RISK ASSESSMENT FOR OCCUPATIONAL EXPOSURE TO ISOFLURANE IN PHARMACEUTICAL RESEARCH AND VETERINARY FACILITIES IN SAN DIEGO, CALIFORNIA

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In Partial Fulfillment
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Master of Public Health
with a Concentration in
Environmental Health

by

Michael J. Checkai

Spring 2014
The Undersigned Faculty Committee Approves the

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Risk Assessment for Occupational Exposure to Isoflurane in Pharmaceutical
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DEDICATION

This thesis is dedicated to my parents, Robert and Mary, who continually support and encourage me to choose education as a priority in my life. Without their love and support I would not have been able to complete this paper. I would like to thank my professional colleagues at Occupational Services, Inc., San Diego State University Public Health professors, and the San Diego research community.
Knowledge is power.

-Sir Francis Bacon
Religious Meditations, of Heresies
ABSTRACT OF THE THESIS

Risk Assessment for Occupational Exposure to Isoflurane in Pharmaceutical Research and Veterinary Facilities in San Diego, California

by

Michael J. Checkai
Master of Public Health with a Concentration in Environmental Health
San Diego State University, 2014

The main objective of this study was to conduct a risk assessment based on exposure data collected from pharmaceutical research scientists and veterinarians in San Diego, CA who are occupationally exposed to isoflurane gas. To achieve the objective, it was necessary to determine the dose-response relationship from literature values on the toxicology of isoflurane, and then through an exposure assessment, perform a risk assessment.

Currently, occupational exposure to anesthetic gases occurs in the health sector, such as hospital operating rooms, veterinary clinics, and pharmaceutical research laboratories. According to California OSHA 5155 Table AC-1 Permissible Exposure Limits for Chemical Contaminants, the employer shall assure that no employee is exposed to an 8-hour time-weighted average (TWA) concentration of airborne isoflurane in excess of 2 parts per million parts of air. Between January 2006 and January 2013 isoflurane exposure data was collected from pharmaceutical research and veterinary facilities located in San Diego, CA. Isoflurane samples were analyzed by modified OSHA Method 07. The results show that the mean time weighted average concentration of isoflurane gas exposure in a pharmaceutical research laboratory was 0.62 ppm. The maximum recorded exposure concentration from the data pool was 1.64 ppm. The mean time weighted average concentration of isoflurane gas exposure for the veterinary clinics was 2.14 ppm. The maximum recorded exposure concentration from the veterinary data pool was 2.77 ppm.

The hazard index (HI) is defined as the ratio of the exposure concentration over the reference dose. The hazard index, calculated from the average daily dose (mean TWA of 1.33 mg/kg-day), of the pharmaceutical research population was 0.06. The hazard index for this population’s maximum recorded exposure concentration was 0.16. The hazard index of the veterinary population (average daily dose mean TWA of 0.21 mg/kg-day) was 0.21. The hazard index for this population’s maximum recorded exposure concentration was 0.28. If the HI is less than 1, then the health effects are assumed not to be of concern, however, if the hazard quotient is greater than 1, then health effects may be assumed to occur. Based on the data collected in this study, the hazard indices for both the pharmaceutical research laboratories and veterinary clinics mean, as well as, maximum concentrations were all below a HI of 1.0, which confirms occupational exposure levels in these populations would not be expected to cause harmful health effects.
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<td>15</td>
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CHAPTER 1

INTRODUCTION

According to a 2009 data survey by the Anesthesia Quality Institute, over 80,000 registered anesthesiologists and certified registered nurse anesthetists administer 30 million anesthetic procedures in the United States each year. (Anesthesia Quality Institute, 2009).

Anesthetic agents possess the ability to produce anesthesia in animals and humans to prevent the sensation of pain during surgical or non-surgical procedures. Isoflurane is an anesthetic agent which can induce anesthesia in minutes when inhaled at a concentrations of 0.5 – 3% producing muscle relaxation and loss of consciousness (NuAire, 2006). These functional properties of anesthetic agents, such as isoflurane, have allowed the advancement of human medical surgery, non-invasive animal research, such as bioluminescence imaging, and veterinarian surgery.

With the growth of the healthcare industry, a rising number of workers, such as hospital operating room personnel, pharmaceutical research scientists, and Veterinarians are exposed to trace levels of anesthetic agents. As a response to this increased use of anesthetic agents, there has been an increased interest in the health and safety implications from occupational exposure to waste anesthetic gases that may leak out of anesthesia delivery equipment and into a procedure room during anesthesia procedures.

Chronic exposures to anesthetic agents have been associated with adverse health effects in humans, such as neurotoxicity, incidence of spontaneous abortion, and congenital abnormalities (Tannenbaum & Goldberg, 1985). Acute high concentration exposures to modern anesthetic agents, isoflurane, enflurane, and sevoflurane have been reported to cause headaches, nausea, drowsiness, impairment of coordination, and fatigue (National Institute for Occupational Safety and Health [NIOSH], 1994). However, there is a lack of research that shows that there is a direct correlation between exposure to trace levels of modern anesthetic agents and adverse health effects in humans. Research studies have attempted to detect genotoxic effects from cohort studies of exposed groups as well as retrospective epidemiological studies, however due to these studies’ limitations (most notably in sample
size), no statistically significant findings have been established (American Society of Anesthesiologists, 1974).

In a more recent literature review of over 200 articles published in MEDLINE®/PubMed®, CINAHL® and Cochrane Library databases from 1980 to 2010, it was concluded that data shows that human patients at hospitals exposed to halogenated anesthetic agents, in combination with nitrous oxide, displays genotoxicity. However, due to multitude of chemical exposures a patient receives at a hospital, determining a direct dose response relationship with an individual anesthetic agent was difficult. Furthermore, the review concluded by requesting additional clinical and experimental support is necessary (Schifilliti, Mondello, D'Arrigo, Chille, & Fodale, 2011).

Even without the full knowledge of the potential health effects of anesthetic agents, it is imperative still that businesses protect their employees who are exposed to these elements by implementing control measures to reduce occupational exposure to waste anesthetic gases. These control measures would include scavenging systems, safety policies, education regarding anesthetic delivery equipment, maintenance procedures, and industrial hygiene air monitoring.

**OBJECTIVE**

The objective of this thesis was to conduct a risk assessment based on industrial hygiene air monitoring collected from pharmaceutical research and veterinary facilities in San Diego, CA who are occupationally exposed to isoflurane gas. To do this, it was necessary to determine the dose-response relationship from literature pertaining to the toxicology of isoflurane, and then through the present exposure assessment, characterize the population’s average daily dose (ADD) of isoflurane through inhalation exposure. Combining the ADD and the dose-response relationship allows a risk characterization to be prepared.

The specific objectives for this study included:

1. Collect occupational exposure data from pharmaceutical research and veterinary facilities in San Diego, CA, and calculate Time Weighted Average (TWA) exposures for isoflurane in these two occupational settings.
2. Determine the dose-response relationship from literature pertaining to the toxicology of isoflurane.
3. Calculate the population’s average daily dose (ADD) of isoflurane inhalation exposure.

4. Develop a population-based risk characterization for exposure to isoflurane exposure in these two occupational settings.

**History of Anesthetic Agents Usage**

During the 1800s, scientific interest in anesthetic agents grew out of the need to improve patient health during and after surgery. Anesthetic agents provided immobilization for the duration of the procedure and a method to control the autonomic response of increased blood pressure and heart rate (Sykes, 2007). During the early 1800s only a few physicians used anesthetic agents to induce unconsciousness. Anesthetic agent use was simply viewed as unsafe and unnatural. Pain was considered a natural part of human physiology and interpreted as a religious construct designed through spiritual creation in the body (Young, 1998). Only until William T.G. Morton in the 1840s, were the properties of diethyl ether as an inhalation anesthetic agent, sufficiently demonstrated to convince the scientific community and the general public of its beneficial use for eliminating pain and immobilizing patients during surgery (Figure 1). However, before Morton would publicly and effectively unveil the unique abilities of anesthetic agents, Horace Wells experimented with nitrous oxide as a way to reduce pain involved with dental surgery. Unfortunately, during Wells public demonstration of his method, the patient only went under partial analgesia and was noted to be grimacing with pain during the operation The performance would not only delayed the advancement of anesthetic agent use but also resulted in Wells losing his reputation in the scientific world. Following in Wells footsteps, was Morton, who was determined not to make similar mistakes. He investigated the use of other anesthetic agents and settled on diethyl ether as a suitable drug. In 1846, he proved his method of anesthesia while performing surgery on a patient in front of medical doctors in Massachusetts (Wolfe, 2001). Analogous to the introduction of many new technologies into the general public, society quickly accepted anesthesia as practical and moral with this proven demonstration, dramatically expanding the amount of what doctors were able to accomplish. As the field of anesthesiology advanced, so did medical and anesthesia procedures, the development of anesthetic delivery equipment, and the safety of surgical procedures (Figure 2). By the end of the 1800s, ether, nitrous oxide, and chloroform were proven as suitable anesthetics and these three inhalation anesthetic agents were used widely for the next
100 years (Sykes, 2007). Table 1 describes the introduction of anesthetic agents during the course of the next 200 years. The 1900s saw the introduction of new anesthetic agents, such as halothane, isoflurane, desflurane, and sevoflurane. In 1936 the American Society of Anesthesiologists and in 1985 the Anesthesia Patient Safety Foundation were created (History of Anesthesia Society, 2011). The purpose of these organizations was to raise the standards of medical practice and improve patient safety associated with anesthesia procedures. Today an estimated 40 million anesthetic agents are administered annually in the United States while the number of complications from anesthesia has been reduced considerably. Surgery deaths attributed to anesthesia procedures were approximately 1 in 1,500 fifty years ago. Today this statistic has improved to approximately 1 in 200,000 for healthy patients suffering a death attributable to anesthesia procedures (American Society of Anesthesiologists, 2013).
Table 1. Dates of Inhaled Anesthetic Agents Development and Current Use

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>Year of Introduction</th>
<th>Currently in Use?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diethyl ether</td>
<td>1842</td>
<td>No</td>
</tr>
<tr>
<td>Nitrous oxide</td>
<td>1844</td>
<td>Yes</td>
</tr>
<tr>
<td>Chloroform</td>
<td>1847</td>
<td>No</td>
</tr>
<tr>
<td>Cyclopropane</td>
<td>1933</td>
<td>No</td>
</tr>
<tr>
<td>Trichloroethylene</td>
<td>1934</td>
<td>No</td>
</tr>
<tr>
<td>Fluroxene</td>
<td>1954</td>
<td>No</td>
</tr>
<tr>
<td>Halothane</td>
<td>1956</td>
<td>Infrequently</td>
</tr>
<tr>
<td>Methoxyflurane</td>
<td>1960</td>
<td>Infrequently</td>
</tr>
<tr>
<td>Enflurane</td>
<td>1974</td>
<td>Yes</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>1980</td>
<td>Yes</td>
</tr>
<tr>
<td>Desflurane</td>
<td>1992</td>
<td>Yes</td>
</tr>
<tr>
<td>Sevoflurane</td>
<td>1995</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**REGULATORY ENVIRONMENT**

Many countries around the world have a health and safety regulatory agency, which establishes occupational exposure standards to protect workers against the adverse effects of occupational hazards. The majority of countries define occupational chemical exposure standards using the time-weighted average (TWA) value. A TWA value is based on an employee’s average airborne exposure in any 8-hour per day, 40 hour per week work shift. The 8-hour TWA permissible exposure limit is the level of exposure established as the highest level of exposure an employee may be exposed to without incurring the risk of adverse health effects. A short term exposure limit (STEL) is the employee’s 15-minute time weighted average exposure. This 15 minute value should not be exceeded at any time during a work day, even if the 8-hour TWA is within the threshold limit value. A ceiling value (C) is the employee’s exposure which shall not be exceeded during any part of the work day (Occupational Health and Safety Administration [OSHA], 2006).

The health and safety regulatory agency in the United States is known as the Occupational Safety and Health Administration (OSHA). OSHA determines occupational exposure limits based on scientific input from research and publication agencies such as the
National Institutes for Occupational Safety and Health (NIOSH) and the American Conference of Governmental Industrial Hygienists (ACGIH). OSHA dictates federal standards that businesses must maintain compliance with, however, individual states are encouraged to develop and implement their own occupational health and safety standards. These state standards must be established at equal or lower levels than the federal OSHA standards. States can also promulgate standards covering hazards not addressed by federal standards (OSHA, 2006). Table 2 displays the permissible exposure limit standards set by various legislative, research, and publication agencies, for various anesthetic agents.

Table 2. Occupational Exposure Standards for Various Anesthetic Agents Established by United States Health and States Regulatory Agencies and Organizations

<table>
<thead>
<tr>
<th>Compound</th>
<th>Exposure Limits</th>
<th>CAL/OSHA</th>
<th>OSHA</th>
<th>NIOSH</th>
<th>ACGIH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoflurane</td>
<td>2 ppm TWA</td>
<td>None</td>
<td>2 ppm REL-TWA (0.5 ppm TWA with N2O)</td>
<td>75 ppm TLV-TWA</td>
<td></td>
</tr>
<tr>
<td>Nitrous oxide</td>
<td>25 ppm TWA</td>
<td>None</td>
<td>25 ppm REL-TWA</td>
<td>50 ppm TLV-TWA</td>
<td></td>
</tr>
<tr>
<td>Halothane</td>
<td>2 ppm TWA</td>
<td>None</td>
<td>2 ppm REL-TWA</td>
<td>50 ppm TLV-TWA</td>
<td></td>
</tr>
<tr>
<td>Enflurane</td>
<td>2 ppm TWA</td>
<td>None</td>
<td>2 ppm REL-TWA</td>
<td>75 ppm TLV-TWA</td>
<td></td>
</tr>
<tr>
<td>Chloroform</td>
<td>2 ppm TWA</td>
<td>2 ppm TWA</td>
<td>2 ppm REL-TWA</td>
<td>10 ppm TLV-TWA</td>
<td></td>
</tr>
</tbody>
</table>

In 1972, the National Institute of Occupational Safety and Health (NIOSH) met with the National Academy of Sciences, National Research Council, and the American Society of Anesthesiologists to gather the current available data and establish a recommendation for the occupational exposure limits to anesthetic agents in use by the medical industry, which were chloroform, trichloroethylene, halothane, methoxyflurane, nitrous oxide, fluroxene, and enflurane.

By 1977, NIOSH published a report of its findings from these institutes conclusions, describing the current anesthetic agents effects of exposure, information on scavenging techniques, methods for monitoring air concentrations, and its criteria for recommended exposure limits. Detailed in the publication, the biologic effects of anesthetic gas exposure.
from human epidemiological, animal, and vivo studies are described. NIOSH was unable to provide any statistically significant conclusions from the available data because the studies either had small population size or contained many confounding factors. Additionally, NIOSH was only able to determine a dose-response relationship for only a limited number of anesthetic agents. Therefore they choose a conservative approach and attempted to minimize any risk to the greatest extent possible, by publishing the recommending exposure limit at the lowest achievable measurable level of analytical detection for anesthetic agents at the time or 2 parts per million (ppm). NIOSH published the recommended exposure limit (REL) at 2 ppm as a 60-minute ceiling for all halogenated anesthetic gases, except nitrous oxide. NIOSH determined that for operations where nitrous oxide is used as the sole inhaled anesthetic agent a REL of 25 parts per million (ppm) measured as a time-weighted average (TWA) based on statistically valid and significant research data. This data revealed that nitrous oxide increases the potential of skeletal abnormalities and reproductive effects of exposed pregnant animals (NIOSH, 1977).

In this 1977 publication, NIOSH did not release a REL for the three modern anesthetics (isoflurane, desflurane, and sevoflurane) because they were not yet developed for institutional use. NIOSH later published an addendum letter requesting additional research and data be collected to demonstrate adverse health effects in workers exposed to these three anesthetic agents.

In 1987, the International Agency for Research on Cancer (IARC) released their conclusion that the modern anesthetics agents are not classifiable as carcinogenic to humans. The IARC review of human cancer incidences among worker populations exposed to anesthetic agents were identified to have inadequacies, such as poor response rates and confounding factors of healthcare workers exposures. The IARC also determined that the carcinogenicity potential from animal studies is insufficient as well, due to limitations in the studies and varying results between control and variable groups (World Health Organization [WHO], 1987).

In 1989, the American Conference of Governmental Industrial Hygienists (ACGIH) designated a threshold limit value (TLV) for nitrous oxide and halothane of 50 ppm over an 8-hour period. ACGIH also established a TLV for enflurane and isoflurane of 75 ppm. The TLV for enflurane and isoflurane is based on the ACGIH’s conclusion that these structural
isomers are safer than the anesthetic gas halothane. ACGIH developed the TLV for halothane based on evidence of human hepatotoxicity and the TLVs of trichloroethylene and chloroform (OSHA, 2000).

In 1994 NIOSH published guidelines in a booklet describing, how workers are exposed to waste anesthetic gases, the use of anesthetic agents in the workplace, the adverse health effects of waste anesthetic gases, how workers are exposed to waste anesthetic gases, how to reduce these exposures, including methods to minimize leakage of anesthesia delivery equipment. In this publication NIOSH included the modern anesthetic agents into a recommended exposure limit, publishing that no employee shall be exposed to concentrations greater than 2 ppm over a sampling period not to exceed one hour (NIOSH, 1994).

Currently, Occupational Safety and Health Administration (OSHA) has not adopted permissible exposure limits (PEL) for the modern anesthetic agents. California Occupational Safety and Health Administration (Cal-OSHA) is the only state administration that has set the regulatory limit for all anesthetic agents at 2 ppm for an 8-hour time-weighted average.

Table 3 displays different permissible exposure limits to modern anesthetic agents for various countries. Very few international regulatory health and safety agencies have adopted the NIOSH recommended exposure limit of 2 ppm for halogenated anesthetic gases. Instead the modern anesthetics have generally been established at higher levels based on these regulatory agencies risk assessment of the compounds (NIOSH and Health Docket Office, 2006).

The Netherlands' 1998 Dutch Expert Committee on Occupational Standards (DECOS) derived an occupational exposure limit of 20 ppm for enflurane on the basis of reproductive toxicological data. Based on the evidence that chemicals with similar structure-related activity have similar health effects, DECOS also recommended an occupational exposure limit of 20 ppm for isoflurane, on the basis of it being an isomer of enflurane (Dutch Expert Committee on Occupational Standard [DECOS], 1998). The United Kingdom has set a 50 ppm isoflurane workplace exposure limit as an 8-hour time weighted average, while the STEL values for isoflurane as a sole anaesthetic agent in European countries vary from 4 to 20 ppm. (United Kingdom Health and Safety Executive, 2013).
Table 3. International Permissible Exposure Limits for Various Modern Anesthetic Agents Established by Regulatory Agencies and Organizations

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Canada – Alberta</td>
<td>75 ppm TWA</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Canada – Quebec</td>
<td>75 ppm TWA</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Belgium</td>
<td>75 ppm TWA</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Ireland</td>
<td>50 ppm TWA</td>
<td>None</td>
<td>50 ppm TWA</td>
<td>None</td>
</tr>
<tr>
<td>Netherlands</td>
<td>20 ppm TWA</td>
<td>None</td>
<td>20 ppm TWA</td>
<td>None</td>
</tr>
<tr>
<td>Spain</td>
<td>75 ppm TWA</td>
<td>None</td>
<td>50 ppm TWA</td>
<td>None</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>50 ppm TWA</td>
<td>None</td>
<td>50 ppm TWA</td>
<td>None</td>
</tr>
<tr>
<td>Sweden</td>
<td>10 ppm TWA</td>
<td>10 ppm TWA</td>
<td>10 ppm TWA</td>
<td>10 ppm TWA</td>
</tr>
<tr>
<td>Finland</td>
<td>None</td>
<td>10 ppm TWA</td>
<td>10 ppm TWA</td>
<td>10 ppm TWA</td>
</tr>
<tr>
<td>Norway</td>
<td>None</td>
<td>20 ppm TWA</td>
<td>None</td>
<td>20 ppm TWA</td>
</tr>
</tbody>
</table>

In 1993, a German commission published a report evaluating isoflurane gas. Based on an extensive review of the literature published at the time, which included human dose response studies and data from animal studies, the committee statistically identified acute isoflurane effects. However, the commission were unable to establish a dose response relationship testing chronic exposures to sub-anesthetic levels (Federal Institute for Occupational Safety and Health, 2010).

In 1998, the Swedish Criteria Group for Occupational Standards evaluated sevoflurane and desflurane. They concluded that there was no scientific information on either compound that could be used as a basis for identifying a critical effect relevant to occupational exposures (Thoustrup & Hougaard, 2009).
In 2000, the Norwegian Labor Inspection Authority concluded that the documentation of the health risks of exposure to isoflurane, sevoflurane, and desflurane was too sparse to identify a critical effect (Thoustrup & Hougaard, 2009).
CHAPTER 2

LITERATURE REVIEW

CHEMICAL STRUCTURE OF ISOFLURANE AND OTHER ANESTHETIC AGENTS

All halogenated anesthetic agents are included together into the chemical group known as ethers, where an oxygen atom is connected to two alkyl groups. The anesthetic agent, isoflurane is a polyfluorinated molecule that is a structural isomer of enflurane, having the same chemical formula, however, a different structure (Figure 3) (Figure 4). It has the chemical name, 2-chloro-2-(difluoromethoxy)-1,1,1-trifluoroethane and has a molecular formula of C₃H₂ClF₅O. Isoflurane is a clear, colorless liquid with a pungent ethereal aroma. It is non-flammable and non-explosive. It has a vapor pressure of 238 mmHg at 20°C, a boiling point of 48.5°C, average molecular mass of 184.5g/mole and is heavier than air with a specific gravity of 1.496 (ChemSpider, 2013). Isoflurane is similar to the other fluranes in being structural ethers. Isoflurane and desflurane are halogenated methylethyl ethers with a difluoromethyl group and a fluorinated ethyl group. Isolfurane has one of the fluorine atoms in the ethyl group substituted with chlorine. Sevoflurane is a polyfluorinated methyl isopropyl ether. All of these fluranes share physical and chemical properties. The anesthesia doses used for human induction are 5,000 – 25,000 ppm for isoflurane and 60,000 – 80,000 ppm for sevoflurane in a mixture of oxygen and nitrous oxide. The corresponding doses to maintain anesthesia are 10,000 – 25,000 ppm for isoflurane, 5,000 – 30,000 ppm for sevoflurane, and 20,000 – 60,000 ppm for desflurane. Desflurane is not used as to induce anesthesia as it is a slight irritant to the upper respiratory tract. In the last decade the trend of these flurane anesthetic gases has changed. Isoflurane gas for human anesthesia practices has diminished while sevoflurane has increased. Alternatively, in veterinary and pharmaceutical research facilities isoflurane has remained the only flurane gas used (Thoustrup & Hougaard, 2009).
**ISOFLURANE MECHANISM OF ACTION**

The purpose of an anesthetic agent is to develop and maintain a balance of anesthetic molecules at the site of the anesthetic action in brain. Much research has been devoted to unlocking the mechanism of how anesthetic agents functionally carry out this phenomenon. According to anesthesiologist James Sonner, with the University of California San Francisco,
"Anesthetics have been used for 160 years and how they work is one of the great mysteries of neuroscience" (Travis, 2004).

The mechanism of action for isoflurane is still today scientifically debated; however, two theories have emerged. The foremost accepted theory suggests that isoflurane acts through a multi-site receptor system that favors an interaction with muscarinic (acetylcholine) binding sites (Figure 5). Here, it acts as inhibitor to the synaptic transmission of acetylcholine in the brain and spinal cord, thus slowing to a halt the communication of response throughout the body (Eger, 1984). Simultaneously, isoflurane appears to bind to the following receptor sites as well: the D-subunit of adenosine triphosphate (ATP) synthase and NADH dehydrogenase, the GABA receptor, the large conductance calcium ion activated potassium channel, the glutamate receptor, and the glycine receptor (Feingold & Holaday, 1977).

Another mechanism of action theory is that isoflurane is mediated by functioning through a specific receptor system that decreases the interactions between cells’ lipids or gap junctions (Figure 6). Studies have shown that it may decrease the formation of channels as well as increase the closing time of gap junctions (Alkire & Gorski, 2004).

As mentioned, isoflurane carries out its pharmacological effect by acting as an inhibitor. For example, isoflurane activates calcium dependent ATP synthase in the sarcoplasmic reticulum and contractile proteins of cardiac muscle. This action slows down
calcium ion uptake and conversely increases calcium ion release. The result floods the cellular gates of the cardiac muscle’s cellular action potentials. This causes a general deceleration in the activated tension development and myocardial contractility in the cardiac muscle (Su & Bell, 1986).

In an overview, anesthetic agents achieve anesthesia by affecting many body systems. The brain, with its high perfusion rate, rapidly equilibrates with anesthetic partial pressure in the blood, causing a regional cerebral metabolic depression. Anesthetics deter mobility chiefly by action on the spinal cord, while amnesia and unconsciousness effects are achieved by action on the brain. Central nervous system depression occurs by slowing the heart rate (Dittmar, Petermichl, Schlachetzki, Graf, & Gruber, 2012).

**PHARMACOKINETICS OF METABOLISM OF INHALATION ANAESTHETICS**

The American Society of Anesthesiologists has promoted the use of isoflurane as its primary anesthetic agent for animals in veterinary clinics and research vivarium laboratories due to its advantageous physical characteristics. Isoflurane is also highly stable, non-
flammable, non-explosive, pleasant to inhale, has a good shelf life, and is relatively inexpensive to manufacture (ChemSpider, 2013). Testing on animals has demonstrated ideal anesthesia properties such as a smooth induction and rapid recovery (Lee, 2010).

The quantity of an inhalational anesthetic that must be administered to cause a desired effect, such as general anesthesia, is referred to as its potency. This potency is measured as the minimum alveolar concentration (MAC), which is defined as the alveolar concentration of anesthetic that prevents muscular movement in 50% of the test subjects in response to a painful stimulus (Aranke, Mashour, & Avidan, 2013). A blood/gas partition coefficient is defined as the ratio of an anesthetic gas between two phases of equal volume and in equilibrium. In other words, it describes how easily the anesthetic passes from gas to blood. The MAC represents the partial pressure or blood/gas partition coefficient at equilibrium in the brain. The anesthetic potency of an inhaled agent is inversely related to the MAC. Studies have shown that the MAC for a specific anesthetic agent is remarkably constant between individuals (Dale & Brown, 1987).

The uptake and distribution of an anesthetic agent depends on the inhaled concentration, pulmonary ventilation, solubility in blood, cardiac output, and tissue uptake. Anesthetic elimination or recovery from anesthesia results from the elimination of the anesthetic chemical from the brain. This process primarily occurs through pulmonary exhalation, however, some anesthetic agent undergo metabolism in various tissues in the body. For example, significant amounts of halothane are removed by hepatic metabolism (Dudziak & Vettermann, 1996). The elimination of anesthetic agents is characterized as the process known as wash-out or the reversal of anesthetic uptake. The prominent factors affecting the induction of anesthetic agents are the same as those for anesthetic recovery (Dale & Brown, 1987).

The greater the blood/gas partition coefficient, the smaller the fraction of anesthetic agent metabolized by the liver during anesthesia. This decreases the risk of hepatitic injury. The liver metabolizes anaesthetic molecules as a function of both the quantity delivered to the hepatic enzymes and the susceptibility of the anesthetic molecules to biotransformation (Aranke et al., 2013).

Isoflurane has been identified as the predominant agent of use in animals, due to its advantageous characteristics that make it such a suitable anesthetic agent. Although
isoflurane has a low potency and a relatively low blood/gas partition coefficient, isoflurane shows quick uptake resulting in rapid induction (Table 4). Isoflurane has a faster induction than anesthetic agents such as halothane and methoxyflurane.

**Table 4. Pharmacokinetics of Metabolism of Inhaled Anesthetics**

<table>
<thead>
<tr>
<th></th>
<th>Partition Coefficient</th>
<th>Arterial concentration during anaesthesia</th>
<th>Millimoles metabolized during anaesthesia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blood</td>
<td>FG</td>
<td>VRG</td>
</tr>
<tr>
<td><strong>Soluble</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diethyl ether</td>
<td>12.0</td>
<td>50.0</td>
<td>13.0</td>
</tr>
<tr>
<td>Methoxyflurane</td>
<td>11.0</td>
<td>87.0</td>
<td>2.4</td>
</tr>
<tr>
<td>Chloroform</td>
<td>8.0</td>
<td>31.0</td>
<td>2.0</td>
</tr>
<tr>
<td><strong>Intermediately Soluble</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Halothane</td>
<td>2.3</td>
<td>60.0</td>
<td>3.1</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>1.4</td>
<td>63.0</td>
<td>2.3</td>
</tr>
<tr>
<td>Enflurane</td>
<td>1.9</td>
<td>58.0</td>
<td>2.0</td>
</tr>
<tr>
<td><strong>Poorly Soluble</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sevoflurane</td>
<td>0.69</td>
<td>13</td>
<td>0.6</td>
</tr>
<tr>
<td>Desflurane</td>
<td>0.65</td>
<td>37</td>
<td>1.3</td>
</tr>
<tr>
<td>Nitrous Oxide</td>
<td>0.47</td>
<td>2.3</td>
<td>1.1</td>
</tr>
</tbody>
</table>

*FG: Fat Group or Adipose Tissue  
*VRG: Vessel Rich Group (brain, heart, liver, kidney, muscle tissue)  
NOTE: A blood/gas Partition Coefficient of 0.5 means that the concentration of inhalant in the blood is half that present in the alveolar gas when the partial pressure of the anesthetic is identical at both sites.  
(Yasuda, Targ, & Eger, 1989)  
Metabolism of an anesthetic agent is a function of the uptake of the agent, the MAC in the body and the duration of exposure.

Isoflurane has a high partition coefficient and is insoluble in fat, resulting in a quick induction and rapid wash-out or recovery. Isoflurane shows a low arterial concentration during anesthesia of 0.081 mg/100ml, due to having low blood solubility. Additionally isoflurane only goes through minimal biotransformation in the body. These two characteristics result in less isoflurane molecules delivered to the liver, which decreases the number of isoflurane secondary metabolites produced. Secondary metabolites are known to produce DNA altering active radicals which are associated with damage to cellular structures and processes. Isoflurane secondary products are generally less lipophilic and more water soluble, which diminishes the amount of tissue saturation attained; however, it is important to note that the amount of isoflurane metabolized to secondary products is further increased as
the duration of anesthesia is increased. Secondary metabolites of isoflurane consist of serum and urinary fluorides, which have been identified as both increased after isoflurane anaesthesia (Mazze, Cousins, & Barr, 1974).

**HUMAN HEALTH RISKS ASSOCIATED WITH INHALED ANAESTHETICS**

In the 1840’s William Morton demonstrated the use of ether as an inhalation anesthetic agent to perform dental surgery without causing any pain to its patients, and soon began the widespread use of anesthetic agents across the medical community. During its early use, medical doctors began to recognize the dangers of anesthetic gas use. The first generation of anesthetic agents, ether and cyclopropane, have low flash points and operating room personnel were at great risk for fires and explosions. Additionally, through retrospective cohort studies, the occurrence of patients suffering toxicity, male anesthesiologists developing health conditions, and female nurse anesthesiologists having reproductive issues were detected. In one chloroform study, human deaths were reported due to acute hepatotoxicity in women after childbirth. The combination of prolonged labor with dehydration and exhaustion contributed to the chloroform-induced hepatic failure (Townsend, 1939).

In 1967, a Russian epidemiologist, created an international stir in the medical health care community when he released the results of a health questionnaire he collected from 300 anesthesiologists. The survey revealed a high incidence of headaches, fatigue, and irritability among the personnel, as well as a peculiar correlation between anaesthetic gas exposure and an adverse reproductive effect in female nurse anesthesiologists. This same survey showed that 18 of the 31 pregnancies among female nurse anesthesiologists ended in spontaneous abortions (Vaisman, 1967).

Epidemiological retrospective studies have been conducted looking back 150 years at nation’s health care worker statistics. In 1974, a large survey by the American Society of Anesthesiologists, found that female operating room personnel, such as registered nurses, had an increased risk of spontaneous abortion and congenital abnormalities in their offspring. Exposed male anesthesiologists showed a greater risk of congenital abnormalities in their progeny as well (American Society of Anesthesiologists, 1974).
In 1985, the American Society of Anesthesiologists commissioned a meta-analysis to review the association between health hazards and exposure to anesthetic agents. The analysis corroborated the findings in the 1974 national survey, by finding a 30% increase in risk of spontaneous abortion and congenital anomalies for women and about a 50% increase in liver disease among men and women. Additionally, a 30% increase in kidney disease was shown in women. However, the review did not distinguish a single anesthetic agent dose response rate (Tannenbaum & Goldberg, 1985).

Additionally, the ASA meta-analysis, pointed out that there were many weaknesses in published literature, including small population size, inconsistencies between anesthetic agent exposure levels, and cofounding variables. Furthermore, the report noted that any increased risk they identified was within the statistical range that it could also be correlated to responder bias or uncontrolled confounding variables, such as radiation exposure, stress, and other pharmaceutical agents. These same results were reproduced in various other studies and additionally emphasized the necessity to perform prospective studies to further determine if hazards exist due to anesthetic gas exposure (Ebi, Rice, & Fish, 1994).

A study attempting to detect genetic damage in humans, who were exposed to anesthetic agents found through analysis of many bodily fluids and blood cells from hospital operating personnel, that there was an increased risk of genetic damage. This genetic damage was demonstrated in additional studies to consist of damage to micronuclei in human lymphocytes, the alteration of sister chromatid exchanges, and oxidative DNA damage in blood, which all result in a rise of toxic metabolites in human blood serum. At the same time a decrease in the urinary excretion of these toxic metabolites occurs (Cascorbi, Blake, & Helrich, 1972).

In scientific studies that measured the urinary excretion of toxic metabolites to determine effects from halogenated anesthetic exposure. Identified three specific metabolites produced in the human body, which suggest the presence of reactive intermediates or toxic biotransformation which could cause hepatotoxicity (Cohen, Bellvill, & Brown, 1971). In a research publication attempting to measure and compare the toxicity of halothane, isoflurane, and diethyl ether, an overall increased level of hepatotoxicity was seen in high chronic exposures in humans and low level chronic exposures in animals (Stevens et al., 1975).
However, discrepancies in these studies such as, bias in data collection, uncontrolled confounding exposure variables in operating room environments, and small study groups have made the significance of these findings still controversial. The debate on whether inhalation of trace levels of anesthetic gases can cause adverse health effects in humans, has stimulated subsequent scientific research to determine (with statistical significance) whether inhalation of trace levels of anesthetic gases were able to cause adverse health effects in humans.

A number of studies commissioned by NIOSH were conducted to determine supplementary data on anesthetic agents toxicity to humans by researching mutagenicity, reproductive, skeletal, and carcinogenicity effects in animals and in vitro cell cultures. The in vitro studies concluded that the modern anesthetics agents, isoflurane, enflurane, desflurane, and sevoflurane have no mutagenic potential (Baden & Simmon, 1980). In animal studies using rodents, only chlororform and trichloroethylene, of all the anesthetic agents, were found to be carcinogenic via oral exposure (Eger et al., 1978). In supplemental animal studies, isofurane and the other modern anesthetic agents did not show any organ toxicity evidence to the kidneys, liver, or other organs (Baden, Kundomal, Mazze, & Kosek, 1988). However, a study published this same year as Vaisman’s publication of his health questionnaire, in 1967, concluded that high exposure of nitrous oxide resulted in significant increase of skeletal defects in exposed rats’ offspring (Fink & Shepard, 1967).

**ANESTHETIC AGENTS DOSE-RESPONSE RELATIONSHIP**

Although a dose-response relationship for halogenated anesthetic agent toxicity, such as isoflurane, has not been defined, studies have attempted to correlate anesthetic gas exposure with a dose response relationship.

In an animal study, the lowest observable adverse effect level of halothane exposure was determined at 10 ppm for 8 hours per day, 5 days per week, for 8 weeks, and chloroform exposure at 300 ppm for 7 hours per day, for 9 days resulted in organ and skeletal abnormalities. Reproductive and teratogenic effects were seen at all levels of anesthetic agents tested (Baden, Mazze, Whattron, Rice, & Kosek, 1979). Other animal studies demonstrated that liver and kidney cells structures are altered at a concentration of 10 ppm halothane indicating that chronic exposure to halothane can cause organ toxicity (Chang,
Dudley, & Katz, 1975). Animal studies involving halothane exposure demonstrated that even low concentrations can cause an increase in the body’s duration of exposure because of halothane’s affinity for fat reservoirs (Stevens et al., 1975). Halothane can be recovered in exhaled breath of humans six days following exposure and measure in the urine and feces even longer (Cascorbi et al., 1972).

In another animal study, a nitrous oxide exposure level of 200,000 ppm had detrimental but reversible effects of spermatozoa production in male rats (Kripke, Kelman, Shah, Balogh, & Handler, 1976).

In an inhalation rat study which lasted for 7 hours per day, 5 days per week, for 104 weeks, exposed rats to 10 ppm halothane and 500 ppm nitrous oxide. The results of the two year study had no indication of behavior or physical appearance, as well as no increase in lesion or tumor growth in organs (Coate, Ulland, & Lewis, 1979).

Biotransformation of inhaled anesthetic agents in the body occurs in the liver, which results in the production of toxic metabolites. These toxic metabolites increase the potential of hepatotoxicity. Animal studies have been conducted to demonstrate the correlation between the toxicity of an inhaled anesthetic and its resistance to metabolism. In a chronic exposure study various animals, mice, rats, and guinea pigs, where exposed to various halogenated anesthetic agents for 35 days. The lowest observable adverse effect level results, which was a detriment to weight gain and liver damage, were seen at a concentration of 50-150 ppm for halothane, 200 ppm for methoxyflurane, 1,500 ppm isoflurane and 10,000 for diethyl ether. The study demonstrated reproducible results that halothane is a dose-related hepatotoxin, present in all tested rodent species (Stevens et al., 1975).

In another animal study which attempted to understand if low levels of anesthetic gas exposures have been recorded to cause decreases in cognition, audiovisual ability, and dexterity in humans. Young rats were exposed to halothane at 8-12 ppm, 8 hours per day, 5 days per week, for 8 weeks. The result found that these low concentrations of halothane were sufficient to cause learning impairments during animal adolescent periods (Quimby, Katz, & Bowman, 1975). The results of a study to measure the carcinogenicity of halothane, concluded that exposure to halothane at 500 ppm is not associated with an increased incidence of tumor growth during the course of mice lifetime (Baden et al., 1979).
The reported lethal concentrations for 50% (LC50) of exposed animals at a single inhalation exposure of 3 – 4 hours duration are described below. Results indicate that sevoflurane exhibits approximately half the acute toxicity of isoflurane. Other effects tested displayed that isoflurane at 1,145 ppm for 30-40 minutes cause a hyperalgesic effect and cause a analgesic effect at higher exposures of 2,920 and 5,840 ppm. Additional results displayed animals exposed to 4,000 ppm caused slower learned reaction to new tasks while exposure to 8,000 ppm caused the complete failure to a stimuli. Isoflurane exposure provided fear conditioning at exposure levels of 3,700 ppm and above (Thoustrup & Hougaard, 2009). Table 5 displays lethal concentration testing results of the anesthetic agents, isoflurane and sevoflurane, conducted during this animal study.

Table 5. Lethal Concentrations for 50% of the Exposed Animals at a Single Inhalation Exposure (LC50)

<table>
<thead>
<tr>
<th>Anesthetic agent</th>
<th>Animal Species</th>
<th>Exposure Duration</th>
<th>LC 50 (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoflurane</td>
<td>Rat</td>
<td>0.5</td>
<td>125, 200</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>Rat/mouse</td>
<td>1</td>
<td>58,000 – 83,000</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>Rat</td>
<td>3</td>
<td>15,300</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>Mouse</td>
<td>3</td>
<td>16,800</td>
</tr>
<tr>
<td>Sevoflurane</td>
<td>Rat</td>
<td>3</td>
<td>28,800</td>
</tr>
<tr>
<td>Sevoflurane</td>
<td>Mouse</td>
<td>3</td>
<td>28,300</td>
</tr>
</tbody>
</table>

* No LC50 was identified for desflurane. (Thoustrup & Hougaard, 2009).

In an inhalation study to document the short term effects of Sevoflurane exposure in humans, results from the study revealed subjects exposed to 4,000 ppm concentration for 68 minutes displayed an increase of tiredness and lightheaded. At the highest exposure concentration tested of 10,000 ppm for 68 minutes, no significant additional symptoms were displayed (Janiszewski et al., 1999). Sevoflurane is metabolized in the kidney raising serum inorganic fluoride concentrations, which can cause renal impairment. However, exposures to sevoflurane at anesthetic dose levels in human patients has not been associated with any recorded renal injury. Sevoflurane is metabolized at a much higher extent than isoflurane and desflurane and therefore reduces the risk associated with their use (Thoustrup & Hougaard, 2009).
Chloroform a known centrilobular hepatotoxin, where liver cells contain a higher concentration of metabolizing enzymes has an established lowest observed adverse effect level (LOAEL) of 12.9 mg/kg-day and a BMDL\textsubscript{10} of 1.0 mg/kg-day (Dog, chronic oral bioassay). Animal studies included the following endpoints, the development of fatty cysts in liver, elevated Serum alkaline phosphatase (SAP), and serum glutamate-pyruvate transaminase (SGPT) levels. Chloroforms published inhalation unit risk is 2.3E-5 per ug/m3 (Intergrated Risk Information System [IRIS], 2012).

The ability of isoflurane and nitrous oxide to alter sister chromatid exchanges and damage micronuclei in human lymphocyte was evaluated by measuring the concentration of these waste anesthetic gases and then the resulting genetic damage to lymphocytes after an eight hour work day. Occupational exposure (Hospital Operating Room) was 8 hour time weighted average of 12.8 ppm nitrous oxide and 5.3 ppm isoflurane. The results of the study displayed that there was an increased risk of genetic damage (rate of chromatid exchanges) when comparing 10 non-smokers working in the operating room and 10 non-smoking controls (matched by age, sex, and smoking habits (Hoerauf et al., 1999). In another genotoxicity study in humans, a survey of the amount of DNA damage between anesthetized hospital patients was made. Isoflurane and sevoflurane were found to not cause any amount of DNA damage, such as strand break or lymphocyte alterations, during anesthesia and after (Braz et al., 2011).

In a retrospective cohort study that examined relations between inherited anomalies in offspring and mothers employed as nurses who were exposed to anesthetic gas (Nitrous oxide throughout, halothane, isoflurane, and enfurane in the early years, and isoflurane, desflurane, and sevoflurane in the later years). An association was discovered that exposure concentration at the PEL of 2 ppm TWA or below, did cause an elevated odds of having congenital anomalies in the offspring of nurses (Teschke et al., 2011).

The ability of isoflurane and nitrous oxide to cause genetic damage in female healthcare workers was evaluated by detecting oxidative DNA damage in blood samples following exposure to waste anesthetic gases after an eight hour work day. Occupational exposure data collected for an average sampling time of 360 minutes resulted in an air concentration 185 – 1,502 ppm nitrous oxide and 0.4 – 15.0 ppm isoflurane. The results of the study displayed that there was a significant positive correlation between an exposure to
anesthetic gases and an increase in oxidative DNA damage when comparing 36 nurses occupationally exposed to anesthetics and 36 nurses not occupationally exposed to anesthetics (Wronska-Nofer et al., 2012).

Long-term occupational exposure to 0.16 and 0.35 ppm isoflurane were not associated with effects on liver function or neurofunction, respectively. Also, no effects on liver function were observed in a short-term study in children, exposed at anaesthetic dose levels for short periods for several days. In rats, no detectable effects on the liver or kidneys were observed after continuous exposure to 20 ppm isoflurane for 30 weeks. In rats, mice and guinea pigs, 35 days of continuous exposure to 150 and 500 ppm did not affect the liver, but at 1 500 ppm, liver lesions were non-significantly increased in all three species. Body weight gains were reduced to a similar extent at and above 150 ppm (overall LOAEL) in mice, and in guinea pigs only at 1,500 ppm (Thoustrup & Hougaard, 2009).

Concerning the duration of exposure to volatile anesthetics, a research questionnaire, given to exposed workers, who had a duration of exposure to anesthetic agents of about 9 years, demonstrated that dizziness, headache, irritability, decreased concentration, anxiety, and fatigue were significantly more prevalent among operating room personnel, than compared to the hospital employees who had no exposure to anesthetic agents (Abd El-Aal, Al-Batanony, & El-Shafiy, 2008).
CHAPTER 3

METHODS

This chapter describes the isoflurane data collection sites, sampling method, and analyses.

DATA COLLECTION FACILITIES

Occupational exposure data to isoflurane gas was collected from pharmaceutical and research companies and veterinarian clinics located within San Diego County. Sample data consisted over the period from 2006 through 2013. Each company was contacted by telephone and data was volunteered to contributed to this study.

SAMPLE DESIGN AND COLLECTION

Each data value represents a sample of isoflurane gas collected during an industrial hygiene survey. Sampling techniques for isoflurane include OSHA method 103, which uses a low flow sampling pump with an (6-mm o.d., 150/75 mg) Anasorb CMS or (6-mm o.d., 140/70 mg) Anasorb 747 tube. The low flow sampling pump is pre and post calibrated with a primary air flow calibration device at approximately 0.050 liters per minute (50 ml/min). Measurements are not valid if the pump air flow varied by greater than plus or minus one percent (+1%) during sampling and the Anasorb tubes do not exceed 12 L in air.

Another approved sampling and analysis method is the modified OSHA 07 method which uses organic vapor monitors. These badges passively collected isoflurane vapors on its activated charcoal media.

Isoflurane can also be measured using a direct reading instrument such as a single beam infrared spectrophotometer. These infrared analyzers continuously sample the air and provide real time concentration of anesthetics in the air. These devices are capable of timed, single-sample and continuous data logging.
CHEMICAL ANALYSES

All laboratories used to analyze samples are accredited by AIHA Laboratory Accreditation Program (AIHA-LAP, LLC) and Industrial Hygiene Laboratory Accreditation Program (IHLAP).

Anasorb tubes are desorbed with CS$_2$ while organic vapor monitors are extracted with an organic solvent. Both are analyzed according to modified OSHA Method 07, which uses an organic solvent for extraction and Gas Chromatography (GC) analysis with a flame-ionization detector (FID). The analytical limit of detection for anesthetic gases is 20 µg. GC is an analytical instrument used for organic compound determinations.

The analysis laboratory used a Hewlett-Packard 5890A Gas Chromatograph equipped with a 7673A Automatic Sampler. A Forma Scientific Model 2006 refrigerated circulator was used to cool the sample tray of the HP 7673A to 10°C to minimize evaporation. A 60-m × 0.32-mm i.d. fused silica Stabilwax-D8419 column with a 1-µm df was used to separate isoflurane from the desorption solvent, internal standard, and any interferences. A Waters 860 Networking Computer System was used to measure peak areas. To prepare a target level standard of isoflurane, 10 µL of a stock solution containing 672 mg/mL of isoflurane was injected into 1 mL of desorption solvent.

STATISTICAL ANALYSES

Each pharmaceutical research laboratory and veterinarian clinic had a different number of samples collected. A statistical summary was prepared for each population data pool to compare all time weighted average concentrations between pharmaceutical research laboratories and veterinarian clinics.
CHAPTER 4

RESULTS

A time weight average (TWA-8Hour) value is based on an employee’s average airborne exposure in any 8-hour per day, 40 hour per week, work shift. The time weighted average concentration is calculated using the following equation:

\[
E = \frac{(CaTa+CbTb+...CnTn)}{480 \text{ minutes}}
\]  

Where:

- \( E \) = exposure for the working shift
- \( C \) = concentration during any period of time \( T \) where the concentration remains constant
- \( T \) = duration in minutes of the exposure at the concentration \( C \)

The time weighted average concentrations \((TWA\pm SD)\) in pharmaceutical research laboratories and veterinarian clinics were calculated using equation 1. The maximum data value was collected from pharmaceutical research laboratory #4. The maximum recorded exposure concentration was collected from veterinarian clinic #1. The total TWA range for the pharmaceutical research laboratory population was 0.04 – 1.64 ppm (1.61 ppm) while among the veterinarian clinics it was 1.65 – 2.77 ppm (1.12 ppm). The maximum TWA value among the pharmaceutical research laboratory population was 1.64 while among the veterinarian clinics it was 2.77 ppm. (Table 6).

The aggregate TWA±SD for the pharmaceutical research laboratory population was 0.62±0.47 ppm while among veterinarian clinics it was 2.14±0.38 ppm.

The California OSHA has established a permissible exposure limit for isoflurane at 2 ppm for an 8-hour time-weighted average. All pharmaceutical research data, which included 64 samples, was never above this limit (Figure 7). While the veterinarian data was above this exposure limit a total of 7 out of 11 samples (Figure 8).
Table 6. Summary of Isoflurane Exposure Data Collected from Pharmaceutical Research Laboratories and Veterinarian Clinics

<table>
<thead>
<tr>
<th>Data Pool Source</th>
<th>N</th>
<th>Mean (ppm)</th>
<th>Median (ppm) (Min - Max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmaceutical Research Laboratory 1</td>
<td>10</td>
<td>0.61</td>
<td>±0.47 (0.04 - 1.60)</td>
</tr>
<tr>
<td>Pharmaceutical Research Laboratory 2</td>
<td>10</td>
<td>0.59</td>
<td>±0.40 (0.11 - 1.37)</td>
</tr>
<tr>
<td>Pharmaceutical Research Laboratory 3</td>
<td>9</td>
<td>0.46</td>
<td>±0.14 (0.13 - 0.65)</td>
</tr>
<tr>
<td>Pharmaceutical Research Laboratory 4</td>
<td>10</td>
<td>0.99</td>
<td>±0.40 (0.44 - 1.64)</td>
</tr>
<tr>
<td>Pharmaceutical Research Laboratory 5</td>
<td>25</td>
<td>0.54</td>
<td>±0.54 (0.10 - 1.53)</td>
</tr>
<tr>
<td>Veterinarian Clinic 1</td>
<td>6</td>
<td>2.26</td>
<td>±0.45 (1.72 - 2.77)</td>
</tr>
<tr>
<td>Veterinarian Clinic 2</td>
<td>5</td>
<td>2.00</td>
<td>±0.11 (1.65 - 2.30)</td>
</tr>
</tbody>
</table>

Figure 7. Pharmaceutical research laboratory occupational isoflurane exposure data.
Figure 8. *Veterinarian clinic occupational isoflurane exposure data.*

The aggregate mean and maximum TWA values were used to estimate adverse health risks associated with isoflurane exposure to these levels. Risk calculations incorporated a human breathing rate of 20m3/day of air and a human body weight of 70 Kg. The level of human exposure resulting from isoflurane gas exposure can be expressed by an estimation of the average daily dose calculated using the following equation:

\[
\text{Average Daily Dose (mg/kg-day)} = \frac{HB \times C \times (MW)}{24.45 \times (BW)}
\]  

(2)

Where:

- C = Time weighted average (mg/m3)
- MW = Molecular Weight of isoflurane or 184.5
- HB = Human Breathing/day of air or 20m3
- BW = Body weight (kg) or 70 Kg

Using the aggregate TWA±SD and the maximum TWA values among pharmaceutical research laboratories and veterinarian clinics, the average daily dose (ADD) and maximum daily dose were calculated. (Table 7).
To calculate a Reference Dose (RfD), the no observable adverse effect level (NOAEL) from a scientific animal or human study is first identified. A NOAEL is the calculated dose at which there was no significant indication of a defined effect.

In a rat study, the NOAEL amongst a group of 60 rats, was determined to be a single exposure to 530 ppm isoflurane, with the observed effect being effects to cognitive function (Alkire & Gorski, 2004). In a study involving fourteen humans, the lowest observable adverse effect level (LOAEL) displayed, was an exposure to 1,000 ppm isoflurane for 40 minutes, which resulted in decreased cognitive function detected by a symbol substitution test (Beckman, Zacny, & Walker, 2006). After reviewing several mice studies, an overall LOAEL can be determined at 150 ppm of isoflurane at continuous exposure for 35 days, which resulted in depressed body weight (Stevens et al., 1975).

Reference Dose (RfD) in mg/m³ values were calculated using the following equation:

\[
\text{Reference Dose (mg/m}^3\text{)} = \frac{C \times MW \times (BR)}{24.45 \times BW \times (UF)}
\]  

(3)

Where:

- \( C \) = TWA concentration (ppm)
- \( MW \) = Molecular Weight of isoflurane or 184.5
- \( BR \) = Breathing Rate of species
  - 20m³ Human breathing/day of air
  - 0.22m³ Rat breathing/day of air (Suckow, Weisbroth, & Franklin, 2006)
  - 0.03m³ Mouse breathing/day of air (Spector, 1956)
- \( BW \) = Species Average Body Weight
  - 70 kg for an adult
  - 0.4 kg Rat (Suckow et al., 2006)
  - 0.03 kg Mouse (Lawson, 1999)

---

### Table 7. Average Daily Dose Pharmaceutical Research Laboratories and Veterinarian Clinics

<table>
<thead>
<tr>
<th>Data Pool Source</th>
<th>Average Daily Dose (mg/m³)</th>
<th>Maximum Average Daily Dose (mg/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmaceutical Research Laboratory</td>
<td>1.33 mg/kg-day</td>
<td>3.54 mg/kg-day</td>
</tr>
<tr>
<td>Veterinarian Clinic</td>
<td>4.61 mg/kg-day</td>
<td>5.97 mg/kg-day</td>
</tr>
</tbody>
</table>
- **UF = Uncertainty Factors (factors of 10)**

Uncertainty factors or safety factors were added to the equation to make allowance for species differences between humans and animals, variation in susceptibility among individuals in a given population, and extrapolation from a LOAEL to a NOAEL. Each identified uncertainty factor was assigned a value of 10. Using the rat study’s NOAEL of 530 ppm isoflurane, the RfD was calculated to be 2.20 mg/kg-day, accounting for three uncertainty factors. Using the human study’s LOAEL of 1,000 ppm isoflurane, the RfD was calculated to be 21.56 mg/kg-day, accounting for two uncertainty factors. Using the mice study’s LOAEL of 150 ppm isoflurane, the RfD was calculated to be 1.13 mg/kg-day, accounting for three uncertainty factors.

Health risks produced by non-cancer causing agents are quantified by calculating the hazard index (HI). This value is a ratio of the exposure divided by the Reference Dose (RfD). Once the ADD and RfD are calculated, the hazard index was determined by the following equation:

\[
\text{Hazard Index} = \frac{ADD}{RfD}
\]

Where:
- **ADD = Average Daily Dose (mg/kg-day)**
- **RfD = Reference dose (mg/kg-day)**

If the ADD of a chemical is the same as or exceeds the RfD, then the HI is equal to or greater than one, which indicates that adverse health effects will potentially be observed.

To calculate the HI the calculated human LOAEL (21.56 mg/kg-day) was selected due to the fact that the value does not include uncertainty factors regarding interspecies differences. The determined HI for pharmaceutical research laboratories was 0.06. The hazard index for their maximum recorded exposure concentration was 0.16. The hazard index for the veterinary population exposed to the average daily dose (0.21 mg/kg-day) was 0.21. The hazard index for the maximum recorded exposure concentration was 0.28 (Table 8).

Figure 9 – 15 display graphical representation of the data from each population. Skewness, a measurement of symmetry, identifies how the data distribution falls within its range. A data set with a distribution to the right has a positive skew, while distribution to the
Table 8. Hazard Indices of Pharmaceutical Research Laboratories and Veterinarian Clinics

<table>
<thead>
<tr>
<th>Data Pool Source</th>
<th>Hazard Index (using ADD)</th>
<th>Hazard Index (using Max ADD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmaceutical Research Laboratory</td>
<td>0.06</td>
<td>0.16</td>
</tr>
<tr>
<td>Veterinarian Clinic</td>
<td>0.21</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Figure 9. Histogram of isoflurane exposure data from pharmaceutical research laboratory 1.

Figure 10. Histogram of isoflurane exposure data from pharmaceutical research laboratory 2.
Figure 11. Histogram of isoflurane exposure data from pharmaceutical research laboratory 3.

Figure 12. Histogram of isoflurane exposure data from pharmaceutical research laboratory 4.

Figure 13. Histogram of isoflurane exposure data from pharmaceutical research laboratory 5.
left has a negative skew. A symmetrical distribution has a skewness of zero. Kurtosis is a measure of how relative the data is bell-shaped or representative of a Gaussian function. Data sets with negative kurtosis tend to be flatter near the mean rather than have a sharp peak (Figures 9 – 15).

The skewness and kurtosis values can be used to determine approximately where the next sampling data value concentration will be. None of the pharmaceutical research laboratory histograms displayed a normal distributed bell curve covering the range from 0 to 2 ppm concentration. Instead four out of the five histograms displayed a skewed data to the right indicating that the next data sample collected will most likely be less than 1 ppm. None of the veterinarian clinic histograms displayed a normal distributed bell curve as well, however, this population’s data pool was higher ranging from 1.5 to 3 ppm concentration.
These histograms are skewed toward the left because veterinarian clinics stopped collecting data once they had an exposure survey which displayed isoflurane levels under 2 ppm. (Table 9).

Table 9. Skewness and Kurtosis Values of Pharmaceutical Research Laboratories and Veterinarian Clinics

<table>
<thead>
<tr>
<th>Data Pool Source</th>
<th>Skewness</th>
<th>Kurtosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmaceutical Research Laboratory 1</td>
<td>0.47</td>
<td>0.95</td>
</tr>
<tr>
<td>Pharmaceutical Research Laboratory 2</td>
<td>0.50</td>
<td>-0.19</td>
</tr>
<tr>
<td>Pharmaceutical Research Laboratory 3</td>
<td>-1.67</td>
<td>4.61</td>
</tr>
<tr>
<td>Pharmaceutical Research Laboratory 4</td>
<td>0.07</td>
<td>-1.04</td>
</tr>
<tr>
<td>Pharmaceutical Research Laboratory 5</td>
<td>0.96</td>
<td>-0.83</td>
</tr>
<tr>
<td>Veterinarian Clinic 1</td>
<td>-0.24</td>
<td>-2.22</td>
</tr>
<tr>
<td>Veterinarian Clinic 2</td>
<td>-0.38</td>
<td>0.28</td>
</tr>
</tbody>
</table>
CHAPTER 5

DISCUSSION AND CONCLUSION

DISCUSSION

Over the last 30 years several animal and epidemiological studies have attempted to quantify the adverse health effects of occupational exposures to trace waste anesthetic gases. From Vaisman’s health questionnaire to the ASA meta-analysis review, early studies analyzed historical anesthetic agents, such as halothane, nitrous oxide, and chloroform. The published research literature prior to 1970s, found associations of health effects in animals and humans exposed to these historical anesthetic agents. Halothane was identified to be associated with reproductive and teratogenic effects (Baden et al., 1979). Chloroform and halothane were both identified to cause hepatotoxicity (Townsend, 1939). Nitrous oxide, typically used in combination with other anesthetic agents, was associated with skeletal defects in rats’ offspring and caused genotoxic and congenital disorders in humans (Teschke et al., 2011). Recent studies, analyzing modern anesthetic agents, including sevoflurane, enflurane, isoflurane, and desflurane, have published results indicating decreased health risks.

Today’s modern anesthetic agents have been developed out of a collection of chemicals which all contain similar chemical structures. This chemical group is different than the older anesthetic agents. The isomer chemical structures have been evaluated and identified to contain less reactive elements which also have bonds with increased stability (ChemSpider, 2013).

After exposure, the metabolism and resulting secondary metabolites, of modern anesthetic agents have been researched. These studies indicate that this group of chemicals are metabolized at a slower rate, due to their insolubility in fat and low blood/gas partition coefficient. Resulting in a reduced anesthetic induction recovery time. As well as demonstrating that a lower fraction of the absorbed modern anesthetic molecules is recovered as toxic secondary metabolites (Mazze et al., 1974).
The modern anesthetic group also has similar low potencies, which is defined by having a lower blood/gas partition coefficient, compared to the older anesthetic agents. The characteristics of modern anesthetic agents have demonstrated that their use is considered safer than the older anesthetic agents such as diethyl ether, trichloroethylene, and halothane (Aranke et al., 2013).

No human epidemiological studies of low-level inhalation exposures to modern anesthetic agents have resulted in long term health effects. Genotoxicity studies using modern anesthetics varied in response to the amount of DNA damage caused by exposure. In studies where modern anesthetic agents were used, in combination with nitrous oxide, results tended to display an increased risk. Comparatively, no DNA damage was observed where modern anesthetic agents were solely used (Braz et al., 2011). Research studies identifying carcinogenic effects of modern anesthetics have found that these agents do not cause carcinogenicity via oral exposure in animals (Eger et al., 1978). Exposure studies evaluating whether modern anesthetics cause hepatotoxicity in humans, demonstrated that low concentrated exposures did not affect the liver or cause impaired liver function. Hepatoxicity was only a statistical finding in studies, where a modern anesthetic agent was used and delivered in combination with nitrous oxide. When modern anesthetic agents were the sole anesthetic agent used, the results did not display any evidence of toxicity to the kidneys in animals (Baden et al., 1988). Mutagenicity research on modern anesthetics was evaluated using in vitro studies. The exposure of each singular agent did not display any mutagenic potential (Baden & Simmon, 1980). Reproduction and teratogenic studies evaluated modern anesthetic agents. A survey to detect these adverse health effects, collected from various medical hospitals, included data from 455 women. No significant differences were found during pregnancy or delivery rates between female employees who had isoflurane exposure during the course of their daily work than those who did not (Beilin et al., 1999). Additionally, numerous epidemiological studies examined the exposure to modern anesthetic agents and reproductive problems in women. These overall results tended to show that exposure may enhance the risk for spontaneous abortions and the incidence of malformations in children, however, these studies were subject to recall biases, confounding factors, and many other methodological flaws (Weinberg & Wilcox, 1998).
This research project used a collection data from 5 pharmaceutical research laboratories and 2 veterinary clinics of employees exposed occupationally to isoflurane gas. The mean time weighted average concentration and maximum recorded exposure concentration from each data population, were calculated to be (pharmaceutical research laboratories TWA-8Hour 0.62 ppm; MAX-8Hour 1.64 ppm) (veterinarian clinics TWA-8Hour 2.14 ppm; MAX-8Hour 2.77 ppm) all under 3 ppm. Exposure levels in animal studies to detect health effects were higher and did not included typical workplace exposure durations. For instance, in a mouse study, the continuous exposure of 20 ppm anesthetic agent was delivered for 30 weeks. In another study, rats, mice, and guinea pigs, were exposed continuously for 35 days to 150 ppm, 500 ppm, and 1,500 ppm to an anesthetic agent. In comparison, the average duration of exposure in the total combined data collected for this risk assessment was approximately 2 hours.

Because of the relatively high exposures and extended duration, the lowest observable adverse effect level (LOAEL) from the mouse study was not chosen to calculate the Reference Dose (RfD). The rat study was discounted, as well, because of the high exposure level and its variability in identifying its LOAEL or cognitive function impairment in rats. Although the LOAEL in the human study was high, it was ultimately selected to calculate the reference dose because it eliminated the interspecies uncertainty factor, which accounted for the fact that humans may be more or less sensitive than an animal population.

Using the Human RfD of 21.56 mg/kg-day, the hazard index for this risk assessment was calculated. The hazard index is defined as the ratio of the exposure concentration over the reference dose. This value is compared to 1.0. If the measure is less than 1, then adverse health effects are accepted to be of concern. If the hazard risk is greater than 1, then adverse health effects are expected to occur (Environmental Protection Agency [EPA], 2012). Based on the data collected in this study, the hazard indices for pharmaceutical research laboratories and veterinarian clinic populations’ mean time weighted average (ADD 1.33 mg/kg-day and 4.61 mg/kg-day) and maximum (MAX 3.54 mg/kg-day and 5.97 mg/kg-day) concentrations were all under a risk ratio of 1.0, which confirms occupational exposure levels in these populations will not cause harmful health effects.

All pharmaceutical research data, which included 64 samples, was never above the Cal/OSHA permissible exposure limit (PEL) for isoflurane of 2 ppm. The veterinarian data
was above this exposure limit a total of 7 out of 11 samples. The maximum concentration recorded in this survey, was 2.77 ppm, which was collected from the veterinarian clinic population. Using this concentration, the hazard risk ratio was under 1.0 and displays no significant risk of adverse health effects. These results confirm that the California OSHA PEL of 2 ppm is set at a limit below the harmful health effect level in humans.

**DIFFERENCES BETWEEN VETERINARIAN CLINICS AND PHARMACEUTICAL RESEARCH LABORATORIES**

Veterinarian clinics and pharmaceutical research laboratories both use isoflurane as their primary anesthetic agent. In pharmaceutical research laboratories the agent is used to anesthetize animals prior to drug injections, surgical procedures, or imaging animals for tumor growth. During each anesthetic laboratory operation there is potential exposure to isoflurane during the initial induction of the animals, typically mice or rats. The potential for gas to escape occurs while the induction chamber’s lid is opened and the animal is placed or removed. Another source of exposure is during procedures to scan animals for tumors. The chamber of a tumor imaging machine provides a concentration of anesthetic gas to maintain anesthesia in the animals. Gas escapes and causes exposure to operators when the door of the chamber is opened to place or remove animals.

Veterinarian clinic animals are typically cats or dogs. Anesthesia is delivered via IV induction followed by anesthetic gas inhalation during procedures to maintain the anesthetized state. In a few cases an animal is masked to induce anesthesia. In both these scenarios the potential for gas to escape comes from around the nose cone if the cuff is not fitted correctly. There is also a potential for gas to escape from the anesthetic delivery and scavenging system itself.

Veterinarian clinics had a higher population average daily dose (ADD) than pharmaceutical research laboratories due to several factors. Pharmaceutical research laboratories in San Diego are inspected by a representative of California Occupational Safety and Health Association on an 18 month basis, therefore, the management team of these facilities implement measures to control all occupational exposures as low as possible. Typically, anesthetic equipment used in laboratory facilities, are conducted within local exhaust ventilation units such as chemical fume hoods, which exhaust any excess or leaking anesthetic gas away from operators. Veterinary operations typically conduct anesthesia
procedures on larger animals which require a larger amount of anesthetic gas to induce and maintain anesthesia in animals. Additionally, work is conducted on open bench tops or surgical tables, with mechanical room ventilation and anesthesia scavenging systems only, to control waste anesthetic gases.

An additional factor is, it appeared veterinary clinics only collected isoflurane sampling data until an exposure measurement was under the Cal/OSHA PEL. Pharmaceutical research laboratories periodically evaluate anesthetic delivery equipment and occupational exposure levels even if exposure levels were below the Cal/OSHA PEL.

**Waste Anesthetic Gas Removal**

Even though the Cal/OSHA permissible exposure limit for isoflurane of 2 ppm was determined to be set at a very conservative limit, it is recommended that facilities lower their occupational exposure to anesthetic agents as reasonably as achievable. The following is a list of control measurements that facilities which perform anesthetic procedures should implement:

- Chemical fume hoods should be used to directly exhaust waste anesthetic gases during animal anesthesia procedures. This has been demonstrated to reduce occupational exposure levels below the limit of analytical detection or less than 20 µg. This technique is only viable if the anesthetic delivery equipment can be placed inside the chemical fume hood, such as bench-top animal induction chambers.

- If an anesthetic delivery device is too large to be placed in a chemical fume hood, installation of a waste gas scavenging device, in connection to an anesthesia delivery machine, is essential. The waste gas scavenging device uses a positive pressure relief valve that siphons away excess anesthetic gas being delivered to a subject. The excess or exhaled gas is directed to an evacuation circuit that redirects the gas to a canister of activated charcoal or to an exhausting ventilation system. This system efficiently reduces the build-up of anesthetic gas conditions in an operating room (Barberio, Bolt, Austin, & Craig, 2006).

- An anesthetic gas delivery machine that also incorporates a waste gas scavenging device may still result in an exposure over the California Occupational Health and Safety Administration (CalOSHA) permissible exposure limit of 2 ppm, if there are leaks in the delivery and exhaust equipment, a larger quantity of gas is delivered than can be removed by the scavenging system, or if the activated charcoal is fully quenched (Smith & Bolon, 2003). Therefore it is important to implement administrative controls such as, a preventive maintenance program to conduct leak tests and change out activated charcoal material, in place to minimize these errors with the equipment. Other imperative administrative controls include filling the vaporizer chambers in areas of high ventilation, adequate operator training on proper
technique to control the anesthetic gas flow system, and increasing the air turnover rate in procedure rooms.

- A high potential point of waste anesthetic gas release is at the mouth. Engineering modifications made to face masks should be made to improve the seal on a subject (Saere, Ambrisko, & Moens, 2011). In a study that evaluated different types of face masks to increase the efficiency of gas delivery and patient uptake, it was determined an addition of a simple latex diaphragm to the conical face mask attached to a Mapleson E circuit significantly reduced Isoflurane emissions to the anesthesia operator (Smith & Bolon, 2006).

- A medical surveillance program should be instituted which includes the segment of a workforce exposed to anesthetic gases. Prospective surveys have demonstrated a vital tool to examine health effects to anesthetic gas exposure. Therefore, it is recommended that a comprehensive medical record be maintained on each employee during employment, by conducting annual physical examinations.

- In addition to a medical surveillance program, periodic air monitoring should be conducted to continually evaluate area room and personal waste anesthesia exposure concentrations.

**CONCLUSION**

- The average aggregate time weighted average exposure for the pharmaceutical research laboratory population was 0.62±0.47 ppm, while the maximum time weighted average value was 1.64 ppm.

- The aggregate time weighted average exposure for the veterinarian clinic population was 2.14±0.38 ppm, while the maximum time weighted average value was 2.77 ppm.

- For all pharmaceutical research data, which included 64 samples, values were never above the Cal/OSHA permissible exposure limit (PEL) for isoflurane of 2 ppm. On the other hand, for the veterinary clinic population levels were above the PEL exposure limit for a total of 7 out of 11 samples.

- The aggregate average daily dose for the pharmaceutical research laboratory population was 1.33 mg/kg-day, while the maximum daily dose was 3.54 mg/kg-day.

- The aggregate average daily dose for the veterinary clinic population was 4.61 mg/kg-day, while the maximum daily dose 5.97 was mg/kg-day.

- Using the calculated human reference dose (21.56 mg/kg-day) the hazard index of all populations average and maximum daily dose levels were determined to be under 1.0, indicating occupational exposure levels in these populations did not cause harmful health effects.

- Even at levels above the Cal/OSHA PEL, exposures not did result in risk ratio above 1, which indicates that the Cal/OSHA PEL may be revised upwards to be more consistent with other international governing agencies.

- This risk assessment concluded that humans exposed to trace concentration levels to modern anesthetic agents are expected to have minimal chronic health risks. It is
recommended that any facility which uses anesthetic agents implement measures to ensure exposure levels are as low as achievable, to further minimize the risk of exposure.
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WORKS CITED


WORKS CONSULTED


