Passive	Small
Heart Ultrasound	2074	2.9	Primary	Passive	Small
Heart Ultrasound	3916	0.41	Secondary	Passive	Small
Humanized Liver Model	6279	0.25	Primary	Active	Small
Humanized Liver Model	4014	0.42	Secondary	Active	Small
Intraperitoneal Injections	4257	9.5	Primary	Passive	Small
Intraperitoneal Injections	1784	3.7	Secondary	Passive	Small

Kidney Ultrasound	2495	1.5	Primary	Passive	Small
Liver Cancer Model	2636	2.10	Secondary	Passive	Small
Liver Cancer Model	2512	2.50	Primary	Passive	Small
Liver Cancer Model	4637	1.80	Secondary	Passive	Small
MRI	4805	0.61	Primary	Active	Small
MRI	6138	0.15	Secondary	Active	Small
Skin Disease Model	9683	0.12	Primary	Active	Small
Telemeter Implantation	5201	2.20	Primary	Passive	Small
Time Course Model	4020	0.25	Primary	Active	Small
Time Course Model	1925	0.25	Secondary	Active	Small

APPENDIX D: ASSESSMENT OF ISOFLURANE EXPOSURE TO RESEARCHERS DURING ANIMAL PROCEDURES (CHAPTER 4)

Cardiac Arrest Treatment in Pig

The research protocol involved one research animal per procedure. A total of 5 to 6 research personnel, two cardiovascular surgeons and four technicians, participated in each procedure. The technicians induced anesthesia in the pigs using injection or cone mask with 5% isoflurane at a flow rate of 1 liter per minute (L/min) for 4 to 5 minutes. Pigs were intubated to maintain anesthesia with isoflurane at 1.5 to 2 % isoflurane at a flow rate of 1 L/min until the completion of the research protocol. Pigs were kept under anesthesia for 6 to 8 hours per procedure. A total of 5 procedures were observed for this study. In four of the studies the animals were recovered, in the final procedure the animal was euthanized with potassium chloride (KCl) and 5% isoflurane for 5 minutes.

The purpose of the research procedures were to injure and occlude either the left or right carotid artery or the left or right descending aortic vessel to create a heart attack in the animal. The research team would treat the animal with standard heart attack treatments and the research treatment. The research treatment was micro-bubbles activated with ultrasound. One technician was in charge of prepping animals for surgery by placing IVs and intubating the animal. After the initial prep time the technician would be in and out of the procedure room. Two other technicians assisted in the preparation. During the procedure one technician recorded notes and another manipulated the camera arm of the fluoroscope. A third technician assisted the main cardiovascular surgeon to occlude the artery or blood vessel and then treat the occlusion. A second cardiovascular surgeon operated the ultrasound during the treatment period.

After treatment the animals were either recovered or moved to an MRI for imaging. Animals that underwent MRI imaging were placed on a cart with a portable isoflurane vaporizer to maintain anesthesia during transport to the MRI facility. At the

facility, the animals were transferred to the MRI chamber and isoflurane vaporizer. During the MRI, scanning research personnel were in a separate room from the MRI, away from isoflurane sources. After the MRI the animals were transported back to the procedure room with the same equipment and recovered or euthanized. During recovery vitals were monitored. After recovery, animals were transported back to their housing area. The concentration for the field blanks were below the limit of detection of 0.04 ppm.

Surgical Robot Test in Pig

One Yorkshire pig was used during the testing of a surgical robotics instrument. Anesthesia was induced using 5% isoflurane at flow rate of 1.5 L/min delivered by cone mask. After induction the pig was intubated to maintain anesthesia. An active vacuum line was run from the building exhaust to the animal during induction and intubation. The pig was then transferred to the procedure room and placed on isoflurane at 2% at flow rate of 1.5 L/min. Two technicians were involved in the preparation and intubation of the pig. Each of the technicians was monitored for isoflurane exposure during the entire time that they were present for the procedure.

A total of 15 individuals observed or participated in the robotics test. The procedure began approximately 1.5 hours after the pig had been prepped for the surgery. The abdominal cavity of the pig was opened and a minor gastrointestinal surgery was performed. The researchers then attempted to perform the same procedure using the robot. The purpose of the study was to further the use of non-invasive surgery techniques using surgical robots. The procedure took approximately 45 minutes to perform. Two researchers were monitored for isoflurane exposure during the procedure. One researcher operated the surgical robot, the other researcher observed the procedure.

Two technicians then began cleaning up the procedure room and euthanizing the pig. The pig was euthanized using KCl and 5% isoflurane. Isoflurane was turned off after approximately 5 minutes. Vacuum scavenging was used during the surgical procedure and turned off with the isoflurane. The technicians finished cleaning the room and disposing of the pig within 1 hour. Both of the technicians were monitored during the cleanup. The concentration for the field blank was below the limit of detection of 0.04 ppm.

Acute Cardiac Disease Model in Rabbit

Anesthesia was induced in the rabbit by injection. The rabbit was intubated with a fully inflated cuff and anesthesia was maintained at 1.5—2% isoflurane at flow rate of 1 L/min. An F/Air™ canister was used to scavenge waste anesthetic gas. A non-sterile technician assisted in taking notes and moving non-sterile instruments. The technician was monitored during the procedure. A surgeon opened the abdominal cavity of the rabbit and placed a blood flow monitor on the renal vein. The surgeon was not monitored due to concerns of maintaining a sterile surgical area. Active vacuum lines were available in the procedure room, but were not used. The procedure took approximately 1 hour to complete. The rabbit was recovered by the technician for 3 hours and then taken back to its housing area. The technician was monitored for the entire procedure and recovery time. The isoflurane concentration for the field blank was below the limit of detection of 0.04 ppm.

Cardiac Arrest Treatment in Rat

Anesthesia was induced using a 1 gallon ice cream container as induction chamber. The anesthesia induction was not monitored. After induction the rat was placed on a nose cone and shaved for procedure. Anesthesia was maintained at 1.5—1.75% isoflurane at a flow rate of 1 L/min for approximately 1.5 hours. During the procedure, a technician tied off left ascending cardiac vessel and prepared rat for

cardiovascular injury. The technician used an active vacuum line and F/Air™ canister to scavenge isoflurane vapors. The technician was monitored for the entire duration of exposure.

A second technician performed the injury to the vessel to induce a heart attack. The technician then treated the rat using ultrasound activated micro-bubbles. A cardiovascular surgeon operated the ultrasound during the treatment. A third technician assisted with the ultrasound and surgery. All participants were monitored for isoflurane during the procedure. The animal was recovered after 1.5 hours and returned to its housing. The concentration for the field blank was below the limit of detection of 0.04 ppm.

Telemeter Placement in Rats

A researcher induced anesthesia in rats using induction chamber with isoflurane at 3% at flow rate 1 L/min for 5—15 minutes. Animals were moved to surgical area on bench and placed on nose-cone to maintain anesthesia. Anesthesia was maintained at 2% isoflurane at a flow rate of 1 L/min.

Animals were secured to the surgical area, shaved, and swabbed with iodine solution. A sterile cover was placed over the animal with a cut-out to expose surgical area. Researcher opened the animal's abdomen and chest, making two separate cuts. Telemeter device was placed in abdomen with leads in the chest. The researcher sutured abdomen and chest openings. Procedure time was approximately 1 hour per animal. The researcher placed one telemeter device in a total of three rats during the monitoring period. A class 2 BSC and active vacuum ports were available, but not used. The researcher used ReFresh canisters to scavenge WAG from the induction chamber and nose-cone. The isoflurane concentration for the field blank was below the limit of detection of 0.04 ppm.

Intraperitoneal Injections in Rat

Twelve rats were injected once daily for seven days. Two researchers were monitored for isoflurane exposure during one of the injection periods. One researcher weighed the rats. The second researcher prepared the injections based on the rat's weight. The first researcher placed the animals in the induction chamber and performed the injections. The researcher did intraperitoneal injections into the peritoneal cavity of the rat.

Anesthesia was induced using 4% isoflurane at a flow rate of 2 L/min for 5 minutes; the flow rate was then reduced to a flow rate of 1.5 L/min. The induction chamber was placed on a bench top and attached to an F/Air™ canister. A class 2 BSC and active vacuum ports were available, but not used. The injections took approximately 15 minutes to perform. The researchers returned the animals to the housing area and cleaned up. The concentration for the field blank was below the limit of detection of 0.04 ppm.

Liver Cancer Model in Rat

A total of four rats and three researchers participated in the protocol. Anesthesia was induced in the rats using an induction chamber placed inside a class II BSC. An F/Air™ canister was attached to the induction chamber. Anesthesia was induced at 4—5% isoflurane at a flow rate of 2 L/min for approximately 5 minutes. Rats were then removed from the induction chamber and placed on the procedure table. Isoflurane was delivered at 1% at a flow rate of 1 L/min using a nose cone on the procedure table. The nose-cone was fitted with a rubber septum and the rat's entire head was placed in the nose cone. An F/Air™ canister was used for scavenging isoflurane from the nose cone. The rat was shaved and secured to the surgical procedure area.

The abdominal cavity was opened and intestines were gently placed to the side of the animal. The researchers clamped the bile duct and then injected cancer cells into the liver. The purpose of this procedure is to induce liver tumors in the rats. The

researchers then suture the inner abdominal wall followed by the outer skin of the abdomen. The researchers held the rats for approximately 3—5 minutes to warm the animals. The rats were placed in the BSC on a heating pad to recover. The procedure was performed on three rats in succession. The researchers then took a 30 minute break and performed the procedure on a fourth animal.

The researchers took turns inducing anesthesia, prepping the animal, performing the procedure, suturing the surgery site, and recovering the animals. One researcher was in and out of the room preparing the cancer cells for injection. This researcher performed the fourth procedure. The other two researchers were in the room for the duration of the research. An active vacuum line for the procedure table was available, but not used. The concentration for the field blank was below the limit of detection of 0.04 ppm.

Body Composition of Mice

Body composition procedures were monitored for isoflurane two separate times with two different technicians. A technician performed body composition measurements on 23 mice using a dual X-ray absorptiometry (DEXA) machine. The technician induced anesthesia in the mice using 2.5—3% isoflurane at a flow rate of 1 L/min in a slide top induction chamber. The induction chamber was placed on a table outside of a nearby class 2 BSC. After anesthesia was induced, the technician transferred the animal to the DEXA machine in the BSC. Anesthesia was maintained using the nose cone of the DEXA machine at 2% isoflurane at a flow rate of 1 L/min. Mice remained on the DEXA machine for approximately 4 minutes for the scan to complete. Mice were then removed and recovered in cages. The technician placed a lead screen in front of the BSC during the scan. Two VaporGuard™ canisters were used to scavenge waste gas. One canister was attached to the induction chamber and one was attached to the DEXA machine and placed in the hood. Both canisters were laid on their sides. The procedure and cleanup

lasted for approximately 1.5 hours total. The technician refilled the isoflurane vaporizer outside of the hood after the procedure. The technician placed the mice in the housing facility after the procedure. The technician was monitored for isoflurane exposure during the entire procedure.

A second body composition procedure was conducted with 15 mice and a separate technician. The technicians each used the same protocols for the body composition measurements. The total procedure time for this procedure was approximately 1 hour and 15 minutes. The technician used 1.5% isoflurane at a flow rate of 1 L/min to induce and maintain anesthesia in the mice. One F/Air™ canister was attached to the induction chamber outside of the BSC and one VaporGuard™ canister was attached to the DEXA machine inside the BSC.

The isoflurane concentration for the field blank was below the limit of detection of 0.04 ppm for this first procedure and less than 0.26 ppm for the second.

Embryo Transfer in Mice

Researchers prepared embryos for transfer. Five female mice were implanted with the embryos. One researcher used the isoflurane in the open-drop method to anesthetize the mice individually. The second researcher performed the transfer surgery in a laminar flow hood. A Pure-Guard™ with an Enviro-Pure™ passive scavenging canister was used to scavenge waste isoflurane gas. Each surgery lasted approximately five minutes. The isoflurane concentration for the field blank was below the limit of detection of 0.04 ppm.

Dissection 1: Mice Pups

Three mice pups were euthanized in a class II BSC with isoflurane using the open-drop method. The mice pups were placed in a plastic canister with 0.1 milliliters (mL) of isoflurane placed on a paper towel. The pups were removed one at a time and dissected. Organs were collected for further analysis. The canister was open for

approximately 30 seconds at a time. The procedure took approximately 15 minutes and was completed in the BSC. The researcher performing the procedure was monitored for isoflurane exposure during the procedure and the cleanup. The isoflurane treated paper towels were left in the canister in the hood. After approximately two hours the paper towels were disposed of in the biohazard waste.

The isoflurane concentration for field blank was below the detection limit of 0.04 ppm.

Dissection 2: Mice

A technician euthanized and dissected a total of ten adult mice. The mice were euthanized using isoflurane and the open-drop method. Five mice were placed in a desiccator jar in a class 2 BSC with paper towels treated with approximately 25 mL of isoflurane. After the mice are euthanized they were removed from the jar as a group and transferred to the bench for dissection. The leg tissue and knee joints were collected from each mouse. Two groups of five mice were euthanized and dissected. The technician was monitored for isoflurane exposure during the entire procedure. The procedure and cleanup time was approximately 45 minutes. The paper towel was removed from the desiccator jar and left in the hood for approximately 1 hour then disposed of in the biohazard waste.

The isoflurane concentration for the field blank was below the detection limit of 0.04 ppm.

Dissection 3: Mice

Four researchers euthanized and dissected a total of four mice. The mice were anesthetized individually using 2.5% isoflurane at a flow rate of 1 L/min in a clip top induction chamber. The induction chamber clip was not secured between animals. A technician and three students sat in a circle around a table to conduct the procedure. The technician sat closest to the induction chamber and placed and removed the mice

from the chamber. A student sat across from the technician. This student weighed the animals and the organs collected from the dissected animals. A second student sat next to the technician and assisted by dissecting the mice with the technician. The final student kept notes for the procedure and placed the collected organs in collection vials. The group worked together in an assembly line like manner. The procedure and cleanup was completed in approximately 40—45 minutes. Isoflurane was actively used for approximately 20 minutes.

A ReFresh passive canister was attached to the induction chamber to scavenge WAGs. There was no access to active vacuum lines or a ventilated hood in the room. The technician and the three students were monitored for isoflurane exposure during the entire procedure. The concentration for the field blank was below the limit of detection of 0.04 ppm.

Drug Pump Implantation in Mice

A technician implanted drug release mini-pumps into three mice. Anesthesia was induced using 2% isoflurane at a flow rate of 1 L/min a clip top induction chamber. The mouse was removed from the chamber, shaved, and placed on a nose cone to maintain anesthesia at 2% isoflurane at 1 a flow rate of L/min.

The technician implanted the mini-pump device in the abdomen of the animal. The technician repeated the procedure for two more mice. Animals were covered the cages and returned to housing. Mice were housed in the procedure room. Two vapor guard passive canisters were used to scavenge waste gas. One canister was attached to the induction chamber and one was attached to the nose cone. A BSC was available in the room but not used during the procedure. No vacuum scavenging lines were available for the room. The technician was monitored for the entire procedure time of 34 minutes.

The isoflurane concentration for the field blank was below the limit of detection of 0.04 ppm.

Heart Ultrasound in Mice

Six mice were used in this study. Two researchers participated in the study, a technician who ran the ultrasound machine, and a second researcher who assisted the technician. Both researchers were monitored for isoflurane exposure during the entire procedure.

The mice were anesthetized using 3% isoflurane at a flow rate of 1 L/min in a clip top induction chamber on the bench top. Anesthesia was maintained with the nose cone attached to the ultrasound machine at 1% at a flow rate of 1 L/min. Each animal was anesthetized individually. Once under anesthesia, the animal was moved to the ultrasound stage and secured using tape. A large dollop of ultrasound gel was placed on the mouse's abdomen. The transducer was lowered and positioned manually by the technician. The heart was imaged and recorded by the technician. The entire procedure for all six animals took 70 minutes to complete. After the procedure, the researchers cleaned the room and returned the mice to the housing facility.

Two F/Air canisters were used to scavenge waste anesthetic gas. One F/Air canister was attached to the induction chamber; the second was attached to the ultrasound nose cone. No active vacuum ports were observed in the room. The researchers worked with the door to the room open to the hallway. The isoflurane concentration for the field blank was below the limit of detection of 0.04 ppm.

Humanization Liver Model in Mice

Two researchers completed a procedure to create humanized mouse liver models in four mice. Anesthesia was induced using a slide top induction box outside of a BSC. A technician shaved mice and placed them in the induction chamber. Mice were

individually induced at 4% isoflurane at a flow rate of 1 L/min by the technician. The mice were transferred to a class 2 BSC for the procedure.

Anesthesia was maintained at 2% isoflurane at a flow rate of 1 L/min on nose cones. The researcher transferred human hepatocyte cells into the mouse spleen. Hepatocyte cells were aspirated into a specialized needle/loop apparatus and transferred to the mouse. While conducting the procedure the sash of the BSC was raised above the maximum height. The researcher ignored the alarm and turned the alarm off several times. The researcher also leaned into the BSC so that the researchers head and shoulders were inside of the BSC while working.

Two F/Air canisters were used to scavenge waste anesthetic gas. One was attached to the induction chamber outside of the BSC and one was attached to the nose cone inside of the BSC. The technician and researcher were monitored for isoflurane during the entire procedure. The total procedure time took approximately 1 hour, including cleanup. The isoflurane exposure for the field blank was less than 0.17.

Kidney Ultrasound in Mice

One researcher performed the ultrasound on the kidneys of 8 mice. Mice were anesthetized individually in a clip top induction chamber using 2.5—5% isoflurane at a flow rate of 1 L/min for approximately 1 minute. The mice were removed from the chamber and placed on the ultrasound stage and secured using tape. Anesthesia was maintained by delivering 2% isoflurane at a flow rate of 1 L/min to the nose cone of the ultrasound machine.

The researcher removed the hair from the abdomen of the mouse and applied ultrasound gel to the abdomen. The researcher then manipulated the ultrasound transducer to image the kidney and related blood vessels. After imaging the animal was placed back in the cage to recover. The procedure was repeated for 8 mice and took place for approximately 2 hours. After the procedure the researcher cleaned up and took

the mice back to the housing area. The isoflurane concentration for the field blank was below the limit of detection of 0.04 ppm.

MRI Imaging in Mouse

Two researchers imaged a mouse using a small animal MRI. The mouse was anesthetized using 1.5% isoflurane at a flow rate of 1 L/min in a slide top induction chamber. The induction chamber was attached to an active vacuum line to scavenge waste anesthetic gas from the top of the chamber. The mouse was in the induction chamber for 20 minutes.

After the mouse was anesthetized, the student researcher placed the mouse in an MRI chamber of the bench top. The chamber with the mouse was then placed in the MRI. The student researcher and technician adjusted the MRI for the procedure. The MRI procedure and animal's vitals were monitored using a computer approximately 4 feet from the MRI. Anesthesia was maintained at 1.5% at a flow rate of 1 L/min using a nose cone within the MRI. The animal was imaged for approximately 2 hours, recovered, and taken back to its housing area. The technician and student researcher were monitored for isoflurane exposure during the entire procedure except when placing the animal in the MRI and adjusting the MRI. The researchers could not be monitored during this time due to the metal clips on the passive monitoring badges. This time was approximately 10 minutes of the entire procedure. The isoflurane concentration for the field blank was below the limit of detection of 0.04 ppm.

Skin Disease Model in Mice

A research student performed skin test procedures on 20 mice. Anesthesia was induced at 3% isoflurane at a flow rate of 1 L/min in a slide top induction chamber placed within a class 2 BSC. After 5 minutes the isoflurane concentration was reduced to 1.5%. Mice were anesthetized 5 at a time.

After induction, mice were removed from the induction chamber and anesthesia was maintained on nose cone inside of BSC. A total of 5 nose cones were setup for the procedure. The researcher applied cream to the ears of the mice using a small spatula. The mice were kept under anesthesia for 30 minutes. Mice were rotated on the nose cones. A maximum of 10 mice were under anesthesia at a time; five mice in the induction chamber and five mice on nose cones. Mice were recovered in cages. The researcher cleaned up the room and placed the animals in the housing area. The isoflurane concentration for field blank was below 0.17 ppm.

Time Course Model in Mice

Two researchers completed a tail vein injection and euthanasia/ dissection of five mice. A large induction chamber was placed in a class 2 A2 BSC. The induction chamber was flooded with 5% isoflurane at a flow rate of 3 L/min for approximately 5 minutes. The isoflurane concentration was reduced to 2.5% at a flow rate of 3 L/min and all five mice were placed in the induction chamber to induce anesthesia.

One researcher placed mice in and removed mice from the induction chamber. This researcher also injected the tail veins of the mice. A technician prepped the tail vein injections. Two mice were euthanized and dissected immediately after the tail vein injection. Mice were euthanized on the bench top CO_2 euthanasia station, which is a specially designed cage lid which fits tightly over the mouse cage and pumps CO_2 into the cage. The technician immediately dissected the euthanized animals and collected tissues in a second BSC. The other three mice were euthanized and dissected in the laboratory at two and four hours after tail vein injection; by the researcher. The tail vein injection and two dissections were completed in the same room in approximately 1 hour. The researchers cleaned up and took the remaining three mice to the lab for holding. A ReFresh canister was attached to the induction chamber and placed in the hood to scavenge waste gas. The vaporizer was flushed with O_2 before being turned off. Both

researchers were monitored for isoflurane exposure during the tail vein injections, the first two dissections, and cleanup from the procedure. Researchers were not monitored in the lab for isoflurane exposure as isoflurane use was completed for the day and the researchers were leaving the procedure room in which isoflurane was used. The isoflurane concentration for the field blank was below 0.35 ppm.