UNIVERSITY OF CINCINNATI

11/05 003

, 2

I, Brent William Edwards,

hereby submit this as part of the requirements for the degree of:
Master of Science

in Environmental and Industrial Hygiene

It is entitled "Effects of Alterations of Blood Lipids by Diet on Solvent Air:Blood Partition Coefficients in:vitro"

Approved by:

Effects of Alterations of Blood Lipids by Diet on Solvent Air:Blood Partition Coefficients *in vitro*

A thesis submitted to the
Division of Research and Advanced Studies
At the University of Cincinnati

In partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE

In the Department of Environmental Health

of the College of Medicine

2003

by

Brent William Edwards

B.S., Bowling Green State University 1999

Committee Members: Glenn Talaska, Ph.D., CIH

Mary Beth Genter, Ph.D.

Paul Succop, Ph.D.

ABSTRACT

There is a wide individual variation in responses to organic solvent exposure. Factors that contribute to this variability are largely unknown. We tested the hypothesis that eating higher fat content meals increases blood lipid levels and results in higher blood levels of lipophilic organic solvents. We investigated the effects of diet and blood lipid levels with solvents having different octanol:water partition coefficients.

Blood was drawn from 11 male and 9 female volunteers on two separate days, before and after low or high fat content meals were served. The subjects, of varying body types, were between the ages of 19-29 and acted as their own controls. A fasting blood sample was taken each day as the baseline for the blood sample taken 4 to 5 hours after the meal was consumed. Three solvents, chloroform, isobutanol, and methanol, were introduced *in vitro* to 1 mL aliquots of whole blood and allowed to equilibrate at 37 degrees C for five minutes. Solvent concentrations in the headspace were then measured using gas chromatography.

Average blood lipid levels increased from 117.3 to 119.7, and 80.1 to 160.3 mg/dL for low and high fat meals, respectively. Headspace chloroform concentrations decreased as blood lipid levels increased. Headspace isobutanol and methanol concentrations slightly increased as blood lipid levels increased. This was not expected for isobutanol. Solvent headspace concentrations and blood lipid levels were all significantly correlated with chloroform having the highest correlation coefficient and lowest p value ($r = -0.38$; $p=0.001$).

Headspace chloroform concentrations decreased when individuals consumed the high fat versus the low fat meal (paired t-test, $p=0.002$). No differences in headspace concentrations were observed for isobutanol and methanol with respect to diet. Thus, data to date suggest that diet may contribute to uptake of a lipophilic solvent and in this way may influence solvent related narcotic effects.

DEDICATION

To my Grandfather, Clarence Edwards

To my Cousin, Alan Edwards

ACKNOWLEDGEMENTS

Thanks are in line to my advisor, Dr. Glenn Talaska for the overall project idea as well as guidance and encouragement throughout. Thanks to my committee members Drs. Mary Beth Genter and Paul Succop for helping with the process and the final product. I would also like to thank those who gave invaluable technical advise throughout the project including Dawn Ramsey, Dr. Ed Burroughs, and especially Shuann Lee, who sacrificed his free time to help set up the gas chromatograph. Thanks are also due to Drs. Grace Faciglia and Audrey Miller for their help with the diet and their generosity in offering their food laboratories. I also would like to thank Dr. Andrew Freeman and Linda Carter for their collaboration with the volunteer blood draws at the Occupational Health Center. Recognition is given to NIOSH ERC Pilot Project Research Training Grant #T42/CCT510420 through the University of Cincinnati Education and Research Center (UC/ERC) for the financial support necessary to carry out this project. I would like to thank Brenda Schumann for logistical help throughout the project.

TABLE OF CONTENTS

TABLES

Appendix A Tables

Tables in Text

FIGURES

Appendix A Figures

Figures in Text

Appendix B Figures

Introduction

Occupational exposure to organic solvents occurs in many industries including the chemical, food, dry cleaning, painting, and plastics industries. Exposure to organic solvents can cause negative acute and chronic health effects (Plog, 1994). Chronic cerebral diseases are believed to be associated with long-term exposure to organic solvents (Cherry, 1993). Short-term effects to exposure include a narcotic effect that can influence the ability to operate heavy equipment or other machinery, and may increase the risk of slips and falls (Farmer, 1989). Disorientation, euphoria, giddiness, confusion, paralysis, and convulsions are all possible adverse health effects at different concentrations and lengths of exposure (Cherry, 1993). All of these narcotic effects can lead to traumatic accidents and injuries in the workplace, from a number of causes.

Opdam and Smolders (1986) saw that body fat was associated with uptake of an inhaled tetrachloroethene dose in a group of workers (Opdam and Smolders, 1986). Currently, the effects of diet on solvent absorption in the blood are poorly understood or considered as it relates to overall worker exposure. Furthermore, there is no current literature regarding the direct effects or relationship between blood lipids and solvent absorption. The in vitro study method proposed explores this area of limited information. It is known that all organic solvents affect the central nervous system acting as depressants and anesthetics (Plog, 1994). Despite this well established information, the mechanisms that cause these effects at the site of action are largely unknown (Chang and Dwyer, 1995). There is a wide range of responses within a population but many of the factors contributing to the variability remain unknown as well (Ulfvarson and Riihimaki, 1985). The research project described herein lays the groundwork for understanding this variability and other pharmacodynamic aspects influencing solvent exposure: specifically, how eating a fatty diet can increase the health risks of persons working with lipophilic organic solvents due to increased solvent absorption and distribution.

Background

Solvent Classes

Organic solvents are a group of low molecular weight liquids that have different physical structures and properties but all have the common property of having a hydrogen backbone dissolving other organic substances (McMartin, et al. 1998). In general, organic solvents have a high vapor pressure and may be found to some degree in the vapor form wherever the liquid form is present. Organic solvents can be broken down by degree and nature of substitution: aliphatic, aromatic, and halogenated hydrocarbons, ketones, esters, alcohols, and ethers (Plog et al., 1996). Refer to Table 1 in the appendix for some examples of the chemicals that are represented by each group. These substitution properties effect the distribution, metabolism, and elimination of the solvent once an exposure takes place. The many different structural variations of organic solvents bring about a variety of specific physiological effects (Franks and Leib, 1990). Factors such as exposure duration, route of exposure such as percutaneous absorption or inhalation, and concentration all contribute to how a solvent elicits a response to an individual (Kumagai and Mutsunaga, 1999). These parameters will be explored later in this paper.

Organic solvent exposures cause a multitude of responses in an individual. Some of these responses are a result of the respective general and specific toxicity of a compound. The general toxicity of a compound is related to the parent compound of the xenobiotic. The parent compound has a direct effect on the biological membrane of the central or peripheral nervous system. This results in narcosis of the CNS. Generally speaking, the narcotic effect increases as the molecular weight of the xenobiotic

increases. This effect is completely reversible, unlike the effects that are a result of the specific toxicity of a compound. The specific toxicity of a xenobiotic is related to the parent compounds' metabolites. These effects are chronic and cumulative. These factors can contribute to organic solvents having a high specific toxicity but a low general toxicity or vice versa. Alternatively, a compound could very well have both a high general and specific toxicity (Goldstein et al., 1974).

Unfortunately, many industrial solvent uses in industry are mixtures of two or more organic solvents. This makes occupational exposure assessment very difficult in some cases. The current occupational safety and health practice is to treat occupational exposures to the different compounds additively. However, research has suggested that both potentiative and synergistic effects can occur with organic solvent mixtures (Ulfvarson and Riihimaki, 1985).

Solvent use in industry

Some uses of solvents in industry include thinners, degreasers, solvents, monomers for polymer production, and extractants (Savolainen, 1977). Organic solvents have been used since the 1840's. Carbon disulfide was documented as one of the first to be used (Arlien-Soborg, 2000). The separation of benzene from coal tar naptha was accomplished as early as 1825 (Gerarde, 1960). The first case of benzene poisoning was reported in 1862 (Winslow, 1926). As industrialism took place around the turn of the 20^{th} century, industrial solvent use increased. Along with this increase, there was also an increased awareness of the apparent neurotoxic effects of these solvents as well. This led to an increase in the number of papers published on the neurotoxicity of solvents. Also,

increased awareness of the specific toxic effects were documented by industries (Arlien-Soborg, 2000). As industry became more aware of the health hazards associated with these compounds, unintentional exposures decreased in industry and cases of acute poisonings decreased as well. During the 1960's and 1970's there were still outbreaks of peripheral neuropathy mainly from n-hexane and methyl n-butyl ketone that were documented despite the overall decrease in exposure since the early 1900's (Spencer and Schaumburg, 1980).

Today, solvent use in industry is ubiquitous, in that most industries use solvents to some degree during some part of a production process. Reports from the Agency for Toxic Substances and Disease Registry (ATSDR) confirmed that in 1993 the US produced 12.3 billion pounds of isobutanol, and in 1995 the US produced 523 million pounds of chloroform (ATSDR, 2003). The Environmental Protection Agency has also reported that 8 billion pounds of methanol are produced each year (US EPA, 2003).

Painting requires the use of various different kinds of organic solvents depending on the type of application. Along with the pigments, film formers, and other additives, different types of solvents are used. Some of these can include ketones, alcohols, glycols, glycol ethers, esters, aliphatic and aromatic hydrocarbons, cresols, chlorinated solvents, and others (Perkins, 1997). Degreasing operations make use of chlorinated solvents such as dichloromethane, tetrachloroethylene, 1,1,1-trichloroethane, and trichloroethylene. N-hexane is used in adhesive preparations. Toluene remains the most widely used solvent in industry today (Wypch, 2001).

There are more than a thousand different solvents that are used in industry today (Wypych, 2001). Some are rare and process specific, and others such as toluene and

xylene are more common. An example of a process specific solvent is the use of styrene in the boat building industry (Baker et al., 1985). Solvents are used for many different types of applications in industry. They can be used to control the rate, selectivity, or equilibrium of reactions. The intermediates and products of these reactions can also be used for the same purpose. They can be used as either the solvent or the solute in a chemical system (Collings and Gluxon, 1982). The actual number of uses for solvents in industry is large enough that entire textbooks are devoted to this topic alone (Zenz, 1994). Because of the large number of uses for solvents, Table 2 has been added in appendix A, listing some of the solvents used in industry. This table was adapted from George Wypych's Handbook of Solvents. For further detail on specific industrial processes and industries please refer to this reference (Wypych, 2001).

Solvent Exposures to workers in industry

Millions of workers throughout the world are commonly exposed to solvents (Zenz, 1994). In 1990 the National Institute for Occupational Safety and Health (NIOSH) estimated that approximately 100,000 workers have had some level of exposure to toluene and approximately 140,000 workers have had some level of xylene exposure in the United States annually (Seedorff and Olsen, 1990; Zenz, 1994). Solvents in industry today continue to be of vital importance to sustain manufacturing operations. The usefulness of solvents leads to many different types of exposures throughout manufacturing and other sectors.

Unfortunately, along with the usefulness of these compounds, there are potential health risks associated with them. In 1836, Delpech was the first to document the

adverse health effects of solvent exposures (Arlein-Soborg, 2000). Delpech observed both acute and chronic effects to occupational carbon disulfide exposures in the workplace. These topics are explored in more detail in the next section of this paper. Solvent exposures inciting both acute and chronic effects to workers still remain a reality in general industry today (Zenz, 1994). The three main routes of exposure to these potentially hazardous compounds include inhalation, dermal absorption, and ingestion. Depending on the process, one, two, or all three routes may be a possible exposure scenario for a worker. Due to the low molecular weight of many organic solvents, the potential for inhalation is the greatest among the three if one were to generalize, followed by dermal absorption and ingestion. These three routes of entry ultimately result in entrance of the solvent into the blood stream (Plog et al., 1996).

Inhalation

The most common of the three routes of exposure is inhalation because the high vapor pressure of most solvents increases the likelihood of vapor to be present in the air (Zenz, 1994). Vapors that are inhaled via the lungs enter the blood stream readily because of the large surface area of the lungs and the small amount of tissue in the alveoli for the vapor to pass through to reach the blood stream (Perkins, 1997). The nature of the gas vapor allows it to penetrate deep into the lungs to the alveolar region where gas exchange takes place. The estimated area of this gas exchange region of the lungs is estimated to be approximately 1.2 square meters. This total area is only two cells thick making gas exchange between the venous blood supply and the oxygenated air supply very efficient (Perkins, 1997). Solvent vapors can easily enter the blood supply through

this route given the concentration gradient of solvent vapor in the lungs is greater than that in the blood (Plog et al., 1996).

Dermal absorption

In some cases it is important to note that the dermal route of exposure may be a greater concern than the inhalation route (Scott, 1995). It has been reported that by submerging ones hand in a particular compound for a short period of time, greater or equal amounts of the chemical can be absorbed into the bloodstream, compared to the amount absorbed into the bloodstream if one were to inhale the same compound for eight hours at a concentration that is equal to the threshold of toxicity for the given compound (Engstrom and Bjurstrom, 1977). Several important factors determine absorption into the blood stream via the dermal route. These factors can be theoretically calculated with the use of Fick's Law relating to passive diffusion. Factors such as thickness of the skin, hydration of the skin, temperature and humidity, number of skin appendages such as hair follicles and sweat glands, and skin condition, all play a role in determining this theoretical amount. Cuts in the skin or other abrasions may significantly affect the overall absorption into the blood (Bird, 1981; Plog et al.1996; Perkins, 1997). The other important aspect of dermal absorption is the nature of the solvent. The molecular weight and structure of the solvent are important as is the water/lipid solubility properties (Bird, 1981). These factors play into the actual permeability in to the skin as well. The dermal route cannot be overlooked as a potential significant contributor to overall blood absorption (Perkins, 1997).

Ingestion

The least likely exposure route for solvents is ingestion. Organic solvents are not likely to be ingested because of their relative high volatility (Perkins, 1997). The possibility of not properly washing solvents off of ones hands or clothing after contamination has occurred. Eating or drinking after this occurs, could lead to small amounts of ingestion (Plog et al., 1996). However, accidental and intentional ingestion also has been known to occur (Perkins, 1997).

Occupational exposure limits

Exposure limits have been established to monitor the level of exposures to workers through these three routes of exposure. The Occupational Safety and Health Administration (OSHA) regulates these airborne exposures through the means of an 8-hour time weighted average called a permissible exposure limit (PEL). The PEL is a federally regulated standard that suggests no adverse health effects should occur in most workers when they are exposed to airborne concentrations of a given solvent at or below the PEL. Along with these standards, exposure recommendations have been established for airborne concentrations by other professional health and safety organizations. These recommendations are called threshold limit values (TLV), recommended exposure limits (REL), and workplace environmental exposure levels (WEEL), and are established by the American Conference of Governmental Industrial Hygienists (ACGIH), National Institute for Occupational Safety and Health (NIOSH), and The American Industrial Hygiene Association (AIHA), respectively. Along with the TLV's, ACGIH has also established the biological exposure indices (BEI). The indices represent measurements of

a biological marker extracted from a potentially exposed worker. The biological marker is usually extracted from a collection of breath, urine, or blood. The BEI's are developed to represent an exposure equal to the representative TLV for an eight-hour airborne exposure. Table 3 in the appendix represents the respective exposure limits for the three solvents used in this experiment. These exposure limits and recommendations can be utilized for both air and biological monitoring in the workplace. Monitoring techniques are discussed in more detail in the later sections of the background.

To complicate the picture of exposure assessment, today's workplaces contain dynamic and sometimes complicated industrial process that may present the worker with mixtures of solvents. This complicates the process of estimating exposures. Little is known today about the mechanisms and health outcomes of overexposure to solvent mixtures. Synergistic, additive, and antagonistic effects can all be possibilities (Ulfvarson and Riihimaki, 1985).

Toxicology and neurotoxic responses to organic solvents

Along with the narcotic effect that solvents may cause an individual after exposure, other neurotoxic effects due to solvent exposure may also occur. A neurotoxic response to an organic solvent is defined by the handbook of neurotoxicology as "an adverse structural or functional change in the nervous system produced by exposure to the solvent" (Chang and Dyer, 1995). Exposure to organic solvents can be broken into two categories. The first exposure category can be defined as acute exposures, which is generally accepted to cause depression of the CNS and in some cases an anesthetic effect. The second, chronic exposure, is related to long term exposures at concentrations lower

than those seen to cause acute toxicity as described in the first category. The two differ in that the acute effect of solvent exposures may arise from a direct contact between the solvent molecules and membranes of the nerve cells, associated proteins, or ion transfer channels, whereas the chronic effect of lower concentration exposures may occur due to the metabolic effect of the parent compound or its metabolites (Savolainen, 1977).

Acute exposures can lead to effects ranging from intoxication to death (Chang and Dyer, 1995). Almost all organic solvents act as depressants to the CNS at certain concentrations. Currently there are no definitive mechanisms that have been found for most solvents. However, the endpoints to the effects that they do cause have been extensively studied for compounds acting as anesthetics. Therefore by analogy, the same paradigm is used to explain the action of acute solvent exposure effects. The traditional mechanism is thought to be the interaction of both lipophilic and non-lipophilic solvents to act non-specifically with the nervous system cell membrane bi-layers. New findings suggest that solvent interaction with certain proteins that have specific site interaction may also play an important role (Franks and Leib, 1990).

Current mechanisms of general anesthesia generally include the definition of "a reversible drug induced loss of consciousness" (Franks and Leib, 1990). It is a simple definition for a complex phenomenon, however, certain accepted principles do exist. These principles can most easily be used when discussing a simple non-polar chemically inert molecule. Currently it is assumed that ionic transfer is interfered with at the neuron from direct contact with the solvent molecule (Shrivastav et al., 1976). It is also believed that some solvents directly effect the mitochondrial energy production (Tarkowski and Sobczak, 1971; Herd and Martin, 1975). Protein synthesis may also slow down or cease

because of direct interaction with the solvent molecules ultimately affecting the overall narcotic effect (Renis et al., 1975).

Accute exposures in an industrial environment are of concern for occupational health professionals because of the narcotic effect that an exposure may illicit (Perkins, 1997). NIOSH estimated in 1990 that annual estimates of U.S. employees exposed to methanol was almost 2 million workers. Other compounds such as methanol and isobutanol were estimated to challenge 135,000 and 300,000 workers respectively, each year (National Occupational Exposure Survey NOES, 1990). Research has also shown that the narcotic effects of organic solvent exposure could lead to an increased risk of accidents because of the loss of motor control and overall depression of the CNS (NIOSH, 1987).

Chronic effects have recently been the area of interest in the occupational health profession, as opposed to acute effects, which have been known since the 1800's. This is largely due to the introduction of hexacarbons such as n-hexane, methyl n-butyl ketone (MNBK) and benzenes. These solvents were linked to causing an outbreak of polyneuropathies in the 1960's and 1970's (Spencer and Schaumberg, 1980). There are many neurobehavioral evaluation tools to detect the neurotoxic effect of long-term solvent exposures. Some of the neuropsychological tools are subject to debate however. Others, such as electrophysiology are very reliable. Many of the current methods used for assessing the neureotoxic effects of solvents have been reapplied to be used with investigations in applied neurology. These methods include computerized tomography (CT) of the brain, and electroencephalography (EMG) (Arlein-Soborg, 2000).

Neuronal metabolism is one of the factors in understanding chronic low-level exposures. This includes oxygen and energy demand, protein synthesis, localized change in fluidity of the vicinity of the sodium ion channels, and the bodies' ability to metabolize the solvent into a more or less toxic agent (Savolainen, 1977).

Documented acute and chronic effects of solvent exposures

Health effects related to solvent exposures are well documented in the literature. Specific effects are not fully understood for all of the hundreds of solvents that are used. Studies involving the long term health effects of specific solvents and mixtures of solvents is currently growing. Despite the current lack of knowledge on the specific health effects of specific solvents, it is generally accepted that solvents act as anesthetics from evoking a narcotic effect. Some solvents such as chloroform, tetrachloroethylene (TCE), and ether were used as some of the first anesthetics (Arlien-Soborg, 2000). Toxic effects to the liver and blood were also noted when solvents were first used. Other less serious effects such as mucus membrane irritation were also initially noted. Research to understand the neurotoxicity of solvents is an ongoing endeavor. Other potential solvent related health effects have been documented based on a compound's carcinogenic, mutagenic, and teratogenic potential (Zenz, 1994).

As stated previously, acute effects of solvent exposure ultimately depress the CNS and result in narcosis or anesthesia, sometimes leading to the point of unconsciousness or even death. Depression of the CNS can result in symptoms such as headache, lightheadedness, dizziness, giddiness, and fatigue. One can also suffer form tightness of chest or general difficulties in breathing. Another symptom is the irritation of mucous

membranes, causing eye, nose, and throat irritation. It is also possible for organic solvent exposures to cause general irritation of the respiratory tract causing additional difficulties in breathing (Ulfvarson and Riihimaki, 1985; Plog et al. 1996).

Occupational dermatitis is also attributed to physical contact to organic solvents. Some cause an irritation effect and others result in the degeneration of fatty tissues in the skin resulting in a compounded health effect. This degeneration of the skin might also act to increase the solvent that reaches the blood stream because of the loss of the skin's natural protection barrier (Plog et al., 1996).

Current literature shows that there may be associations with many occupationally related diseases of long term low level (chronic) solvent exposures. Although the mechanisms are not fully understood yet, and there is debate over what neuropsychological assessment tools should be used to clinically define a specific adverse neurobehavioral effect, there are many studies that show correlations between solvent exposures and disease (Zenz, 1994).

Some solvents have been associated with carcinogenicity, mutagenicity, and teratogenicity through animal experiments. In general, epidemiological evidence does not exist to either support or refute the animal experiments because the nature of solvent exposures in industry are so complex. Solvent mixtures and other factors contribute to this problem. Nonetheless, compounds such as the chlorinated solvents have been shown to exhibit carcinogenic properties in animal experiments (Zenz, 1994). Other studies have linked solvents such as percloroethelyene and trichloroethylene (TCE) to carcinogenicity in humans based on the results of animal studies. These solvents are listed by the ACGIH as A2 carcinogens. This means that they are suspected human

carcinogens based on the results compiled from animal tests. Other evidence shows that carcinogenicity of solvents is difficult to extrapolate from animals to humans, with the exception for benzene. Benzene is biotransformed to create benzene epoxide as one metabolite early, and further into the process, diolepoxide is formed. Benzene is currently the only solvent that is designated as a confirmed human carcinogen by the ACGIH with an A1 designation (ACGIH, 2003). ACGIH has designated other chemicals as A1 through A5, including solvents. These designations are explained in more detail in Table 4 in Appendix A. It is currently theorized that carcinogenic metabolites such as epoxides or radical cations are formed from the metabolism of chlorinated solvents such as chloroform, TCE, tetrachloroethylene, or carbon tetrachloride (IARC, 1987).

Benzene has been confirmed as a human carcinogen because of the evidence that has been shown to date with existing research. Several organic solvents fall into the "Suspected human carcinogen" classification (A2). Some of these solvents include benzotrichloride, carbon tetrachloride, diazomethane, 1,4-dichloro-2-butene, vinyl bromide, and vinyl fluoride. Other organic solvents are labeled with the (A3), (A4), and (A5) classification as well (ACGIH, 2003).

Studies have shown that other solvents show mutagenic characteristics, such as styrene. For this reason it is suspected as a possible carcinogen but strong animal and epidemiological evidence have been limited thus far (Zenz, 1994). In some bacterial tests other solvents have been shown to be mutagenic including methyl chloroform, methylene chloride, perchloroethylene, and TCE (Vainio et al., 1985). These mutagenic properties are a concern because of a mutagenic compound's inherent capability to cause some cancers (Toftgaard and Gustafson, 1980, Zenz, 1994).

Epidemiological studies have linked an increase in cancer risk to certain occupations but the link to a particular solvent has currently not been found. Studies have found an increased incidence of Hodgkin's and non-Hodgkin's lymphomas with exposures to solvent groups of aliphatic and aromatic hydrocarbons, as well as the chlorinated solvents (Olsson and Brandt, 1980; Hardell et al., 1981). Other more recent studies have found an increase in cancer mortality for cancers of the esophagus, larynx, lung, and cervix for a large cohort dry cleaners (Blair et al., 2003). This study suggests that there is an increased mortality of cancer linked to the occupation but evidence suggesting organic solvent exposure variables were causally related was limited, even though solvent exposures did occur to some degree on a regular basis for this population. Other studies have shown an increased odds ratio (OR) for breast cancer in women who work around industrial solvents as compared to their controls (Hansen, 1999). Some solvents have been found to cause mammary gland tumors in animal assays. These solvents include benzene, 1,2-dibromomethane, 1,1-dichloroethane, methylene chloride, styrene, 1,2,3-dichloropropance and vinyl chloride (Lynge et al., 1997; Labreche and Goldberg, 1997). Again, the link to identify a particular solvent that causes breast cancer in humans is currently lacking.

Other studies have linked non-carcinogenic effects of long term exposure to organic solvents as well. These include effects to the lung, kidney, liver, and may also contribute to other diseases. Organic solvents are largely metabolized through the liver and the kidneys. Some undergo oxidization or reduction which in some cases may result in bioactivation (Labreche and Goldberg, 1997). Studies have shown that there may be

associations with hydrocarbon exposure and an increased risk of glomerulonephritis (Bierne and Brennan, 1972; Largrue, 1976; Ravnskov et al.,1979).

Hepatotoxicity of some organic solvents, including the chlorinated solvents such as chloroform, has been known for approximately 100 years (Lundqvist et al., 1999). A recent study has linked organic solvent mixtures to fatty liver disease (FLD). This particular study controlled for alcoholism, obesity, drug use, and other diseases. Results showed that the relative risk of developing such a disease was increased over the control group (OR 4.3) for mixed solvent exposure within the study cohort (Lundqvist et al., 1999). Liver damage is linked specifically with carbon tetrachloride. Studies have shown that hepatotoxic damage can occur only a few days after exposure and some indicate that cirrhosis and hepatic tumors may develop later (Johnson et al., 1977). An interesting exposure paradigm suggests that alcohols and chlorinated solvents, such as isopropyl alcohol and trichloroethylene, may interact to increase the risk of cirrhosis in painters and other occupations with such mixtures (Traiger and Plaa, 1974; Cornish and Adefum, 1966; Toftgaard and Gustafson, 1980).

Cardiovascular disease and respiratory effects have also been linked to solvent exposures. Carbon disulfide was one of the first solvents to be used in industry as well as one of the first linked to neurotoxic effects. Studies have associated the use of carbon disulfide in the rayon-viscose industry with links to cardiovascular diseases (Hernberg et al., 1973; Tiller et al., 1968). Loss of respiratory function has been found to be associated with mixtures of organic solvents from even low concentrations. An example of this is exposure to mineral spirits among painters and floor layers (Harving et al., 1991).

Along with the potential for long term exposure effects stated above, there are other possible neurological disorders such as parkinsonism, polyneuropathy, cognitive function decline, and chronic toxic encephalopathy (Hageman et al., 1999). Other documented neurobehavioral effects mainly include the decline of psychomotor function, reduced short-term memory capabilities, and mood disturbances (Baker et al., 1988).

Organic solvent air monitoring (vapors)

The capability to assess organic solvent exposure in industry has greatly improved over the past 20 years (Guillemin, 1985). This section covers some of the most commonly used analytical methods available for solvent vapor detection today.

Traditional monitoring for solvents requires air sampling techniques including both personal and area measurements, direct reading measurements, and passive diffusion measurements (Plog et al., 1996). Several methods currently exist for the collection of solvent vapors to be analyzed for concentrations. Activated charcoal is used to collect solvent vapors through both active (using a pump) and passive samplers (Guillemin, 1985). After the sampling period, the solvents that were adsorbed by the activated charcoal are removed by washing it with another solvent and the contents analyzed. Passive diffusion uses a diffusion coefficient to determine the approximate concentration after the mass of specific solvents are determined analytically (Rose, 1982). Active samples utilize the known flow rate and analytically determined mass of a specific solvent to estimate the concentration. These are the two most widely used exposure assessment methods for estimating personal exposures today (Cohen and McCammon, 2001).

The National Institute of Occupational Safety and Health (NIOSH) publishes the NIOSH Manual of Analytical Methods (NMAM) which consists of current sampling methodologies for hundreds of different compounds. These methods are accepted by the occupational health and scientific community as reliable and accurate assessment tools to quantitatively assess concentrations of contaminants in air (Cohen and McCammon, 2001). Solvents, falling under the seven different chemical composition groups described previously, have different chemical substitutions. As different substitutions cause different health effects, they also require different sampling methodologies to ensure accurate quantitation. For instance, the three solvents that were used in this study all require different sampling methodologies. Chloroform is analyzed by NIOSH Method 1003 "Halogenated Hydrocarbons", isobutanol by method 1401 "alcohols II", and methanol by method 2549 "Volatile Organic CPDS" (NIOSH, 2003).

Some methods used to determine real time solvent concentrations include solid sorbent tubes and direct reading instruments. Solid sorbent tubes are best used as a qualitative measurement of a specific solvent. Each tube is filled with an activated compound specific to a certain solvent or group of solvents. A known volume of air is drawn through the tube using a hand or electric pump and a colorimetric reaction takes place. The reaction travels a given distance in the tube dependent upon concentration. The concentration is read with graduations on the side of the tubes which represent a higher concentration the farther the colorimetric reaction travels in the tube. The tubes are graduated This colorimetric change is sometimes difficult to interpret and a 25% margin of error is needed (Plog et al., 1996). Direct reading instruments, however, are more accurate and can be used for quantitative measurements of solvent concentrations.

There are, however, negative aspects to these instruments as well. The apparent ease of using these instruments can lead to improper use (Guillemin, 1985). Also, most direct reading instruments are non-specific. For instance, a photoionization detector (PID) is a good instrument for determining aromatic hydrocarbon vapors but it is not sensitive to other solvent vapors that have a high ionization potential (Plog et al., 1996). The flame ionization detector (FID) is used the same way the PID is used but has a flame to ionize the vapors instead of ultraviolet light energy. This instrument has yet a different range of sensitivity for each of the solvent classes. Other direct reading quantitative measurement devices are used for combustible gas monitoring (including certain solvent vapors) (Perkins, 1997). With all of these methods available to estimate solvent vapor concentrations to approximate exposure, especially the direct reading methods, it is essential that the user know the limitations of each instrument. It is also a must to understand which instrument should be used for a particular solvent vapor or mix of solvent vapors, since one instrument may not be able to accurately identify all of the solvent vapors that are present in a given situation (Perkins, 1997).

Other methods, including gas and liquid chromatography, are used with a wide spectrum of detectors including electron capture, flame ionization, photo ionization, and nitrogen phosphorus. Some gas chromatographs are equipped with mass spectrometers which can very accurately identify compounds (including solvents) and estimate concentrations (ACGIH, 2001). The use of field portable gas chromatographs equipped with mass spectrometry, is currently a very useful tool for many industrial applications, especially when the identity of mixtures of solvents are unknown (Perkins, 1997).

Uptake distribution and metabolism of organic solvents

After occupational exposures to organic solvents occur, the xenobiotic is taken into the body. Uptake is the amount of solvent that enters the body via the three exposure routes explained earlier (inhalation, dermal absorption, ingestion). The rate at which a solvent enters the body depends on its biosolubility and the rate at which it is distributed to the tissues. Biosolubility includes partition coefficients between tissue or body fluid (blood) and air, as well as other factors such as the solvent's lipophilicity (Ulfvarson and Riihimaki, 1985; Astrand and Gamberale, 1978; Zenz, 1994). Once a solvent enters the blood stream, the partition coefficient of the particular solvent directly affects uptake in certain tissues (muscle, fat, liver, etc.). Hydrophilic solvents will be dispersed evenly throughout the body, and have been shown to have a tissue/blood partition coefficient of almost one. Lipophilic solvents have an affinity for lipid containing tissues and membranes, depending specifically on the degree of solvent lipophilicity (Ulfvarson and Riihimaki, 1985). The majority of organic solvents that are used in industry are lipophilic as opposed to hydrophilic (Labreche and Goldberg, 1997). A particular compartment (tissue) that has a high affinity for the solvent will continue to absorb the solvent at a specific rate until the solvent partial pressures are equal in the blood and the tissue. The blood flow rate of the tissue has a direct effect on the uptake of the solvent as well (Zenz, 1994). Half-lives for lipophilic solvents are also known to be directly correlated with obesity. Concentrations throughout the adipose tissues depend on the overall volume of adipose tissue for each individual. Lean persons will have higher concentrations associated with their adipose tissues as compared to an obese person because an obese person has a higher overall volume of adipose tissue. These factors

cause solvent half-lives to be longer in obese individuals than in lean individuals (Ulfvarson and Riihimaki, 1985).

Transportation mechanisms of organic solvents in blood is another area where there is limited information available (Lam et al., 1990; Pang et al., 1980). Studies have been completed with rats to determine uptake of these solvents in components of the blood with specific solvents such as carbon disulfide. Carbon disulfide, which is highly lipophilic and highly volatile, is mainly transported by the red blood cells (RBCs), and not the blood plasma. Approximately 90% of the solvent by volume is found in the RBCs regardless of the exposure time (Lam et al., 1986). Also, in 1990 Lam et al. found that anywhere from 50 – 95% of any organic solvent is taken up by RBCs. Very little information is available about the role of triglycerides in blood for transporting lipophilic organic solvents. Some studies have measured uptake for triglycerides as lower than that of hemoglobin (the carrier of oxygen contained within RBC's) but much higher relatively speaking than the blood plasma (Astrand and Gamberale, 1978). The specific role of uptake of lipophilic organic solvents by triglycerides is unknown (Pang et al., 1980). It is possible that as these lipid containing compartments in the blood that bind and transport lipophilic organic solvents, do not have an adverse effect on the nervous system because of this reason. For example, carbon disulfide binds irreversibly with the RBC's and therefore does not act as a sufficient transport mechanism to enhance interaction with the body's neural network (Lam, et al., 1990).

Variations in physiological conditions for workers are important to consider when discussing uptake. For instance, exercise will increase cardiac output and ventilation

rate, which will increase the rate of metabolism by transporting the solvent more quickly to the area of metabolism (Bergert and Nestler, 1991).

Other considerations, such as increased workload, may affect overall uptake into the blood. It is known that ventilation rates increase as an individual is subjected to an increased workload. Considering this, Johanson has found that solvent uptake is nearly proportional to alveolar ventilation for highly fat soluble solvents such as styrene and xylene. Uptake into fat or other tissues for these solvents is very high relative to other, more hydrophilic solvents. This was found when subjecting workers to three different workloads including 50, 100, and 150-W. The workers' ventilation rate increased in relation to the increased workload. Unlike the styrene and xylene, less fat soluble solvents such as methylene chloride and trichloroethylene (TCE) were found to double in uptake when comparing rest to a 50-W workload. A threshold was found at the two higher workloads (100-W and 150-W), in which uptake did not increase higher than that of the 50-W workload (Johanson, 1995). This threshold was postulated to be directly related to the fat solubility of the compounds used for this study.

As stated previously, the specific mechanisms resulting in clinical effects from solvent exposures are still yet to be fully understood. Possible theories on some commonly used solvents including methanol, ethanol, benzene, carbon disulfide, and toluene, are generally accepted and have been defined in the literature (Wypych, 2001). Nonetheless, internal and effective dose relies on other factors such as solvent uptake, metabolism, distribution, and elimination. Solvent toxicity is related to the amount of the compound, whether parent or its metabolite, that reaches the site of action (Ulfvarson and Riihimaki, 1985).

Several metabolic systems exist within the body to metabolize the parent compound solvent that enters the body. High metabolic activity results in a large amount of biotransformation, resulting in a large amount of metabolites being formed proportional to that of the internal dose. These metabolites may be either more or less toxic than the parent compound. Low metabolic activity results in a small amount of biotransformed compound, and the toxic effect of the solvent is mainly related to the toxicity of the parent compound (Ulfvarson and Riihimaki, 1985; Browning, 1965; Collings and Gluxon, 1982). Typically, the lipophilic, non-polar solvents are metabolized to a more polar state, thus facilitating their elimination through the urine. This ultimately reduces the toxicity of lipophilic solvents in general because it lowers their ability to cross membranes, including those of the central and peripheral nervous system (Astrand, 1985; Astrand, 1975). Metabolism of lipophilic compounds to make them more polar does not reduce the toxicity of all lipophilic solvents. Some compounds such as benzene, n-hexane, and methanol are metabolized to more toxic substances. For instance, benzene metabolite formation is directly related to its chronic toxicity while its parent compound causes acute toxicity (Wypych, 2001). In general, the metabolism of organic solvents is only well defined for solvents encompassing certain groups, such as the aromatic hydrocarbons. Other solvent groups such as the ketones, ethers, and esters are yet to be fully understood when compared with aromatic hydrocarbons (Ulfvarson and Riihimaki, 1985; Zenz, 1994).

Excretion of both hydrophilic and lipophilic solvents is driven by their specific physiochemical properties. Hydrophilic solvents are usually biotransformed in some way and eventually excreted through the urine. Lipophilic solvents that are not

biotransformed will eventually be eliminated through the lungs. If they are biotransformed, their metabolites will eventually be eliminated through the urine as well (Ulfvarson, 1985).

Organic solvent biological monitoring

Unlike traditional air sampling methods used for assessing occupational exposures, biological monitoring can be used for a more representative estimate of the total body burden by calculating internal dose. Biological monitoring not only takes the inhalation route into consideration, but also the dermal and ingestion route. It is also a method that takes factors such as physical work load, an individuals metabolic rate, and other factors such as wearing personal protective equipment (PPE) into consideration when estimating exposures (Angerer, 1985).

Almost all organic solvents can be estimated via biological monitoring. The use of headspace gas chromatography enables the investigator to draw blood at designated times within the exposure cycle to be used as the matrix (Angerer and Heinrich, 1982). Specific analytical methods are used that take metabolism, distribution, and the pharmacokinetics of the solvent in the body to make measurements of uptake and distribution (Angerer, 1985). For example, biological monitoring for methanol can be performed using both urine and blood. Methanol is metabolized and formic acid and formaldehyde are formed as metabolites. These end products can be analyzed in the urine and the blood to estimate the internal dose of methanol if the kinetics of the methanol and its metabolites are understood (Sedivec et al., 1981). Analyzing the methanol directly in the blood and urine by gas chromatography is also an option (Arlein-

Solberg, 2000). Other methods may use a subject's alveolar air by capturing exhaled alveolar air in a Tedlar® bag for gas chromatographic analysis. These air samples are taken at specific times using established techniques that allow the worker to exhale for a certain period of time before the last part of the breath is captured in the tedlar bag representing the alveolar portion of the exhaled breath (Opdam and Smolders, 1986).

There are inherent differences among the different types of biological monitoring. The half-lives of solvents are relatively short in exhaled air due to their typically high vapor pressures. This is the most difficult medium to use as compared to blood or urine, particularly because of the exhalation techniques described in the previous paragraph, and the short half-lives. Urine is the most stable of the three mediums. The kinetics of metabolites due to solvent exposure are better understood and more stable in the urine. Also, the half-lives of the metabolites are longer in urine than are those of the parent compounds that are measured in exhaled air. These principles ultimately cause urine to be the most widely used medium for solvent biological monitoring. Blood is also used as a medium but it can be slightly more complex and more expensive to analyze than urine. Blood is used as a confirmatory method to supplement the other two mediums in determining accuracy and precision (Baselt, 1998).

Hypothesis

We hypothesize that solvent uptake will be greater in the blood of individuals who have consumed a high fat meal versus those who have consumed a low fat meal of the same caloric content. We will estimate this effect by comparing average headspace concentrations for each solvent. We also hypothesize that the solvent in the headspace will have a larger decrease in the samples collected after consumption of a high fat meal, as compared to control samples, than the decrease after consumption of a low fat meal as compared to the control. We anticipate that the greatest effect will be chloroform and a lesser effect will be with isobutanol and methanol, consistent with their octanol:water partition coefficients. We also anticipate that changes in headspace concentrations of the three will be correlated with the amount of triglycerides that are present in the blood. The magnitude of this effect will be based on the octanol:water coefficient for each respective solvent, thus, the correlation will be greatest for chloroform, followed by isobutanol, and least for methanol.

The following null hypotheses will be tested:

1) Average triglyceride levels will be significantly lower for the high fat meal compared to the low fat meal.

2) Headspace chloroform and isobutanol concentrations will have a positive correlation with respective triglyceride levels and headspace methanol concentrations will have a negative correlation with respective triglyceride levels.

3) There will be no decrease in headspace chloroform concentrations from the high fat category blood samples relative to the headspace chloroform concentrations from the low fat category blood samples.

4) There will be no difference between the headspace solvent concentrations for the three solvent categories relative to their respective controls.

5) Solvents with increasing octanol:water partition coefficients will be less highly correlated with triglyceride levels relative to the other two solvents.

* All statistical comparisons will use an alpha-level of 0.05

Materials and Methods

Materials

Chloroform, isobutanol, methanol, acetone, 1000 uL pipette tips, and 100 uL pipette tips were purchased from Fisher Scientific (Atlanta, GA). Two mL clear glass vials with screwtop hole cap PTFE/silicone septa, Hamilton 10 uL cemented needle syringes, and Hamilton 10 uL gas-tight removable needle syringes were purchased from Supelco (Bellfonte, PA). Compressed gases for gas chromatography, including nitrogen, hydrogen, and ultra-pure air, were purchased from Wright Brothers (Cincinnati, OH). Ten mL sodium heparin Vaccutainers® were obtained from Becton Dickenson (Franklin Lakes, NJ). Swagelock Snoop® was purchased from Nupro Co. (Willoughby, OH). Molecular Technologies (St. Louis, MO) supplied 1000 uL and 200 uL pipettes. Lastly, the DB-WAX .45 millimeter diameter column used for gas chromatography was manufactured by J and W Scientific, and purchased from Agilent Technologies (Willmington, DE).

Human subject treatment and recruitment

The study was carried out according to the requirements of the University of Cincinnati Institutional Review Board (IRB Study # 02-11-4-4). A total of 20 non-smoking students between the ages of 20 and 29, 10 male and 10 female, were recruited through flyers that were posted at various locations throughout the University Medical Center. The age range was selected to decrease confounding based on the possibility of an unexpected age related effect. Actual study participation included 11 males and 9 females due to difficulty in recruiting women that did not meet the exclusion criteria. One additional male was recruited to make 11 total to keep the total number of

participants to 20. Persons less than 19 years old or more than 29 years old were not asked to participate in this study. Also, current, regular tobacco smokers, diabetics, alcoholics, women taking oral contraceptives, and persons with known renal or liver disease were excluded.

Blood was drawn from the subjects twice, on two different days for a total of four blood samples throughout the experiment. Each blood sample consisted of a total of 10mL, collected in three separate tubes. All blood samples were taken by the same registered nurse in a clinical setting. The subjects were asked to fast for 8 hours before the first (control) blood sample was taken in the morning. They were instructed to drink plenty of water the evening before blood sampling occurred to help facilitate blood collection. After the fasting sample was collected, the subject was provided either a low or high fat meal and allowed to leave the premises. The subjects then returned to the laboratory between 4 and 5 hours after the meal was consumed and the second blood sample for that day was taken. This time course was chosen because triglycerides have been shown to peak and be relatively stable between four and five hours following a meal (Kylin et al., 1967). The next sampling day, one week after the first, the same procedure was followed but the alternative meal was provided to each subject. The contents of the low and high fat content meals are described in "diet preparation" of the materials and methods section. Subjects were compensated with a cash payment of $50 for their participation in this study. They were provided with the results of the lipid panels run on each blood sample taken. The subjects received the information either via e-mail or hard copy (at the preference of each participant). The lipid panel included triglyceride, HDL, LDL, and cholesterol levels.

Diet preparation

The two diets that were used in this experiment were chosen with the help from a registered dietician from the College of Allied Health Sciences at the University of Cincinnati. The goal of the diet was to create meals that were similar in calorie content but significantly different in fat content. The diet was also chosen for simplicity of preparation. We were able to measure the portions easily and quickly to facilitate all 20 subjects. In general, the subjects were staggered by about 3-5 minutes to distribute the meals. Both meals were selected with the help of Bowes and Churches Food Values of Proportions Commonly Used, 17th Edition, for fat and calorie content estimation (Bowes and Churches, 1998). The low fat content diet consisted of 2 slices of Klosterman Honey Wheat Bread (toasted), two tablespoons of Kroger jam, 12 oz. of IGA skim milk, 1.5 cups of Kellogs Shredded Wheat, and 8 oz. of Minute Maid 100% Orange Juice. The high fat content diet consisted of 2 slices of Klosterman Honey Wheat Bread (toasted), 1 Tablespoon IGA Butter, 12 oz. IGA vitamin D milk, ¾ cup of Kellog's Kracklin Oat Bran, 6 oz. of Minute Maid 100% Orange Juice. Total fat and calorie content for the low fat meal was 697.5 calories and 3.5 grams of fat respectively. The high fat content meal consisted of 747.5 calories and 32 grams of fat respectively. Please see Tables 5 and 6 in the Appendix for between meal calorie and fat comparisons.

Blood sample collection and preparation

For the remainder of this thesis, the four samples will be referred to as low control, low, high control, and high for the samples that were taken between 4 and 5 hours after either the low or high fat content meal was consumed and the first sample that

was taken each morning as a control for those respective meals. Each of the four blood samples were treated identically for both meals and their respective controls, except for storage time. The blood collection period lasted three weeks with 15 subjects coming on the first sampling day, 20 on the second and 5 on the last. All collection days were on Tuesdays. This caused some of the samples to have longer storage periods than the other sample sets. Three blood collection tubes were collected during each of the four sample periods. Two 10 mL green top sodium heparin Vaccutainers® were used to collect the blood that was used for the headspace gas chromatograph analysis explained later in this section. Sodium heparin was used as an anticoagulant. These two tubes were labeled after collection and refrigerated with the other tubes that were collected that sampling day at 2.78 degrees C. The third tube was a 5 mL purple top serum separator tube that was spun within 1 hour after the blood sample was taken. It was then labeled and sent to a certified laboratory for a lipid panel analysis.

After all samples were collected, one mL aliquots of the whole blood samples were pipetted into four 2.0 mL glass tubes with air-tight septum tops. The vials were pre-labeled with number codes that referred back to one of the four samples (low control, low, high control, high) and a subject identification number with the treatment type. The treatment type refers to one of three solvents or an air blank that was added to the blood in the vial. The air blank was used as a method blank for quality control during the headspace gas chromatographic analysis described later in this section. The blood samples for these treatments were separated into the three solvent categories and the controls were randomly split up between them. The samples were again stored at 2.78 degrees C, until the headspace gas chromatographic analysis was conducted.

Headspace Gas Chromatography

Samples were analyzed randomly after they were separated into the three solvent categories or runs. Each sample category was analyzed independently of one another at different times. Chloroform was analyzed first followed by methanol and isobutanol. The samples in each treatment category were taken from the 2.78 degree C refrigeration unit and placed under a laboratory hood at room temperature for 1 hour before the first sample was analyzed. Each sample was then independently analyzed using a Hewlett Packard 5890 Series II gas chromatograph equipped with a flame ionization detector (FID). The chromatograph was used in conjunction with a Hewlett Packard 3396 Series II integrator.

The chromatographic conditions included a DB-WAX mega bore column, 0.45 millimeter diameter, 0.85 micrometer film, 15 meters in length. Oven temperature was held constant at 45 degrees C with no temperature ramping program. Both injector and detector temperatures were held constant at 200 degrees C. Split sample injection was used at a split ratio of 10:1. Split port flow was 100 mL/min. Nitrogen was used as the carrier gas at a flow of 10 mL/min under 4.6 psi head pressure in an electronically controlled pressure system. Hydrogen and air flow rates were set at 30 and 400 mL/min respectively for the FID. Auxillary nitrogen flow was 0.0 mL/min.

Acetone was used as a carrier solvent to create standard stock solutions of the three solvents by pipetting known amounts of the solvents into volumetric flasks filled with known amounts of acetone. Seven solutions were prepared containing each of the three solvents as the solute and acetone as the solvent. The volume of chloroform was half of the others when applicable (density of chloroform is approximately twice that of

isobutanol and methanol), to make the total mass of each solvent similar in each solution per volume. The starter solution was made to contain 50 mL of acetone and 1 mL each of the three solvents to make the concentrations shown in Table 7 of appendix A.

An example of this calculation for methanol follows:

ρ Methanol = .79g/mL

$$\frac{0.79g}{50mL} \times \frac{mL}{1000uL} \times \frac{1000mg}{g} \times \frac{1000ug}{mg} \times \frac{1000ng}{ug} = 15{,}800 \text{ ng/uL}$$

Concentration = solute/solvent:

15,800 ng MeOh / uL Acetone or

15.8 ug MeOh / uL Acetone

This equation was used to find the concentrations of all standard solutions. Solutions numbers 7,6,1 and 2 were made the same way the starter solution was made with changes to the volumes of acetone and the three solvents. Table 7 in appendix A shows the exact volumes used and the associated concentrations that result from making these four solutions. In solution numbers 3 and 5 the stock solution was used in a serial dilution to add to other volumetric flasks filled with a known volume of acetone. These volumes and associated concentrations are also shown in Table 7 in Appendix A. Finally solution number 5 was made by adding a known amount of solution number 1 to a known amount of acetone. These volumes and concentrations are shown in Table 7 in Appendix A as well. This method was used to have a total of seven points on a calibration curve having a similar range and similar point locations for all three solvents. The actual calibration curve that was used only included five points from each respective curve

excluding two end points. This was done to better fit the range of sensitivity of the solvent. Also, the curves were designed to be linear in nature with the scale of the concentrations exponential.

After the GC was calibrated before each complete solvent set was run, samples were treated with one of the three solvents depending on the run being analyzed. Either 5 uL of chloroform, or 10uL of isobutanol or methanol, or an air blank was added to the vial using the technique described in Kolb and Ettre (1997). The vials were then transferred from room temperature into a glass bath at 37 degrees C and allowed to equilibrate for five minutes. Then, a 2 microliter sample of the headspace was removed using a gas tight syringe and injected into the gas chromatograph. Headspace is defined as the volume left at the top of the glass tubes filled with the 1mL blood sample before sealing (Kolb and Ettre, 1997). This volume was controlled by the accurate addition of 1 milliliter of blood. A total of three sample injections were used for chloroform and methanol while two injections were made for isobutanol because of its relatively longer retention time. Each sample extraction and injection was performed using the technique described in Kolb and Ettre (1997). After each sample injection, data were gathered from the previous samples' chromatogram and documented. Also during this time, the next sample vial was treated with the relevant solvent and added to the glass bath, which acted as a cue, to begin to thermostat. The five minute equilibration time was monitored with the use of a stopwatch.

Sample concentrations were determined by using the regression equation that was developed by the calibration curves found by analyzing the standard solutions. These calibration curves can be found in Figures 1-3 in the apendix. The mean area of the three

samples taken from each individual sample vial was used to determine the concentration in each sample vial headspace. This value was then corrected by adjusting for the method blank by subtracting the relevant method blank value if applicable. Using chloroform as an example, a mean area of three samples taken from a vial is 165,000. The following regression equation was used to determine the mass of chloroform in ng that was detected by the instrument per 2uL's of headspace air injected.

165,000 / 100000 = 1.65

ng = -45.96 + 100.27 x (mean area) = -45.96 + 100.27 x 1.65 = 119.48 ng

The area was divided by 100,000 for each solvent to set the scale of the calibration curve. This was done such that whole numbers were created in both the slope and regression coefficient for the regression equation. Thus, the average area for the three runs for each sample (two runs for isobutanol) was divided by 100,000 to match the scale of the calibration curve. The injection volume for each sample was 2uL. Therefore, the amount of solvent in ng that was found using the calibration curve, was represented as ng/2uL. This quotient represents the headspace solvent concentration for the 2mL vial.

It is important to also point out that the methanol calibration curve regression line did not pass directly through the origin of the X and Y axes. This resulted in some negative numbers because the methanol headspace concentrations were very low. The concentrations were so low that the electronic signal in millivolts from the FID created a total area that was below zero. To perform certain statistical analysis of the methanol

sample results, the data was not converted into a concentration as isobutanol and chloroform were. This allowed the methanol data to be a positive number but in a different scale. Positive numbers allowed us to perform the simple regression to explore the relationship between area and triglyceride levels. It also allowed us to make comparisons between average headspace concentrations for each respective diet, as well as average headspace concentrations for each sample vs. the fasting sample (control). Therefore, the comparisons that were made had the same significance when looking at all three solvents and all three regression models had the same significance even though methanol is on a different scale.

Samples were analyzed blindly, to minimize the potential for bias. Air blanks were analyzed every 4 samples which comprised the method controls. The three runs for each solvent included 27, 27 and 26 air blanks respectively. Also, calibration standards were run and analyzed approximately every 12 samples for quality control purposes. Concentrations were used that were within the range of the concentrations found for the specific solvent being analyzed in the run at that time. The gas chromatograph was both pre- and post- calibrated each day to ensure there was no change in sensitivity, retention time, accuracy, and precision for each respective solvent.

Triglyceride analysis

The triglyceride analysis was conducted by Alliance Laboratory Services, which is part of the Health Alliance at University Hospital in Cincinnati, OH. A lipid panel was performed on each sample and results were sent within a few days. The lipid panel included triglycerides, HDL, LDL, and total cholesterol for each individual sample.

Statistical analysis

Data were entered into a Microsoft Excel 2000 spreadsheet and SPSS 11.5 was used to perform statistical tests. Normality was examined for each data set and data were transformed as needed for statistical analysis. Data sets for methanol, chloroform, and isobutanol were logtransformed because the visual plots for each were interpreted as a lognormal distributions. All triglyceride data sets were interpreted as lognormal distributions as well, therefore, these data sets were also logtransformed before statistical analysis took place.

Headspace concentrations of chloroform and isobutanol, and the area of methanol were each plotted against triglyceride levels to develop three separate correlation coefficients. An alpha level of 0.05 was used to determine statistical significance of the correlation coefficient for each model. Paired sample T-tests were performed to determine differences between group means. An alpha level of 0.05 was used to determine statistical significance for group mean differences. An analysis of covariance was conducted to determine covariance between group means and their respective controls for triglyceride levels. Microsoft Excel 2000 Chart Wizard and SPSS 11.5 was used to create tables and bar charts to visually compare group means.

Results

As Tables 8 and 9 suggest, the analysis of covariance for the four categories of triglyceride levels shows that the controls do not have a statistically significant effect on the triglyceride outcome for each meal for both the low and high fat diets (F-test, 0.896). As we determined the effect of the study factors on the triglyceride levels adjusted for each respective control, we found that the control is not a determinant of the triglyceride level after the meal is consumed. This tells us that the actual fat content of the meal determines the final triglyceride levels. The control values are not a predictor of the final triglyceride levels for each person for each respective meal.

Summarizing the subjects' triglyceride level results, we saw that consuming the low and high fat content meals did have an effect on the amount of triglycerides present in the blood when sampling takes place between 4-5 hours after the meal is consumed. The average blood lipid (triglyceride) levels increased from 116.8 to 119.7, and 80.1 to 156.9 mg/dL for low and high fat meals, respectively (Figure 4, Table 10). Results were statistically significant at the 0.05 level when comparing the low fat meal lipid levels with the high fat meal lipid levels using a paired t-test, $p=0.001$ (Table 11). The same significance was found when comparing lipid levels for fasting vs. postprandial samples for the high fat meal (paired t-test, $p=0.045$) (Table 11). No difference was seen when comparing lipid levels for fasting vs. postprandial samples for the low fat meal (paired t-test, $p=0.710$) (Table 11).

In reference to Figures 5,6,and 7, we see the following results when comparing headspace solvent concentrations (ng/2uL) in relation to the amount of triglyceride levels (mg/dL) in the blood. Headspace chloroform concentrations decreased as blood lipid

levels increased. The correlation between these two variables was statistically significant ($r = -0.30$; $p=0.007$). Headspace isobutanol and methanol concentrations slightly increased as blood lipid levels increased. The correlations between methanol and isobutanol concentrations and their associated triglyceride levels were also significant at the $\alpha=0.05$ level (methanol $r=0.231$; $p=0.039$, isobutanol $r=0.237$; $p=0.039$).

We also looked at the differences between average headspace solvent concentrations (ng/2uL) with respect to the meal that was consumed. As Figure 8 and Table 12 suggest, the results also showed that headspace chloroform concentrations decreased when individuals consumed the high fat versus the low fat meal (paired t-test, $p=0.001$). Since the *in vitro* headspace gas chromatography method utilized a sealed source environment in the 2mL vials, we can extrapolate to say that the chloroform concentration in the blood increased for the high fat content meal vs. the low fat content meal. There was also no significant difference ($\alpha=0.05$) in the headspace concentrations for methanol and isobutanol when comparing the low and high fat diet concentrations for each, respectively (Figure 8, Table 12).

Headspace chloroform concentrations also decreased (chloroform concentrations in the blood increased) when individuals consumed the high fat content meal versus the fasting sample, or control (paired t-test, $p=0.045$) (Table 13, Figure 9). All other differences between the meal types and their respective fasting samples (controls) were not significantly different ($\alpha=0.05$) for each respective solvent.

Table 8: Analysis of Covariance (ANCOVA) for triglyceride levels

Without Interaction

SAS GLM Procedure

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	13295.9178	6647.9589	1.56	0.2228
Error	37	157246.0822	4249.8941		
Corrected Total	39	170542			

R-Square	Coeff. Var.	Root MSE	Y Mean
0.077963	41.1301	65.1912	158.5

Source	DF	Type I SS	Mean Square	F Value	Pr > F
group	1	112.50627	112.50627	0.03	0.8716
contro.	1	13183.41152	13183.41152	3.1	0.0865

Source	DF	Type III SS	Mean Square	F Value	Pr > F
	1	3225.6303	3225.6303	0.76	0.3893
group	1	13183.41152	13183.41152	3.1	0.0865
control					

Table 9: Analysis of Covariance (ANCOVA) for triglyceride levels cont'd

With Interaction

SAS GLM Procedure

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	13372.4931	4457.4977	1.02	0.3948
Error	36	157169.5069	4365.8196		
Corrected Total	39	170542			

R-Square	Coeff. Var.	Root MSE	Y Mean
0.078412	41.68729	66.07435	158.5

Source	DF	Type I SS	Mean Square	F Value	Pr > F
group	1	112.50627	112.50627	0.03	0.8716
control	1	13183.41152	13183.41152	3.1	0.0865
control*group	1	76.57527	76.57527	0.02	0.8954

Source	DF	Type III SS	Mean Square	F Value	Pr > F
group	1	160.89134	160.89134	0.04	0.8488
control	1	3985.378568	9385.378568	2.15	0.1513
control*group	1	76.575268	76.575268	0.02	0.8954

Table 10: Raw triglyceride data

tryglylc	tryglyhc	tryglylo	tryglyhi
81	58	93	117
95	61	81	133
89	103	95	299
101	90	163	174
156	63	136	90
170	56	163	112
92	116	76	176
95	120	114	327
50	82	37	105
228	68	273	156
194	57	112	76
65	143	126	213
91	78	62	117
193	50	205	79
156	59	183	95
84	86	52	152
96	79	97	163
69	54	70	169
114	82	137	216
	64		181
	114		145
Mean levels (mg/dL):	116.7895	80.14286	119.7368	156.9048
Standard deviation:	50.28649	25.84044	58.54613	65.85583

"tryglylc" = triglycerides for low fat diet control (fasting sample)
"tryglyhc" = triglycerides for high fat diet control (fasting sample)
"tryglylo" = triglycerides for low fat diet (postprandial sample)
"tryglyhi" = triglycerides for high fat diet (postprandial sample)

Table 14: Raw triglyceride data for analysis of covariance (ANCOVA)

0 = high fat meal
1 = low fat meal
Y = end triglyceride level

Results are represented in (mg/dL)

control	Y	group
58	117	0
61	133	0
103	299	0
90	174	0
63	112	0
56	176	0
116	327	0
120	156	0
82	76	0
68	213	0
57	117	0
143	79	0
78	95	0
50	152	0
59	169	0
86	216	0
79	181	0
54	163	0
82	90	0
64	117	1
114	133	1
81	299	1
95	174	1
89	90	1
101	112	1
156	176	1
170	327	1
92	105	1
95	156	1
50	76	1
228	213	1
194	117	1
65	79	1
91	95	1
193	152	1
156	163	1
84	169	1
96	216	1
69	181	1
114	145	1

Table 15: Raw methanol data by diet

amehi	ngmehi	amelow	ngmelow
238635	-63.6	898554	137.59
107823	-103.49	89857	-108.96
473529	8.01	124098	-98.53
229392	-66.42	154811	-89.16
137286	-94.5	173967	-83.32
11855	-132.75	433471	-4.2
280560	-50.82	83324	-110.96
224055	-68.05	182124	-80.83
227888	-66.88	16062	-131.46
320961	-38.51	135515	-95.04
603437	47.62	98168	-106.43
170398	-84.41	151965	-90.03
325914	-37	130081	-96.7
139068	-93.96	261003	-56.79
182763	-80.64	153163	-89.66
324863	-37.32	38139	-124.73
148467	-91.1	173677	-83.41
78967	-112.28	210765	-72.1
187844	-79.09	246229	-61.29
94644	-107.5		
144907	-92.18		
Mean Concentration (ng/uL):	-68.8033		-76.1058
Standard deviation:	41.37736		58.71227

"amehi" = headspace methanol area response for high fat diet
"ngmehi" = headspace methanol concentration for high fat diet
"amelow" = headspace methanol area response for low fat diet
"ngmelow" = headspace methanol ocncentration for low fat diet

Table 16: Raw chloroform data by diet

aclohi	ngclohi	aclolow	ngclolow
611793	567.43	828394	784.6
309833	264.67	1187873	1145.03
590904	546.48	2002848	1962.16
969927	926.51	524764	480.17
646339	602.07	723261	679.19
465870	421.12	1928279	1887.39
198735	153.28	3023430	2985.43
1437355	1395.17	1954700	1913.88
1929954	1889.07	2978197	2940.08
629280	584.96	1153647	1110.71
887079	843.44	1691299	1649.78
523277	478.68	555615	511.1
416065	371.18	785928	742.02
1703603	1662.12	1335838	1293.38
763438	719.47	2389262	2349.59
1070395	1027.24	528521	483.94
620916	576.58	2314415	2274.55
388504	343.55	2316833	2276.97
457725	412.95	1192554	1149.72
Mean concentration(ng/uL):	725.5774		1506.299
Standard deviation:	471.5041		817.0318

"aclohi" = headspace chloroform area response for high fat diet
"ngclohi" = headspace chloroform concentration for high fat diet
"aclolo" = headspace chloroform area response for low fat diet
"ngclolo" = headspace chloroform concentration for low fat diet

Table 17: Raw isobutanol data by diet

aisohi	ngisohi	aisolow	ngisolow
181938	31.27	666082	92.35
708577	97.71	1104206	147.63
1317420	174.53	4200000	538.19
865566	117.52	573550	80.68
649867	90.31	1245674	165.47
726546	99.98	510191	72.69
458197	66.13	677420	93.78
735363	101.09	581228	81.65
851956	115.8	1627420	213.64
1479376	194.96	894206	121.13
503084	71.79	1100000	147.1
2304206	299.02	695335	96.04
731490	100.6	185531	31.73
1015674	136.46	578103	81.25
874084	118.59	853979	116.06
393376	57.95	191112	32.43
4139376	530.54	423904	61.8
887143	120.24		
1430000	188.73		
1110000	148.36		
Mean concentration (ng/uL):	143.079		127.86
Standard deviation:	108.5353		115.5999

"aisohi" = headspace isobutanol area response for high fat diet
"ngisohi" = headspace isobutanol concentration for high fat diet
"aisolow" = headspace isobutanol area response for low fat diet
"ngisolow" = headspace isobutanol concentration for low fat diet

Table 18. Raw solvent data by diet for control (fasting) samples

ngclolc	ngclohc	ngmelc	ngmehc	ngisolc	ngisohc
509.58	798.51	-102.11	-108.93	122.46	100.49
1603.87	1146.74	-94.47	-63.42	144.37	89.34
920.47	1378.09	-105.65	-36.59	217.2	77.94
1679.43	878.11	-94.37	-73.34	136.68	111.69
480.15	714.53	-68.66	-97.35	98.74	10.26
319.38	1574.14	-89.17	-91.31	86.4	56.28
2567.16	718.78	-118.78	-28.58	60.87	122.36
936.57	382.26	-115.21	32.55	72.06	91.37
531.39	552.94	-46.27	-130.54	37.49	110.73
1716.14	1081.4	-98.87	-63.46	75.85	145.83
1116.78	889.22	-66.76	-3.91	388.03	71.63
1548.56	488.48	-111.62	-59.19	81.69	142.22
635.63	698.95	-105.49	-77.78	106.74	101.77
1040.3	1388.22	-93.47	-54.76	118.26	227.14
642.76	1899.6	-58.71	18.68	82.41	54.63
772.45	2254.11	-111.53	-103.46	79.32	92.75
2397.91	1016.44	-62.85	-61.36	370.22	82.09
1948.31	1527.69	-2.43	-95.18	54.44	110.68
986.97	1028.36	-65.23	-114.1	42.78	36.47
	1055.8		-106.95		148.18
	397.31		-99.52		101.22
Mean concentration(ng/uL):	1176.516	1041.413	-84.8237	-67.5476	125.0532	99.1925
Standard Deviation:	662.0268	490.1761	29.39554	43.79128	98.71369	46.53075

"ngclolc" = headspace chloroform concentration for low fat diet control (fasting) sample
"ngclohc" = headspace chloroform concentration for high fat diet control (fasting) sample
"ngmelc" = headspace methanol concentration for low fat diet control (fasting) sample
"ngmehc" = headspace methanol concentration for high fat diet control (fasting) sample
"ngisolc" = headspace isobutanol concentration for low fat diet control (fasting) sample
"ngisohc" = headspace isobutanol concentration for high fat diet control (fasting) sample

Table 19: Analytical methanol data and associated blood panel data

MeOH

number	approx. area	area minus control	trigly	choles.	HDL	LDL
2	124098	124098	95	151	35	96
3	187844	187844	216	140	39	58
7	256083	228289	194	171	49	83
9	120819	120819	114	233	40	170
10	439287	439287	69	149	54	81
14	148348	137286	90	176	66	92
16	137409	137409	95	154	44	91
18	165831	154769	170	182	48	100
27	274023	246229	137	220	37	156
32	206706	206706	90	161	39	104
34	173967	173967	136	231	68	136
38	323295	295501	50	140	64	66
45	147873	135515	273	179	50	74
46	140694	140694	193	186	38	109
47	69384	69384	95	150	42	89
49	102751	81433	84	122	51	54
55	135332	122974	228	172	50	76
57	472624	434419	57	132	60	61
58	324863	324863	152	172	42	100
65	266093	227888	105	200	41	138
68	172701	144907	145	222	42	151
81	111175	89857	81	161	46	99
82	318765	280560	176	223	67	121
84	327249	327249	103	142	33	88
85	182124	182124	114	160	45	92
88	261083	233289	114	219	39	157
99	125962	98168	112	182	56	104
100	38139	38139	52	123	51	62
104	95682	83324	76	191	38	138
111	224390	11855	112	191	71	98
114	130081	130081	62	147	43	92
115	261003	261003	205	190	39	110
122	170398	170398	213	160	55	62
123	241095	241095	96	122	43	60
126	325914	325914	117	169	53	93
129	246013	246013	79	122	33	73
137	148467	148467	163	130	31	66
140	101251	101251	91	149	41	90
143	391737	353532	116	218	65	130
144	192138	192138	78	162	54	92
145	359166	320961	156	177	46	100

156	57310	19105	82	195	44	135
172	176129	154811	163	168	37	98
173	182763	182763	95	133	34	80
183	70019	57661	92	185	38	129
188	89962	89962	58	174	66	96
190	107823	107823	133	168	46	95
198	165538	147764	56	179	71	97
203	139015	127953	63	177	61	103
209	592214	554009	120	171	53	94
213	112353	112353	81	183	68	99
226	173677	173677	97	126	45	62
230	160355	137719	101	162	38	104
232	107912	107912	86	179	45	117
239	267643	267643	50	149	61	78
240	210765	210765	70	149	53	82
242	473529	473529	299	156	31	65
252	78967	78967	169	140	55	51
256	508538	508538	59	135	41	82
259	262260	224055	327	193	48	80
260	238635	238635	117	189	63	103
264	72998	72998	82	134	41	77
286	239237	239237	61	160	47	101
293	253116	253116	143	154	56	69
308	234402	222044	156	219	63	125
311	461265	433471	163	178	45	100
320	164323	151965	126	144	49	70
321	135061	135061	54	141	62	68
324	94644	94644	181	160	54	70
330	641642	603437	76	144	63	66
337	277309	239104	68	163	47	102
338	254680	254680	156	132	30	71
339	139068	139068	79	152	60	76
349	93521	81163	65	137	49	75
350	153163	153163	183	143	31	75
352	229392	229392	174	169	37	97
355	43856	16062	37	146	65	74
364	96471	96471	64	148	55	80
376	100727	100727	89	152	35	99
378	89854	89854	93	186	70	97

Table 20: Analytical chloroform data and associated blood panel data

CHLORO

number	approx. area	area minus trigly control	choles.	HDL	LDL	
5	679817	679817	91	149	41	90
15	511228	457725	90	176	66	103
19	1928279	1928279	163	178	45	100
22	2661710	2606257	92	185	38	129
25	921656	921656	90	161	39	104
33	1669523	1669523	163	130	31	66
36	2011666	1940461	59	135	41	82
39	1661550	1590345	65	137	49	75
41	1691299	1691299	112	182	56	104
42	688326	629280	213	160	55	62
43	1179699	1124415	68	163	47	102
48	2341470	2316833	70	149	53	82
51	398560	364395	170	182	48	100
54	3063628	3023430	76	191	38	138
56	785928	785928	62	147	43	92
60	791328	762749	116	218	65	130
64	1014125	979960	95	150	42	89
78	657316	620916	181	160	54	70
80	604262	533057	143	154	56	69
86	476291	442126	114	233	40	170
87	1194547	1153647	273	179	50	76
89	1263759	1192554	137	220	37	156
90	1515182	1420317	103	142	33	88
92	963906	963906	89	152	35	99
93	320923	309833	133	168	46	95
101	1703603	1703603	152	172	42	100
107	631508	597343	82	195	44	135
108	1030227	1030227	114	219	39	157
112	2437452	2437452	96	122	43	60
118	3014597	2978197	37	146	65	74
120	2044494	1989041	69	149	54	81
121	580028	524744	156	219	63	125
127	937136	842271	58	174	66	96
128	575848	575848	50	140	64	66
133	447550	388504	163	222	42	151
135	646188	590904	299	156	31	65
139	2002848	2002848	95	151	36	96
142	783866	742966	78	162	54	92
146	1755027	1720862	101	162	38	104
147	1189578	1189578	61	160	47	101
149	1135275	1098875	64	148	55	80
165	1335838	1335838	205	190	39	110

177	611793	611793	117	189	63	103
179	2369699	2314415	97	126	45	62
187	486163	427117	120	171	53	94
189	506770	465870	176	223	67	121
199	646339	646339	112	191	71	98
202	1615850	1615850	56	179	71	97
206	232900	198735	327	193	48	80
208	932735	932735	57	132	60	61
214	862559	828394	93	186	70	97
215	1138705	1083421	193	186	38	109
218	1437355	1437355	156	177	46	100
219	821439	816274	84	122	51	54
224	1852341	1757476	228	172	50	76
225	757426	723261	136	231	68	136
234	812010	758507	63	177	61	103
246	1929954	1929954	76	144	63	66
248	1193866	1159701	194	171	49	83
249	528521	528521	52	123	51	54
250	1259078	1187873	81	161	46	99
258	803636	763438	169	140	55	51
263	2389262	2389262	183	143	31	75
265	887079	850679	117	169	53	93
266	969927	969927	174	169	37	97
275	1088201	1059622	79	122	33	73
278	503677	503677	105	200	41	138
281	588258	554093	81	183	68	99
328	558929	524764	163	168	37	98
334	2294038	2294038	86	179	45	117
340	1699009	1645506	95	154	44	91
357	592015	555615	126	144	49	70
359	1628568	1569522	54	141	62	68
362	1142716	1071511	82	134	41	77
365	416065	416065	95	133	34	80
367	551277	523277	79	152	60	76
370	1954700	1954700	114	160	45	92
371	723322	686922	156	132	30	71
374	1104560	1070395	216	140	39	58
379	1430425	1430425	50	149	61	78

Table 21: Analytical isobutanol data and associated blood panel data

ISO

number	approx. area	area minus control	trigly	choles.	HDL	LDL
8	871418	871418	193	186	38	109
12	666082	666082	93	186	70	97
20	716698	716698	156	219	63	125
24	561832	273146	114	219	39	157
30	1710000	1479376	156	177	46	100
31	1960000	1627420	37	146	65	74
37	60340	-228346	x	x	x	x
40	726546	726546	112	191	71	98
62	3490000	903904	116	218	65	130
70	654283	365597	69	149	54	81
73	1650000	1317420	299	156	31	65
74	835138	573550	163	168	37	98
75	1500000	1104206	81	161	46	99
79	642235	642235	61	160	47	101
83	1110000	1110000	145	222	42	151
91	492833	231245	50	140	64	66
94	551839	551839	103	142	33	88
95	3360000	2868577	96	122	43	60
97	1350000	1017420	101	162	38	104
98	851447	562761	84	122	51	54
102	811732	811732	68	163	47	102
106	588390	510191	163	178	45	100
110	714576	223153	64	148	55	80
116	735363	735363	327	193	48	80
125	2700000	2304206	213	160	55	62
134	982447	649867	90	176	66	92
141	506828	15405	63	177	61	103
151	930840	669252	79	122	33	73
153	619974	618864	170	182	48	100
154	539524	505198	95	150	42	89
155	731490	731490	79	152	60	76
160	1090000	1090000	57	132	60	61
161	4370000	4139376	169	140	55	51
163	658304	658304	82	195	44	135
168	1010000	677420	76	191	38	138
186	829446	780146	91	149	41	90
193	1290000	894206	273	179	50	74
196	1690000	1655674	89	152	35	99
197	578103	578103	183	143	31	75
200	695335	695335	126	144	49	70
204	873445	584759	54	141	62	68

216	393376	393376	163	130	31	66
217	501792	501792	143	154	56	69
231	733708	503084	76	144	63	66
238	817917	587293	156	132	30	71
247	1100000	1100000	112	182	56	104
255	971336	740712	50	149	61	78
257	x	x	120	171	53	94
267	581542	581542	65	137	49	75
268	1340000	1078412	95	154	44	91
270	367078	367078	86	179	45	117
271	1430000	1430000	181	160	54	70
272	851956	851956	105	200	41	138
279	647195	416571	92	185	38	129
280	581228	581228	114	160	45	92
283	452700	191112	70	149	53	82
285	1600000	1108577	114	233	40	170
289	673361	181938	117	189	63	103
290	474217	185531	62	147	43	92
291	1820000	1734431	59	135	41	82
294	458197	458197	176	223	67	121
315	1280000	1245674	136	231	68	136
318	936443	887143	216	140	39	58
323	1200000	708577	133	168	46	95
325	3480000	3009775	194	171	49	83
331	1100000	811314	82	134	41	77
332	730580	730580	58	174	66	96
335	535263	535263	228	172	50	76
342	865566	865566	174	169	37	97
343	380176	380176	56	179	71	97
344	1350000	1061314	78	162	54	92
345	853979	853979	52	123	51	62
353	1260000	998412	x	x	x	x
354	959653	874084	152	172	42	100
363	4200000	4200000	95	151	36	96
366	1050000	1015674	95	133	34	80
368	1050000	819376	90	161	39	104
369	3010000	423904	137	220	37	156
372	904754	904754	81	183	68	99

* Denotes statistical significance ($\alpha = 0.05$)

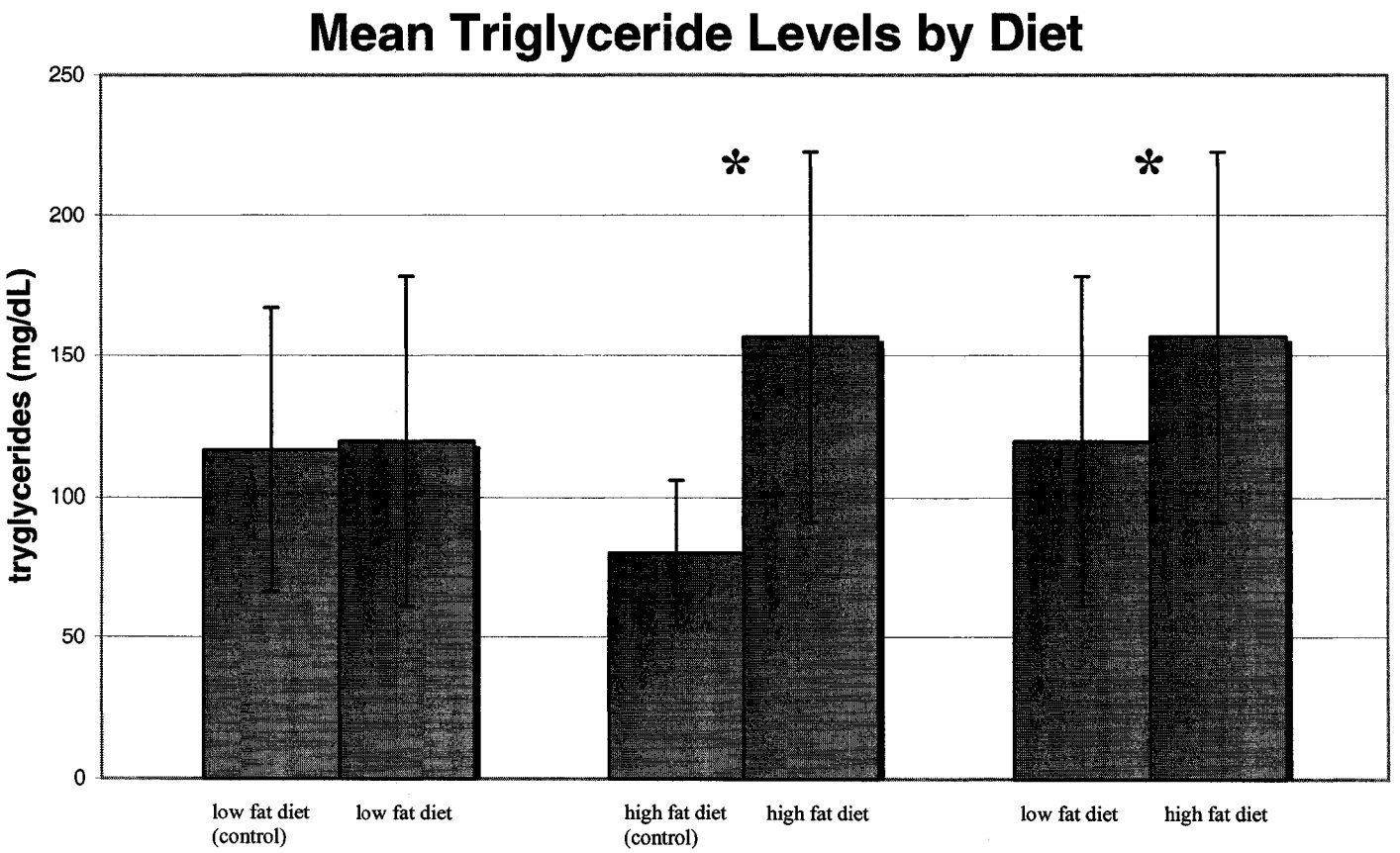
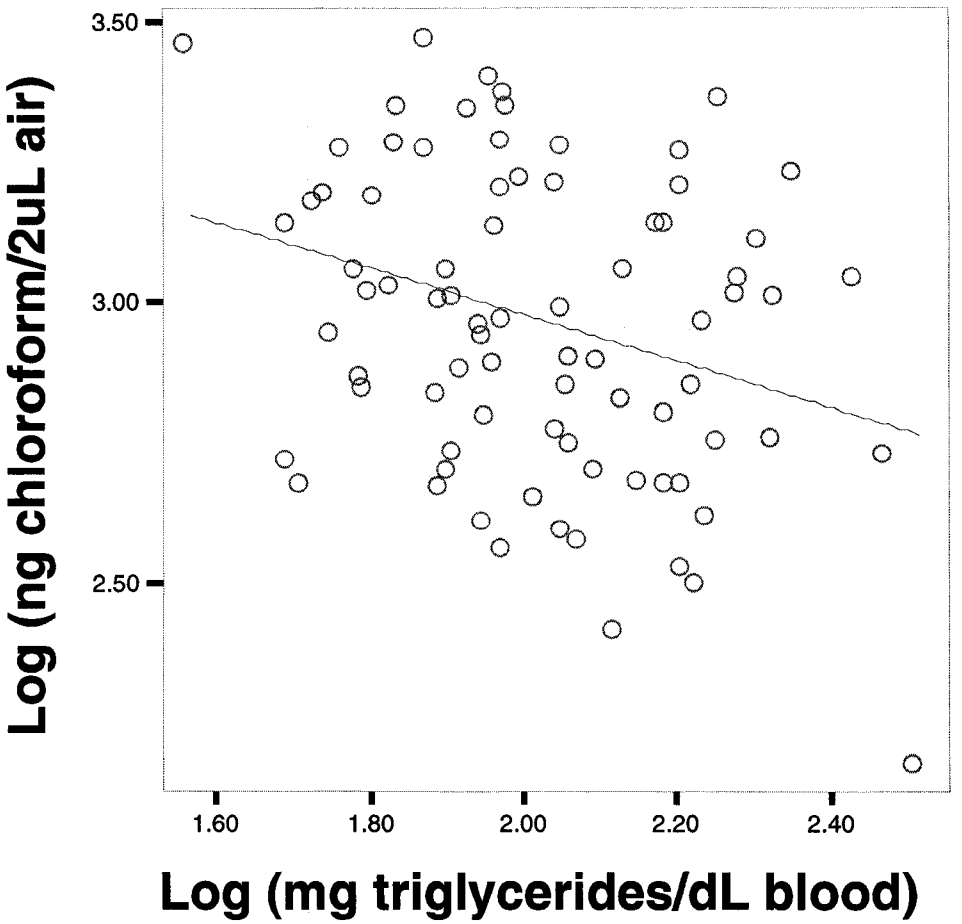


Figure 4: Histogram of mean triglyceride levels

Table 11: t-test, triglyceride levels by diet

GROUPS COMPARED	t-value	d.f.	sig. (2-tailed)
triglyceride levels for low fat diet vs. triglyceride levels for high fat diet	-2.035	18	0.05
triglyceride levels for low fat diet vs. triglyceride levels for low fat diet control	-0.376	18	0.711
triglyceride levels for high fat diet vs. triglyceride levels for high fat diet control	-5.211	18	0.001



R = - 0.30
n = 80
p = 0.007

Figure 5: Relationship between blood triglycerides and headspace chloroform concentrations

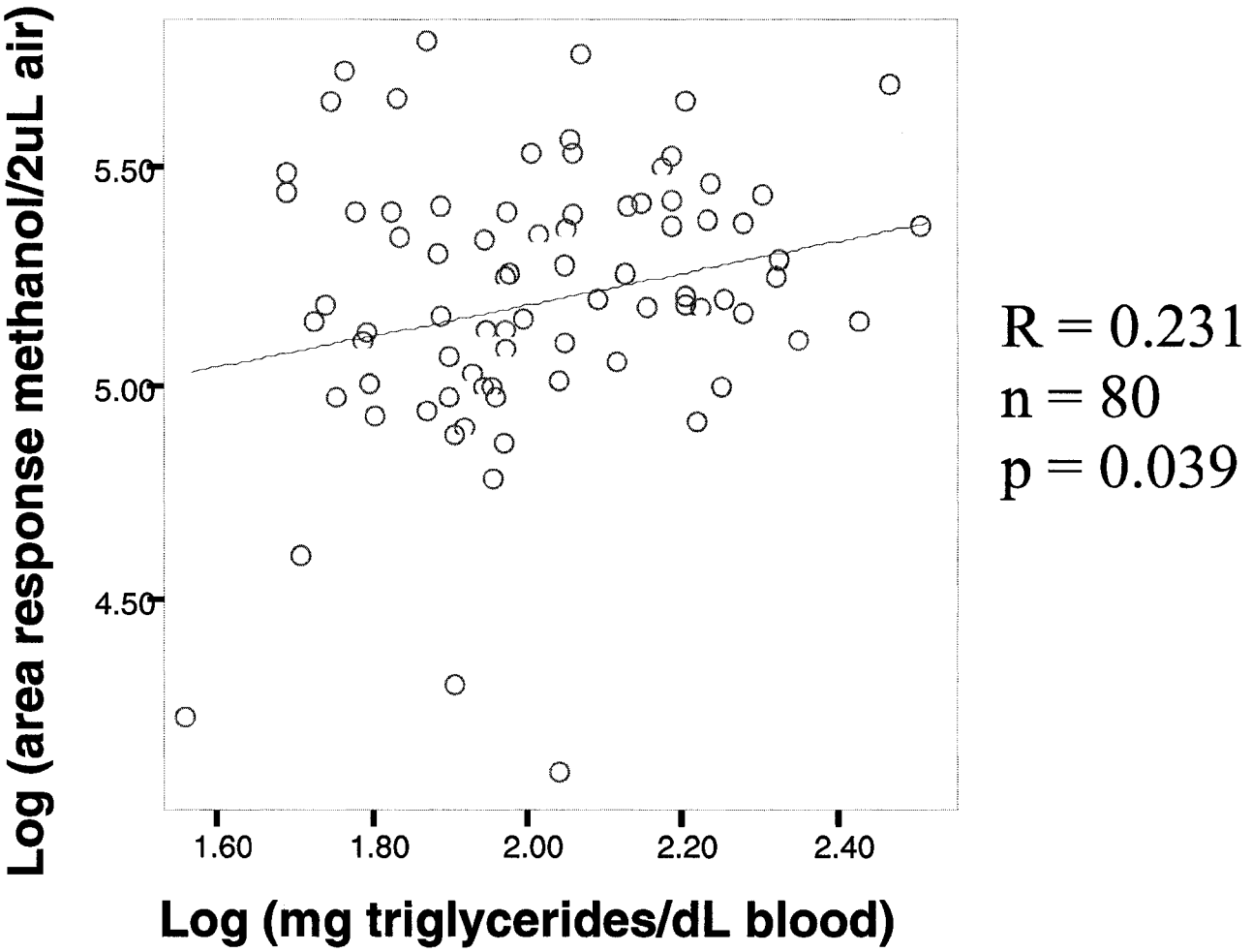


Figure 6: Relationship between blood triglycerides and headspace methanol concentration area response

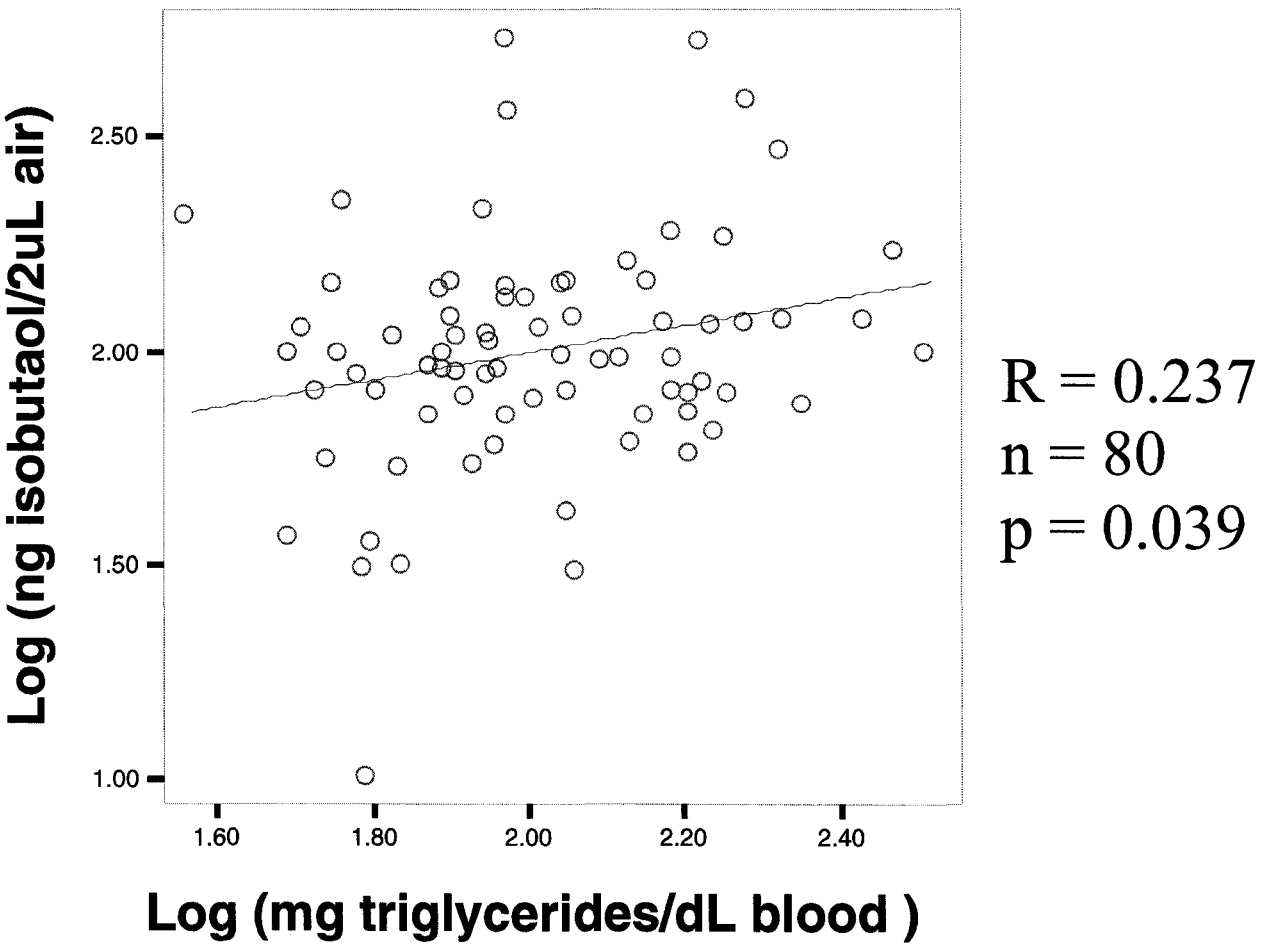
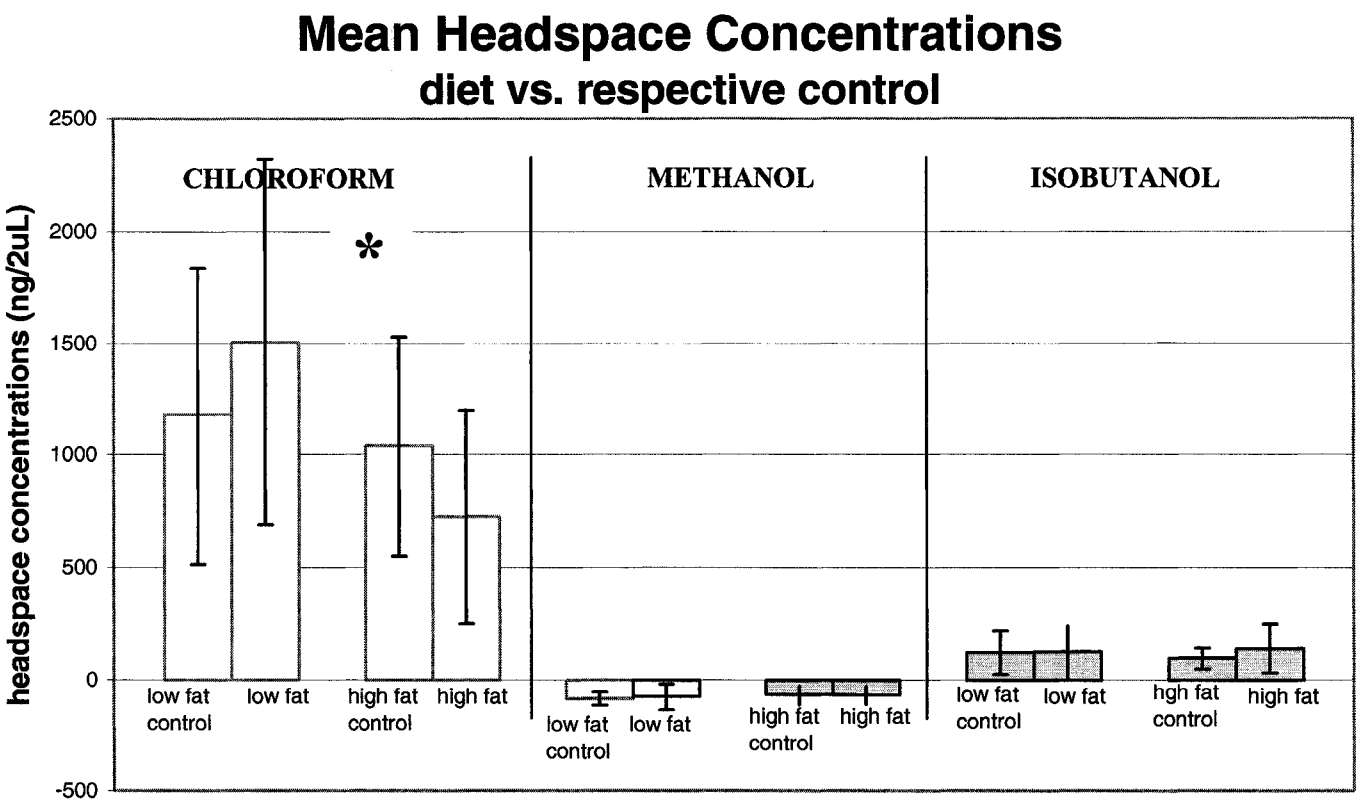


Figure 7: Relationship between blood triglycerides and headspace isobutanol concentrations

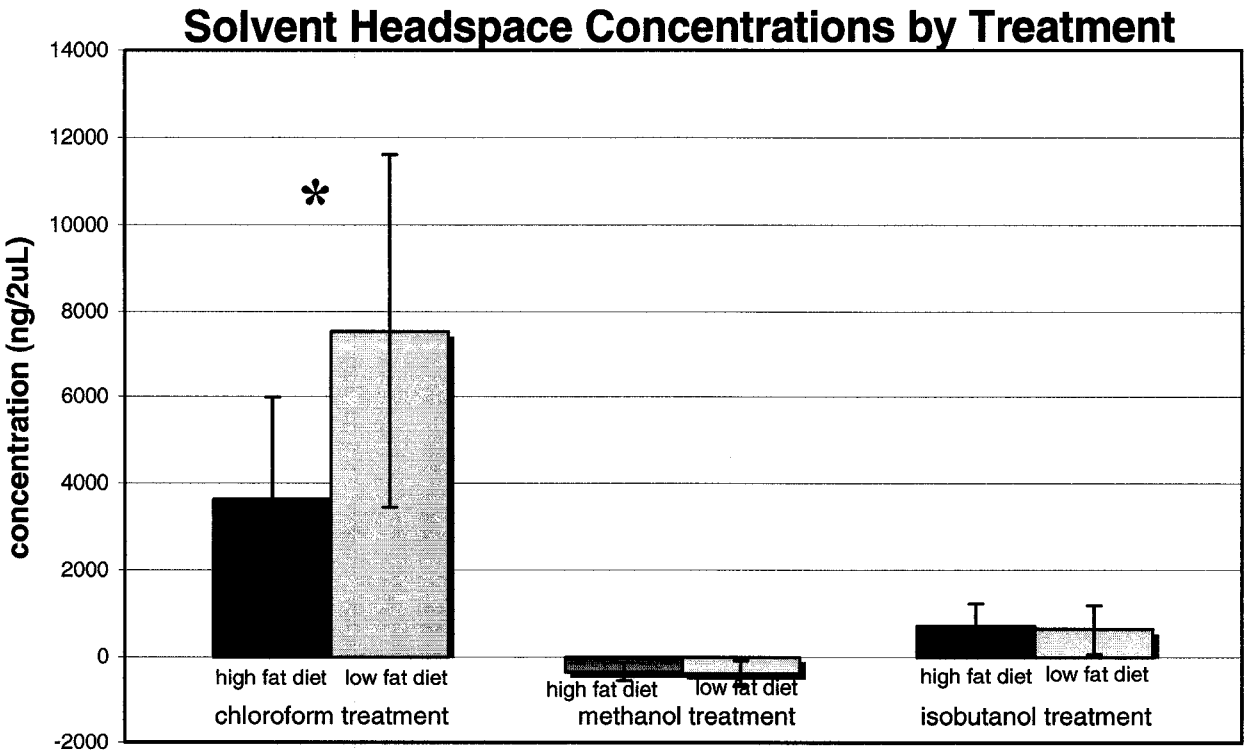


* Denotes statistical significance ($\alpha = 0.05$)

Figure 9: Histogram of mean headspace concentrations, diet vs. control

Table 13: t-test, diet vs. control headspace concentrations

GROUPS COMPARED	t-value	d.f.	sig. (2-tailed)
chloroform conc. for low fat diet vs.	1.568	18	0.134
chloroform conc. for low fat diet control			
chloroform conc. for high fat diet vs.	-2.153	18	0.045
chloroform conc. for high fat diet control			
methanol conc. for low fat diet vs.	0.562	18	0.581
methanol conc. for low fat diet control			
methanol conc. for high fat diet vs.	-0.12	20	0.906
methanol conc. for high fat diet control			
isobutanol conc. for low fat diet vs.	-0.184	16	0.857
isobutanol conc. for low fat diet control			
isobutanol conc. for high fat diet vs.	1.704	19	0.105
isobutanol conc. for high fat diet control			



* Denotes statistical significance ($\alpha = 0.05$)

Figure 8: Histogram of mean headspace concentrations, low vs. high fat

Table 12: high fat vs. low fat headspace concentrations

GROUPS COMPARED	t-value	d.f.	sig. (2-tailed)
chloroform conc. for high fat diet vs. chloroform conc. for low fat diet	-3.186	18	0.001
methanol conc. for high fat diet vs. methanol conc. for low fat diet	0.573	18	0.574
isobutanol conc. for high fat diet vs. isobutanol conc. for low fat diet	0.32	15	0.754

Discussion

The three different solvents chosen for the study were based on their solubilities. These chemicals have different octanol:water partition coefficients but similar gas:blood partition coefficients, within a range of 200 to 400. The solvents chosen for this study were chloroform, isobutanol, and methanol, which have octanol:water partition coefficients (P) of 93.33, 6.92, and 0.18 respectively (Libe, 2002). These values were found by calculating P based on the standard unit of measure for fat solubility, Log P. Chloroform, with an octanol:water partition coefficient of 93 is the most lipophilic of the three solvents. These solvents were chosen because of their wide spread use in industry and their representative differences in lipophilicity. From this widespread range, it was expected that the most lipophilic solvent, chloroform, would be the most likely solvent to be affected by the amount of lipids present in the blood. This turned out to be the case with uptake of chloroform being influenced by the greater amounts of lipids in the blood as seen by simple regression (Figure 5).

The study design of this project included 20 subjects. These subjects represented an age group between 19 and 29 with 9 women and 11 males. The exclusion criteria were important because each factor involved the possibility of influencing the triglyceride levels within each individual. The exclusion criteria acted as a control that reduced the possibility of a subject having triglyceride levels outside the normal range for the general population. Persons with known kidney or liver disease, smokers, and women using oral contraceptives were not eligible for the study. Each potential subject was questioned to determine whether or not they met the exclusion criteria before more information was given to them about the study. All of the above mentioned exclusion

criteria have different influential factors on the regulation of triglyceride levels in the blood (Lab. Corp. of America, 2001). Despite this control, the study design also incorporated the use of a secondary control, in that each subject acted as his/her own control. As the methods indicated, a pre-meal blood sample was drawn for each subject before each respective meal was consumed. This gave us data that was used to investigate differences in pre and post triglyceride levels, and aid in understanding how the diet effects the overall levels of triglycerides that are present in a subject's blood sample at a given time. When considering the analysis of covariance (Tables 8 & 9), we were able to determine that the baseline level of triglycerides that were present in each subject was not a factor that influenced the end level of triglycerides. The end level of triglycerides, measured between 4 and 5 hours after the meal was consumed, proved to be driven by the meal itself, not by the level of triglycerides in the blood before the meal was consumed. In other words we were not able to predict the approximate level that triglyceride levels would increase in each subject. For example, a pre triglyceride level of 100 did not mean that by eating the high fat meal there would be an end level of 200. Since people have different rates of metabolism and a certain degree of inter-individual variability, the end triglyceride amounts were determined directly by the meal itself.

The results in Figure 4 and Table 11 showed that the average baseline (fasting) triglyceride levels for the high and the low fat diets were significantly different from one another. This result was unexpected since each individual was instructed to fast for the same amount of time before each baseline blood sample that was drawn in the morning. In other words, the protocol was the same, just carried out one week later. Given this finding, we looked into the possibility that an individual had not followed the protocol as

was asked of them when they agreed to participate in the experiment. We visually looked for outliers within the control groups for each day and there were no measurements that were beyond two standard deviations from the mean of the group. This may suggest that average triglyceride level inter-individual variation was very high for the subjects tested.

Figure 4 and Table 11 also showed that there was a difference between the low and high fat meals. The mean triglyceride levels for the low fat diet was approximately 115 mg/dL while the mean for the high fat diet was approximately 155 mg/dL. There was a statistically significant difference between these two different measurements at the alpha = 0.05 level. As Tables 5 and 6 show in the appendix, fat content in the two meals increased from 3.5g to 32.0 g, respectively. The calorie content stayed relatively the same for each meal that was consumed. As expected, the high fat meal led to higher levels of triglycerides present in the blood when samples were taken between 4-5 hours after the meal was consumed. This is an important aspect of the overall project because the data suggests that blood triglyceride levels increase from eating the meals with the higher fat content.

The gas chromatographic method used was based on current literature that describes the basic fundamentals of gas chromatography (GC) in general, and more specifically, the fundamentals of headspace gas chromatography. The parameters of the GC method were chosen based on the retention time of each respective solvent, the sensitivity of the detector, and the range of concentrations that were anticipated (Baugh, 1993). Based on the partition coefficients of the chosen solvents, the amount of solvent directly injected into each vial, the temperature, and the volume of air in the vial, we were able to estimate the concentrations that we thought we would see in the headspaces

for each respective solvent (Kolb, 1997). However, this did not take into consideration how other variables such as the triglyceride levels would effect this concentration, if at all.

There were some limitations with the GC method that was used. After the addition of isobutanol and methanol, we found that the samples started to solidify after a short amount of time, usually within 20 minutes. This phenomenon happened much quicker when the samples were heated in the 97.8 degree F glass bath. Based on these observations, smaller amounts of isobutanol and methanol were added to the samples. This corrected the problem of the solidifying blood matrix before the headspace samples were run by the GC. But in doing so, this created another problem in that the methanol samples had a very low concentration present above the blood matrix in the headspace. This is consistent with what we anticipated because of methanol's hydrophilic properties. We anticipated that the majority of the methanol sample that was injected into the sample vial would stay in the plasma of the blood because of this property. Nevertheless, the headspace concentrations were even lower than anticipated. Coupled with this, the calibration curve that was developed with standard solutions did not pass directly through the origin (Figure 1). Since the calibration curve did not pass directly through the origin and the concentrations were so low, the corresponding concentrations that were estimated from the calibration curve were negative. This did not limit our statistical analysis however, because the relationship between the level of triglycerides, and the area of response can still be made. This means that the simple regression line that was produced from plotting triglycerides versus the area of response has the same relevance as the isobutanol and chloroform regression lines. The correlation coefficients and the p values

that were determined have the same statistical weight for each solvent. The only difference is that methanol has a different scale. The same can be said for the comparisons between mean area of responses with t-tests. The comparisons have the same weight in relation to the comparisons between isobutanol concentrations and chloroform concentrations. We did not make comparisons between solvents types so this was not an area of concern.

Additional methodology items that need to be mentioned are syringe problems throughout the sample runs. Two types of syringes were used to perform the analysis. The stock solutions were injected with a cemented, 5uL syringe. The headspace samples were injected with a side port, removable needle, 5uL gas-tight syringe. The gas tight syringes became blocked at times from the septa that covers the injection chamber of the injection heater block. This created a problem with the analysis because it caused inaccurate readings. Usually this error resulted in the form of a very small response but sometimes resulted in the opposite. A very large response would occur other times because the needle would become unplugged during the injection. This inherently caused the injection volume to enter the injection chamber abruptly and inconsistently, ultimately inaccurately changing the signal of the FID. To ameliorate this problem, careful attention was given to each injection and its associated response. If any given response showed a suspect area from the integrator, the needle was checked for defects. A beaker of distilled water was used to ensure bubbles were coming out of the end of the syringe after ambient air was drawn into the needle. If there were no bubbles, the syringe was replaced and the plugged syringe was set aside and properly cleaned for later use.

Also, if it was determined that the needle was plugged, the previous sample was disregarded and an additional sample was taken after the syringe was replaced.

Other considerations with the GC method are the inherent realities of operator error and inter-injection variability. The operator in this study performed headspace chromatography with solvents in different matrixes until the operator injection error was within two standard deviations from the overall mean. Also, each part of the method was practiced many times and headspace samples were practiced on the same vials until reproducibility became accurate.

The blood samples were collected with sodium heparin vaccutainers and allowed to incubate anywhere from four to seven weeks before the analysis took place. The length of blood storage time is not thought to not have a significant impact on the nature of the triglycerides in the blood because they are not thought to readily break down over time if refrigerated (Angerer, 1993). There are however some unknowns with the storage time, for instance, how the blood proteins were effected. It is also unknown whether the red blood cells were affected during the storage time, such as a breakdown in structure, etc. If these types of changes in the composition in the blood took place, it could have theoretically affected the results. The results could have been different particularly for the chloroform samples. Previously discussed studies have found that lipophilic organic solvents have an affinity for red blood cells (Lam, 1986; Lam, 1990). If these red blood cells were changed in any way it may have affected the overall uptake of the solvent.

Despite all of the limitations mentioned above, we are confident that the results reflect a legitimate description of how the triglyceride levels affected the uptake of the given organic solvents. These conclusions, and the triglyceride level changes seen

between the low and high fat content meals, allowed us to accurately test the hypotheses developed for the project.

Referring to Figure 5, we see that the correlation coefficients for the linear regression analysis is negative. This was anticipated for the chloroform samples but also for the isobutanol samples. We also anticipated that the chloroform regression coefficient will have a larger negative correlation coefficient than isobutanol. Figure 7 however shows that the isobutanol model has a slightly positive correlation coefficient. The oil:water partition coefficient for isobutanol is 2, therefore the solvent is two times more likely to be in fat than in water. Based on this physiochemical property, we anticipated that as the triglyceride levels increased in the blood, the headspace concentration would slightly decrease, because there would be a larger reservoir of membranes available (triglycerides) to attract the slightly lipophilic isobutanol. The reason that the correlation coefficient was actually positive is unknown. However, there are a few things that may explain this result. First, due to the limitations that were discussed previously, the variability in the GC method could have been large enough to increase the correlation coefficient when in reality it should have been lower. This could result in the overall error of the correlation coefficient to be actually closer to a straight line than the current results have shown. If there was no error in the method and the variability was very low, we could say that the p-value of 0.039 is suitable in determining the statistical significance of the model. In this case we see that the current model shows that only 23.7% of the variability is explained in the model (Kleinbaum, 1998).

Contrary to this result, we see that the other two models followed what we anticipated based on our hypothesis. We were able to reject the null hypothesis for

chloroform and methanol. Figure 5 shows that chloroform had the highest degree of correlation with triglyceride levels with an r value of -0.30. Chloroform is 93 times more likely to be in the fat than in water, therefore, as the fat reservoir increased in the blood samples, we saw that more chloroform stayed in the blood, attracted to this reservoir. The opposite is true for methanol. Methanol is 18 times more likely to be in the water than in fat. As figure 6 shows, there is a positive correlation with triglyceride levels as they increase. Since methanol is hydrophilic, as opposed to lipophilic, we anticipated that there would be no change on the headspace concentrations as triglyceride levels increased, or if anything, there would be a slightly positive correlation. The slightly positive correlation that we found for methanol was interestingly almost identical to that of isobutanol. This may suggest that both the hydrophilic and lipophilic properties of methanol and isobutanol respectively are low enough that they do not make a difference in the headspace concentrations as the fat in the blood increases. The slightly positive correlation in each model may simply be attributed to the variability in the GC method. Another parameter to consider is the incubation period. Did the variability in the incubation period (from 4-7 weeks) change the nature of the blood such that the blood that was incubated longer had a tendency to retain more of a given compound? This is an item that will be discussed in more detail in the future directions section of this report.

One of the most significant findings in this experiment are the changes in mean headspace concentrations when comparing the two different diets and their respective controls. Figure 9 and Table 13 show that there are no differences in the mean headspace solvent concentrations between meal type and the respective controls except for chloroform. The difference in the high fat headspace concentrations and the high fat

control samples for chloroform shows that there is a difference in the amount of uptake in the high fat meal samples vs. the high fat meal control samples. When looking back at Figure 4 and Table 11, the triglyceride levels for the high fat and the high fat control blood samples, we see that there is also a significant difference between these measurements. These two observations suggest that there is a correlation between uptake of chloroform in the blood and triglyceride levels present in the blood due to the type of meal that was consumed. The figures and tables mentioned show that there is a difference in tirglyceride levels based on the meal that was consumed. We also see that there is a corresponding difference in chloroform headspace concentrations for the high fat content meal vs. its control. Therefore, these tests suggest that the increased amount of triglyceride levels in the blood after a high fat content meal is consumed leads to an increase in lipophilic solvent uptake, in this case, chloroform.

The other substantially important finding is the headspace concentration differences that were found between the high fat and the low fat diet. Figure 8 and Table 12 show that the differences in the headspace concentrations between low and high fat meals are not different for methanol and isobutanol. There was a significant difference seen between the low and high fat meals for chloroform. These results suggest that the diets make no difference in the solvent uptake for methanol and isobutanol. Due to the small lipophilicity of isobutanol (oil:water partition coefficient = 6.9) we did not anticipate that we would see a significant difference between the two diets. If there was a notable difference, we anticipated there would be a slightly lower headspace concentration for the high fat meal vs. the low fat meal samples because of the higher amount of triglycerides in the high fat meal blood. Nonetheless, the data show that there

is a significant difference between the two diets for the samples that were treated with chloroform. These results support the previous findings suggesting that increased triglyceride levels in the blood occur from eating the high fat content meal. The increase in triglyceride levels in turn, directly effects the uptake of chloroform in the blood. A lower average headspace concentration of chloroform was found for the high fat meal, which has a larger statistically significant amount of triglycerides than the low fat meal. These findings show that eating a higher fat meal may increase uptake of an organic solvent, in this case chloroform, but has no impact of uptake of a slightly lipophilic or hydrophilic organic solvent.

Along with the limitations that were discussed previously in this section, it is important to point out several things that add to the validity of the overall results. Several quality control measures were followed when performing the analysis. As discussed briefly in the methods section of this report, method blanks were used approximately every four samples to ensure that there was no residual solvent in the syringe from the previous injection. If the method air blank showed a response, the syringe was immediately cleaned with acetone and de-ionized water. After this procedure, the syringe was checked for its gas-tight capabilities by injecting a small amount of ambient air into a glass beaker of de-ionized water. After the small bubbles were seen, an air blank of ambient air was injected into the GC. If there was no response, the sample run was continued with the same syringe. If there was any type of response the syringe was set aside and a new syringe was used to continue the run. The responses that were documented for the method blanks were deducted from the previous samples to correct for the baseline if there was a response from that particular method blank injection. Also,

as previously mentioned, the gas chromatograph was both pre-and post calibrated. Pre and post calibration area responses were ran for several solvent calibration mixtures within the approximate range of detection for the solvent headspace run that was being determined at the time. This was performed to ensure that there was not a significant change in the responses for a given calibration mixture throughout the course of the run.

It is important to understand other parameters of the method in general to ensure that consideration was given to each facet that might interfere with the reliability of the experiment. As described in the methods section of this report, each sample was injected sequentially multiple times. The average of the responses were calculated for each sample. Attention was given to the responses relative to one another for each sample. If any one of the responses were seen to be illegitimate, that particular injection was treated as an outlier and the remaining sample injections were taken as the average. This procedure served as a secondary control and increased the reliability of the data by decreasing the chances that a bad injection would have influenced the calculated response for a given sample (Kolb, 1978; Ioffe, 1984).

Averaging the area responses for the samples was also a tool that helped increase the sensitivity of the method because of the possibility of inaccurate readings for any given injection when performing gas chromatography. Averaging the responses reduced the possibility of an inaccurate reading based on the possibility of the same type of operator error, detector error, or similar error happening two or three injections in a row (Kolb, 1997). If the method used only one injection, it would have been impossible to detect operator or other similar errors based on the one response. This could have resulted in inaccurate readings.

Another parameter that is worth noting is the fact that one operator performed all of the sample runs. This factor eliminates the possibility of inter-operator errors that could have taken place if there were more than one operator. Also, the operator practiced the method many times before the actual analysis was performed. Numerous headspace GC injections were performed until good reproducibility was documented with each of the solvents. Also, the calibration standards were run numerous times before the start of the analysis to increase accuracy and precision when running these samples during the experiment (Kolb, 1997).

Given these parameters, the findings suggest that there is a relationship between the lipophilic solvent (chloroform, oil:water partition coefficient P=93) and uptake in blood determined by triglyceride levels. The findings to date also suggest that there is no relationship between the hydrophilic solvent (methanol, P=0.18) or slightly lipophilic solvent (isobutanol, P=6.9) that was used in this experiment. These findings are very interesting given the fact that triglyceride levels were found to significantly increase after the high fat meal was consumed.

Conclusions

The null hypotheses stated earlier were tested during the course of this experiment. The first null hypothesis was rejected where average high fat meal triglyceride levels were significantly higher ($p<0.05$) than the average for the low fat meal. The null hypothesis that headspace isobutanol and chloroform concentrations will not have a negative correlation with triglycerides, and methanol headspace concentrations will not have a positive correlation with triglycerides at the $\alpha=0.05$ level of significance was accepted. However, only one part of this hypothesis caused the acceptance. Chloroform headspace concentrations did decreases as tyrglyceride levels increased and methanol headspace concentrations did not decrease as triglyceride levels increased. Both of these findings were expected and would have caused a rejection of the null hypothesis, but isobutanol headspace concentrations did not decrease as tyrglyceride levels increased. This was not expected. All other null hypotheses were rejected. The results showed that there was a significant decrease ($p<0.05$) in headspace chloroform concentrations from the high fat meal when compared to the low fat meal. There was also a significant difference ($p<0.05$) in the solvent headspace concentrations for chloroform when compared to its control. Finally, the solvent with the highest lipophilicity (chloroform) was more highly correlated ($r = -0.30$) with triglyceride levels relative to isobutanol and methanol ($r = 0.23$, $r=0.23$).

The findings to date suggest that high fat content meals contributed to increased levels of blood triglycerides between 4 and 5 hours after the meal was consumed. Increases in these blood triglyceride levels from consuming a high fat content meal may increase the blood concentration of lipophilic, but not hydrophilic organic solvents. Data

from this project suggests that diet may contribute to uptake of a lipophilic solvent and in this way may influence solvent-related health effects.

Future Direction

Future work is necessary to determine what degree of lipophilicity starts to have an impact on solvent uptake in a given matrix of blood with a known level of triglycerides. To the same extent, more information is needed to model what levels of triglycerides start to influence solvent uptake in blood for a given solvent with known lipophilicity. This particular experiment only uses one solvent with a high degree of lipophilicity (chloroform). Current literature suggests that the toxicity of a solvent is directly related to its lipophilic properties (Arlien-Soborg, 1992). Based on this information, it would be beneficial to look at compounds that have a higher degree of lipophilicity. The lowest lipophilicity in the range should be above that of isobutanol, which in this project showed that is not affected by the amount of triglycerides that are present in the blood. Therefore, solvents chosen should have a range that is higher than chloroform but also higher than that of isobutanol as well.

More data needs to be collected to model the lipid levels versus the lipophilicities of certain solvents. This could be done by using methods similar to the ones used for this project. Gathering this data could be much simpler because in this study, the meals could be eliminated. Based on the triglyceride data from this study, we see that the range of triglyceride levels have large variability and a large range within a study group. Therefore, simply gathering blood to use as a matrix for headspace gas chromatography should be sufficient. This method should provide a large enough range of triglyceride levels to develop a simple regression line similar to the one in this study, showing the relationship between triglyceride level and uptake of a given solvent. In this study, many different solvents could be used to represent the range of lipophilicities that are used in

industry. From the information gathered, one could feasibly develop a model showing the relationships between the correlation coefficients for each solvent and their respective lipophilicities. A study of this capacity would also eliminate the possibility of confounding due to long and inconsistent incubation periods between the time that the blood is collected and the time of the analysis.

Reference key

Agency for Toxic Substances and Disease Registry. Atlanta, GA Centers for Disease Control and Prevention. Retreived June 27, 2003 from the World Wide Web: http://www.atsdr.cdc.gov/toxprofiles/tp6-c4.pdf

Agency for Toxic Substances and Disease Registry. Atlanta, GA Centers for Disease Control and Prevention. Retreived June 27, 2003 from the World Wide Web: http://www.atsdr.cdc.gov/toxprofiles/tp91-c4.pdf

American Conference of Governmental Industrial Hygienists. (2003). 2003 TLVS and BEIs. Cincinnati, OH: ACGIH.

Angerer J. Occupational Chronic Exposures to Organic Solvents, Metabolism of toluene in Man *Int. Arch. Occup. Environ. Health* **43,** 63-67 (1979)

Angerer J. Occupational Chronic Exposures to Organic Solvents, Phenolic Compounds *Int. Arch. Occup. Environ. Health* **43,** 145-150 (1979)

Angerer, J., Heinrich, R. *Int. Arch. of Occup.l and Env. Health* Vol. 50, No. 4 (1982) 341-9

Angerer J. Biological Monitoring of Workers Exposed to Organic Solvents – Past and Present *Scand. J. Work Env. Health* **11**, 45-52 (1985)

Arlien-Soborg, P. (2000) Solvent Neurotoxicity. Boca Raton, FL: CRC Press

Astrand, I., Uptake of Solvents in the Blood and Tissues of Man, a review. *Scand. J. Work Env. Health* Vol. 1, (1975) 199-218

Astrand, I., Gamberale F. Effects on Humans of Solvents in the Workplace, Method for Estimation of Uptake *Env. Research* **15**, 1-4 (1978)

Astrand, I., Uptake of Solvents from the Lungs. *British J. of Ind. Med.* Vol.42 (1985) 217-218

Baker, E., Smith, T., Landrigan, P. The Neurotoxicity of Industrial Solvents: A review of the literature. *Am. J. of Ind. Med.* Vol. 8 (1985) 207-217

Baker E. *et al.* Neurobehavioral Effects of Solvents in Constructiona Painters *J. of Occ. Med.* **30,** (2) (1988)

Baker E., Smith T., Landrigan P. The Neurotoxicity of Industrial Solvents: A Review of the Literature *Am. J. of Ind. Med.* **8**, 207-217 (1985)

Baker E., Fine L. Solvent Neurotoxicity: The Current Evidence *J. of Occ. Med.*, (2) (1986)

Baselt, R.C. Biological Monitoring Methods for Industrial Chemicals, Second Edition, PSG Publishing Co., Littleton, MA (1998)

Baugh P.J. (1993) Gas Chromatography. "A practical Approach" New York, NY: Oxford University Press

Beirne, G., Brennan, J. Glomerulonephritis Associated with hydrocarbon solvents. *Arch. of Env. Health* Vol. 25 (1972) 365-369

Bergert, K.D., Nestler, K. Solvent Uptake in Relation to Physical Activity. *Sci. Tot. Env.* Vol. 101 (1991) 111-119

Bird M.G. Industrial Solvents: Some Factors Affecting Their Passage Into and Through the Skin *Am. Occ.. Hyg.* **24,** (2) 235-244 (1981)

Blair A., Petralia S. Stewart P. Extended Mortality Follow-up of a Cohort of Dry Cleaners *Ann. of Epi.* **13,** 50-56 (2003)

Bowes and Churches, Food Values of Proportions Commonly Used "7th Edition", 1998

Browning, E. (1938) Toxicology of Industrial Organic Solvents. New York, NY: Chemical Publishing Co. of N.Y. Inc.

Browning, E. (1965) Toxicology and Metabolism of Industrial Solvents. Amsterdam, The Netherlands: Elsevier Publishing Company

Chang, L.W., Dyer, R.S. (1995) Handbook of Neurotoxicology. New York, NY: Marcel Dekker, Inc.

Cherry, N. "Neurological Effects of Solvents: The role of alcohol." *Env. Research* vol.62 (1993): 155-8

Cohen, Dr. Beverly S., McCammon, Charles S. Jr. Air Sampling Instruments ACGIH. 2001

Collings, A.J., Gluxon, S. (1982) Safe Use of Solvents. Oxford, UK: Academic Press

Cornish, H., Adefum , J., Ethanol Potentiation of Halogenated Alaphatic Solvent Toxicity. *Am. Ind. Hyg. Assoc. J.* Vol. 27 (1966) 57-61

Elinder, C., Vesterberg, O. Reports from Workshops "Environmental and Biological Monitoring *Scand. J. Env. Health* **11,** 91-103 (1995)

Elofsson S. Exposure to Organic Solvents *Scan. J. of Work. Env. Health* **6,** 239-273 (1980)

Engstrom, J., Bjurstrom, R. Exposure to Methylene Chloride. Content in Subcutaneous Adipose Tissue. *Scan. J. of Work. Env. Health* Vol. 3 (1977) 215-224

Farmer, T. "Solvent-Health, Safety and Evironmental Aspects.: *Chemistry and Industry* **8,** 247-250 (1989)

Franks N.P., Leib W.R. Molecular Mechanisms of General Anesthesia *Nature (London)* **300,** 487-493 (1982)

Franks N., Leib W.R. Mechanisms of General Anesthesia *Env. Health Perspectives* **87,** 199-205 (1990)

Gargas M., *et al.* Partitiion Coefficients of Low Molecular Weight Volatile Chemicals in Various Liquids and Tissues *Tox. and Applied Pharm.* ***98,*** 87-99 (1989)

Gerarde, H.W. (1960) Toxicology and Biochemistry of Aramatic Hydrocarbons. New York: Elsevier North-Holland

Goldstein A., Aronow L., Kalman, S. Principals of Drug Action John Wiley and Sons, New York, NY (1974)

Guillemin M. Air Contamination Exposure Measurements of Organic Solvents – Past and Present *Scand. J. Work Env. Health* **11,** 33-43 (1985)

Hageman, G., Van Der Hoek, J., Van Der Laan, G. et al. Parkinsonism, Pyramidal Signs, Polyneuropathy, and Cognitive Decline after Long-term Occupational Solvent Exposure. *J. of Neurology* Vol. 246, No. 3 (1999) 198-206

Hansen, J. Breast Cancer Risk Among Relatively Young Women Employed in Solvent-Using Industries *Am. J. of Ind. Med.* **36,** 43-47 (1999)

Hardell, L., Eriksson, M.,Lenner, P., et al. Malignant Lymphoma and Exposure to Chemicals, Especially Organic Solvents, Chlorophenols and Phenoxy Acids. A case control study. *British J. of Cancer* Vol. 43 (1981) 169-176

Harving, H., Dahl, R., Molhave, L., Lung Function and Bronchial Reactivity in Asthmatics During Exposure to Volatile Organic Compounds. *Am. Rev. of Respiratory Dis.* Vol. 143 (1991) 751-754

Heinrich R., Angerer J., Occupational Chronic Exposure to Organic Solvents *Int. Arch. of Occ. Env. Health* **50,** 341:349 (1982)

Herd P., Martin F. Effect of 1,1,1-trichloroethane on Mitochondrial Metabolism *Biochemistry Pharmacology* **24** (1975)

Hernberg, S., Nurminen, M.,Tolonen, M. Excess Mortality from Coronary Heart Disease in Viscose Rayon Workers Exposed to Carbon Disulfide. *Workers Env. Health* Vol. 10 (1973) 93-99

International Agency for Research on Cancer: Some industrial chemicals and dye-stuff, *IARC Mongr. Eval. Carcinog. Risks Hum* **29**:1-146 (1987)

Ioffe B.V., Vitenberg A.G. (1984) Head-Space Analysis and Related Methods in Gas Chromatography. John Wiley & Sons

Jennings W. (1987) Analytical Gas Chromatography. Orlando, FL: Academic Press, Inc.

Johanson, G. Reports from Workshops "Inhalation – Experimental techniques and toxicokinetics" *Scand. J. of Work. Env. Health* **11**, 91-103 (1995)

Johnson, B., Setzer, J., Lewis, I., et al. Effects of methyl-butyl ketone on behavior and the nervous system. *Am. Ind. Hyg. Assoc. J.* Vol. 38 (1977) 567-579

Kleinbaum, D., Kupper, L., Muller, K., Nizam, A. (1998) Applied Regression and Other Multivariable Methods. "Third Edition" Pacific Grove, CA: Duxbery Press

Kolb B. (1978) Applied Headspace Gas Chromatography. London, England: Heyden & son, Ltd.

Kolb, B., Ettre L. (1997) Static Headspace Chromatography. "Theory and Practice" Wiley-VCH

Kumagai S., Matsunaga I. Within-Shift Variability of Short-Term Exposure to Organic Solvent in Indoor Workplaces *Am. Ind. Hyg. Assoc. J.* (60), 16-21 (1999)

Kylin B., Axell K., Samuel H., Lindborg A. Effect of Inhaled Trichloroethylene on the CNS *Archives of Env. Health* **15,** 1967

Laboratory Corporation of America (2001). Directory of Services, an Interpretive Guide. Hudson, OH: Lexi Comp. Inc. p. 372

Labreche F., Goldberg M. Exposure to Organic Solvents and Breast Cancer in Women: A Hypothesis *Ind. Med.* **32,** 1-14 (1997)

Lam C.,Galen T., Boyd J., Pierson D. Mechanism of Transport and Distribution of Organic Solvents in Blood *Tox. and Applied Pharm.* **104,** 117-129 (1990)

Lam C., DiStefano V., Morken D. The Role of Red Blood Cells in the Transport of Carbon Disulfide *J. of Applied Tox.* **6**(2), 81-86 (1986)

Largrue, G., Hydrocarbon Exposure to Chronic Glomerulonephritis. *Lancet* Vol. 1 (1976)

Leaf G., Zatman L.J., A Study of the Conditions Under Which Methanol May Exert a Toxic Hazard in Industry *Brit. J. Ind. Med.* ***9***, 19-31 (1952)

Leib D., (2002) Handbook of Chemistry and Physics "83rd Edition" Boca Raton, FL: CRC Press

Lundqvist G., Flodin U., Axelson O. A Case-Control Study of Fatty Liver Disease and Organic Solvent Exposure *Am. J. of Indust. Med.* **35**, 132-136 (1999)

Lynge E., Antilla A., Hemminki K., Organic Solvents and Cancer. *Cancer Causes Control* **8,** 406-419 (1997)

Mangold S., Laubli T., Krueger H. Effects of a Low Alcohol Dose on Static Balance, Fine Motor Activity, and Mental Performance *Neurotox. and Terat.* **18,** (5) 547-554 (1996)

McMartin K., Chu M., Kopecky E., Einarson T., Koren G. Pregnancy Outcome Following Maternal Organic Solvent Exposure: A Meta-Analysis of Epidemiologic Studies *Am. J. of Ind. Med.* **34,** 288-292 (1988)

McNair H.M., Bonelli, E.J. (1969) Basic Gas Chromatography. Boca Raton, FL:Varian

NIOSH ALCOHOLS II-1401. Atlanta, GA: National Institute for Occupational Safety and Health. Retrieved June 30, 2003, from the World Wide Web: http://www.cdc.gov/niosh/nmam/pdfs/1401.pdf

NIOSH HALOGENATED HYDROCARBONS-1003. Atlanta, GA: National Institute for Occupational Safety and Health. Retrieved June 30, 2003, from the World Wide Web: http://www.cdc.gov/niosh/nmam/pdfs/1003.pdf

NIOSH Manual of Analytical Methods (NMAM) 4th Ed. DHHS (NIOSH) Publication 94-113 Aug. (1994) Cassinelli, ME
Retrieved June 30, 2003, from the World Wide Web:
http://www.cdc.gov/niosh/nmam/pdfs/2000.pdf

NIOSH Recommendations for Occupational Safety and Health Compendium of Policy Documents and Statements. Atlanta, GA: National Institute for Occupational Safety and Health. Retrieved April 7, 2003, from the World Wide Web:
http://www.cdc.gov/niosh/92-100.html

NIOSH VOLATILE ORGANIC CPDS-2549. Atlanta, GA: National Institute for Occupational Safety and Health. Retrieved June 30, 2003, from the World Wide Web: http://www.cdc.gov/niosh/nmam/pdfs/2549.pdf

NOES National Occupational Exposure Survey. Atlanta, GA: National Institute for Occupational Safety and Health. Retrieved July 2, 2003, from the World Wide Web: http://www.cdc.gov/noes/noes1/agtindex.html

Olsen E., Seedorff L., Exposure to Organic Solvents. II. An Exposure Epidemiology Study *Ann. of Occ. Hyg.* **34,** 379-389 (1990)

Olsson H., Brandt, L. Occupational Exposure to Organic Solventsand Hodgkin's Disease in Men. A case-referent study. *Scand. J. of Work. Env. Health* Vol. 6 (1980) 302-305

Opdam J., Smolders J. Alveolar Sampling and Fast Kinetics of Tetrachloroethene in Man. *British J. of Ind. Med.* **43,** 814-824 (1986)

Organic Solvent Neurotoxicity. Atlanta, GA: National Institute for Occupational Safety and Health. Retrieved July 2, 2003, from the World Wide Web: http://www.ncchem.com/niosh.htm

OSHA Regulations (Standards-29 CFR) Part 1910 Occupational Safety and Health Standards. Washington, D.C.: Occupational Safety and Health Administration. Retrieved April 7, 2003, from the World Wide Web: http://www.osha-slc.gov/OshStd_toc/OSHA_Std_toc_1910.html

OSHA Regulations Table Z-1 Limits for Air Contaminants. Washington, D.C.: Occupational Safety and Health Administration. Retrieved April 7, 2001, from the World Wide Web: http://www.osha-slc.gov/OshStd_data/1910_1000_TABLE_Z-1.html

Pang Y.C., Reid P.E., Brooks D.E. Solubility and Distribution of halothane in human blood. *Br. J. of Anaesthesia.* **52,** 851-861 (1980)

Patty, Frank A. Fassett, David W. Irish, Don D. (1966) Industrial Hygiene and Toxicology John Wiley and Sons, Inc. Chapter XXVII

Perkins, J. (1997) Modern Industrial Hygiene. "Recognition and Evaluation of Chemical Hazards" New York, NY: Van Nostrand Reinhold

Plog, B, Niland, J., & Quinlan, P. (1996). Fundamentals of Industrial Hygiene. Itasca, Illinois: National Safety Council.

Ravnskov, U., Forsberg, B., Skerfving, S. Glomerulonephritis and Exposure to Organic Solvents. *Acta Med. Scand.* Vol. 205 (1979) 575-579

Renis M., Giovine A., Bertoline A. Protein Synthesis in Mitochondrial and Microsomal Fractions From Rat Brain and Liver After Acute Ethanol Administration *Life Sciences* **16,** (1975)

Rose V.E. Passive Dosimetry – State of the Art Review *Am. Ind. Hyg. Assoc. J.* **43,** 602-621 (1982)

Savolainen H. Some Aspects of the Mechanisms by which Industrial Solvents Produce Neurotoxic Effects *Chem-Biol. Interactions* **18,** 1-10 (1977)

Sedivec V., Mraz M., Flek J. Biological Monitoring of Persons Exposed to Methanol Vapours *Int. Arch. Occup. Env. Health* **48,** 257-271 (1981)

Seedorff L., Olsen E. Exposure to Organic Solvents. I. A Survey On the Use of Solvents. *Ann. of Occ. Hyg.* **34,** 371-378, (1990)

Scott, R.M. (1995) Introduction to Industrial Hygiene. Boca Raton, FL: CRC Press, Inc.

Spencer, P., Schaumburg, H. (1980) Experimental and Clinical Neurotoxicology. Baltimore, MD: William and Wilkins

Spencer P., Schaumburg H. Organic Solvent Neurotoxicity *Scand. J. Work Env. Health* **11,** 53-60 (1985)

Shrivastav B.B., Narahashi T., Kitz R.J., Roberts J.D. Mode of Action of Trichloroethylene on Squid Axon Membranes *J. of Exp. Ther.* (1976)

Steinkopff V., Parkinsonism, Pyramidal Signs, Polyneuropathy, and Cognitive Decline After Long-term Occupational Exposure *J. Neorology* **246,** 198-206 (1999)

Tarkowski S., Sobczak H. Oxidation and Phosphorylation Processes in Brain Mitochondria of Rats Exposed to Carbon Disulfide *J. Neurochemistry* **18,** 177 (1971)

Taskinen H.K., *et al.* Reduced Fertility Among Female Wood Workers Exposed to Formaldehyde Studies *Am. J. of Ind. Med.* **36,** 206-212 (1999)

Technical Information About Solvents. Washington, DC. United States Environmental Protection Agency. Retreived June 27, 2003 from the World Wide Web: http://es.epa.gov/techinfo/facts/ceramic.html

Tiller, J., Schilling, R., Morris, J. Occupational Toxic Factor from Coronary Heart Disease. *British Med. J.* Vol. 6 (1968) 407-411

Toftgard, R., Gustafson, J. Biotransformation of Organic Solvents, A review. *Scand. J. of Work. Env. Health* Vol. 6 (1980) 1-18

Traiger, G., Plaa, G., Chlorinated Hydrocarbon Toxicity. Potentiation by Isopropal Alcohol and Acetone. *Arch. of Env. Health* Vol. 28 (1974) 276-8

Ulfvarson, R., Riihimaki, V. (1985) Safety and Health Aspects of Organic Solvents. "Progress in Clinical and Biological Research Vol. 220" New York, NY: Alan R. Liss, Inc.

USEPA United States Environmental Protection Agency. Washington, D.C. Retrieved April 21, 2003, from the World Wide Web: http://www.epa.gov

Vainio H., Waters M., Norppa H. Mutagenicity of Selected Organic Solvents *Scand. J. Work Env. Health* **11,** 75-82 (1985)

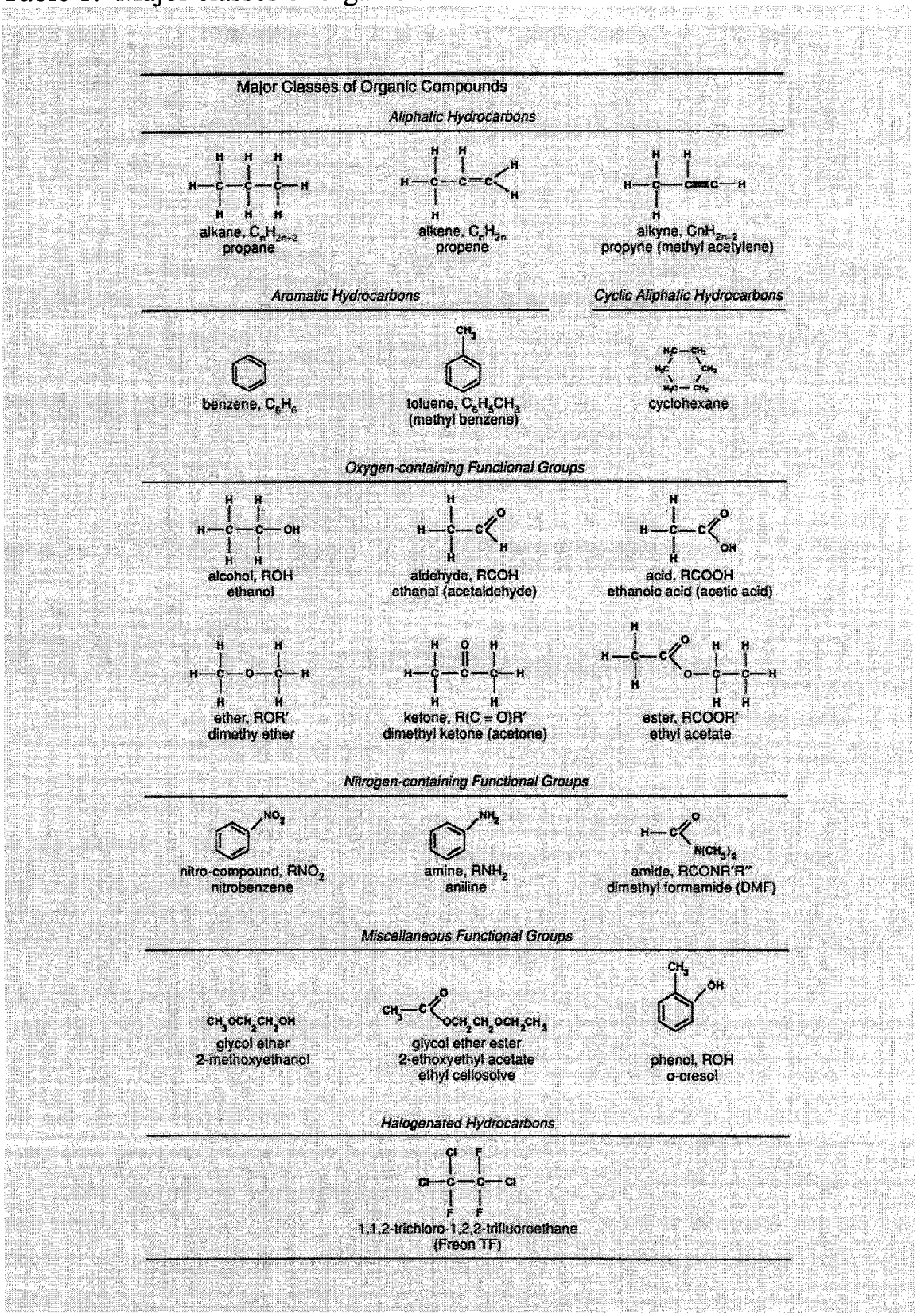
Winslow, C. (1926) Fresh Air and Ventilation.
New York : Dutton

Wypych, G. (2001) Handbook of Solvents. Toronto, ON: William Andrew Publishing

Zenz, C. (1994) Occupational Medicine. "Third Edition" St. Louis, MO: Mosby

APPENDIX A

Table 1: Major classes of organic solvents



Obtained from Plog, et al. (1996)

Table 2: Organic solvents used in industry

Industry	Typical Organic Solvents Used
Adhesives and Sealants	methyl ethyl ketone (MEK), acetone, mineral spirits, toluene, xylene
Asphalts	mineral spirits, fuel oil, xylene, toluene
Cosmetics and Cleaners	acetone, methyl acetate, ethyl acetate, butyl acetate, methyl glycol acetate, MEK, methyl isobutyl ketone (MIK), toluene, xylene, isopropyl alcohol, methyl chloroform, naptha
Dry Cleaning	perchloroethylene
Electronics	isopropanol, alkylpropanols, siloxanes, chlorinated solvents, hydrocarbons terpenes, other alcohols, and ethers
Metal Fabrication	1,1,1-trichloroethane, acetone, toluene, xylene, kerosene, glycols, benzene, MEK, MIK, tetrachloride, dichloromethane, perchloromethane
Iron and Steel	MEK, toluene, xylene, trichloroethylene
Medical Field	Almost all solvents listed to date can be included in this group
Metal Castings	methanol, benzene, toluene, cresol, naptha, some chlorinated solvents
Mortor Vehicle Assembly	acetone, xylene, toluene, 1,1,1-trichloroethylene, butanol, isobutanol, methanol, heptane, mineral spirits, butyl acetate, ethyl acetate, hexyl acetate, MEK, methyl amyl ketone, dimethlylbenzene, 4-methyl-2-pentanone, butyl acetate, naptha, ethyl benzene, 2-butanol, toluene, and 1-butanol.
Paintings and Coatings	Most every type of solvent group is represented here depending on the product
Pharmaceutical Industry	Most every type of solvent group is represented here depending on the product
Printing Industry	isopropyl alcohol, n-butyl alcohol, dichloromethane, 1,4-dioxane, ethylene glycol, ethylbenzene, hexane, isopropyl alcohol, methanol, MEK

*Table constructed with information from George Wypych's Handbook of Solvents, 2001

Table 3: Exposure limits

Contaminant	PEL	TLV	REL	BEI
methanol	200ppm	200ppm	200ppm	15mg/L urine
chloroform	C 50ppm	10ppm	ST 2ppm	X
isobutanol	100ppm	50ppm	50ppm	X

C - represents maximum or ceiling limit
ST - represents 60 minute short term exposure limit
X - BEI does not exist for that compound

Table 4: ACGIH Carcinogenicity Notations

Notation	Category
A1	Confirmed Human Carcinogen
A2	Suspected Human Carcinogen
A3	Confirmed Animal Carcinogen with Unknown Relevance to Humans
A4	Not Classifiable as a Human Carcinogen
A5	Not Suspected as a Human Carcinogen

Table 5: High fat meal constituents

High Fat Content Meal

	calories	fat(g)	sat. fat(g)
2 slices bread (toast)	140	2	0
1 T. butter	100	11	7
3/4 cup Kracklin Oat Bran	200	7	2
6 oz. orange juice	82.5	0	0
12 oz. vitamin D milk	225	12	7.5
Totals	747.5	32	16.5

Table 6: Low fat meal constituents

Low Fat Content Meal

	calories	fat(g)	sat. fat(g)
2 slices bread (toast)	140	2	0
2 T. jam	100	0	0
1 1/2 cup Shredded Wheat	255	1.5	0
6 oz. orange juice	82.5	0	0
12 oz. skim milk	120	0	0
Totals	697.5	3.5	0

Table 7: Calibration table

solution number	volume of acetone	volume of sol. # 1	volume of stock sol.	volume of methanol	volume of isobutanol	volume of chloroform	(ng/uL) Methanol	(ng/uL) Isobutanol	(ng/uL) Chloroform
7	25mL	X	X	30uL	30uL	30uL	948	960	947.2
6	25mL	X	X	15uL	15uL	8uL	474	480	473.6
1	50mL	X	X	15uL	15uL	8uL	237	240	236.8
2	100mL	X	X	15uL	15uL	8uL	118.5	120	118.4
3	25mL	X	100uL	X	X	X	63.2	64	59.2
4	25mL	X	50uL	X	X	X	31.6	32	29.6
5	25mL	500uL	X	X	X	X	4.8	4.74	4.74
stock sol.	50mL	X	X	1mL	1mL	1mL	15,800	16,000	14,800

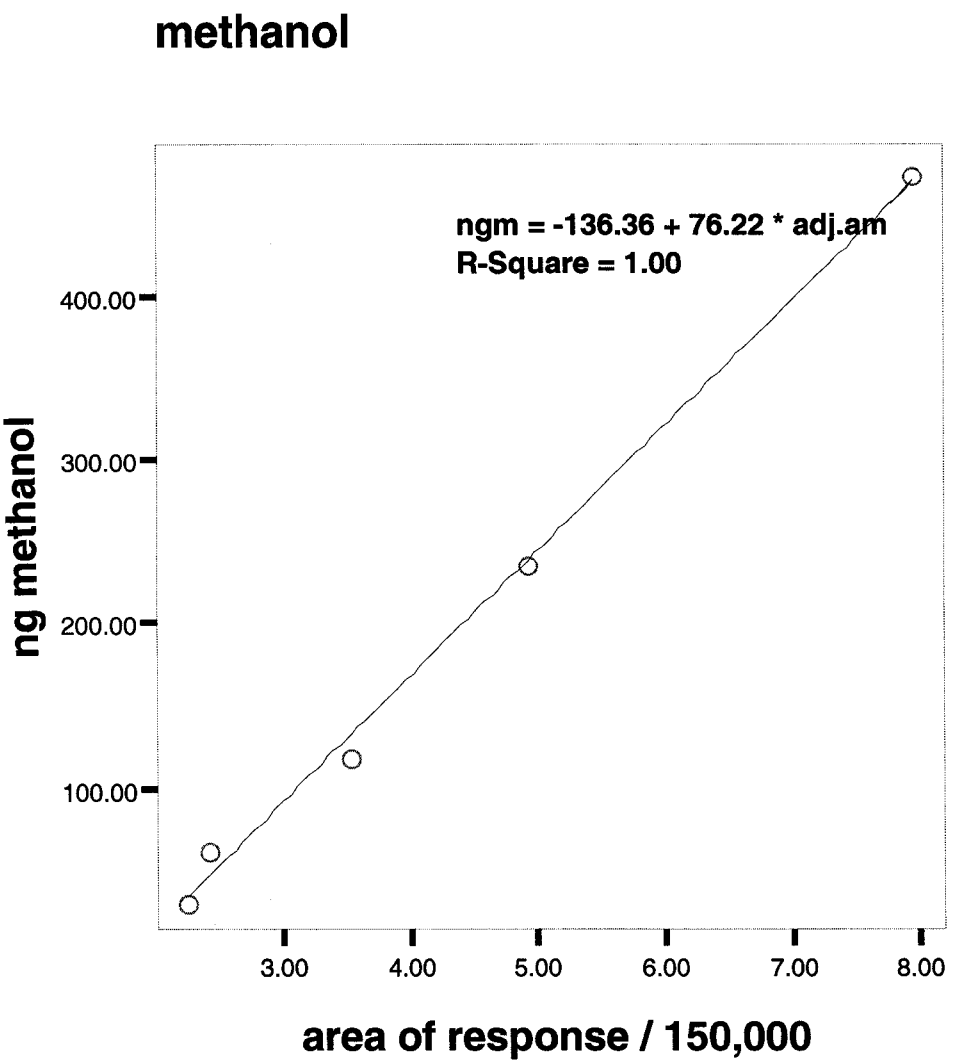


Figure 1: Methanol calibration curve

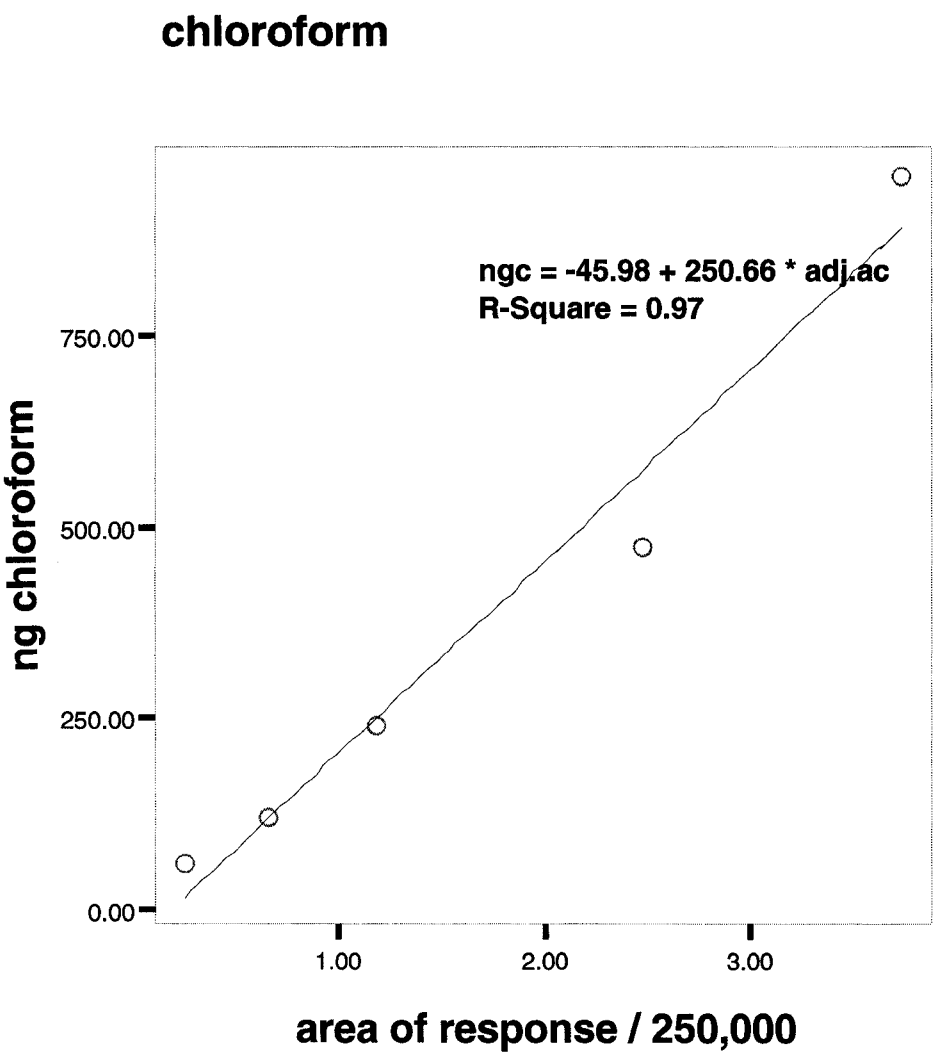


Figure 2: Chloroform calibration curve

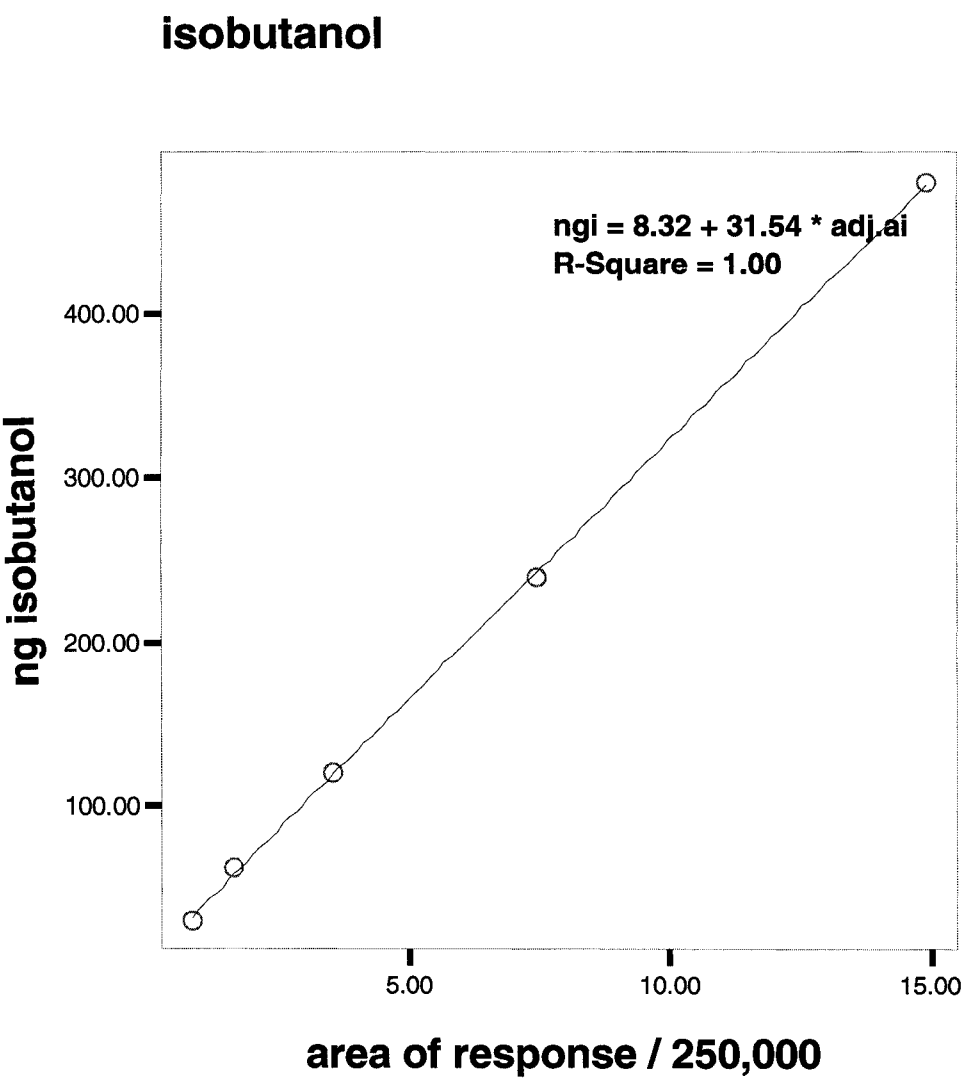
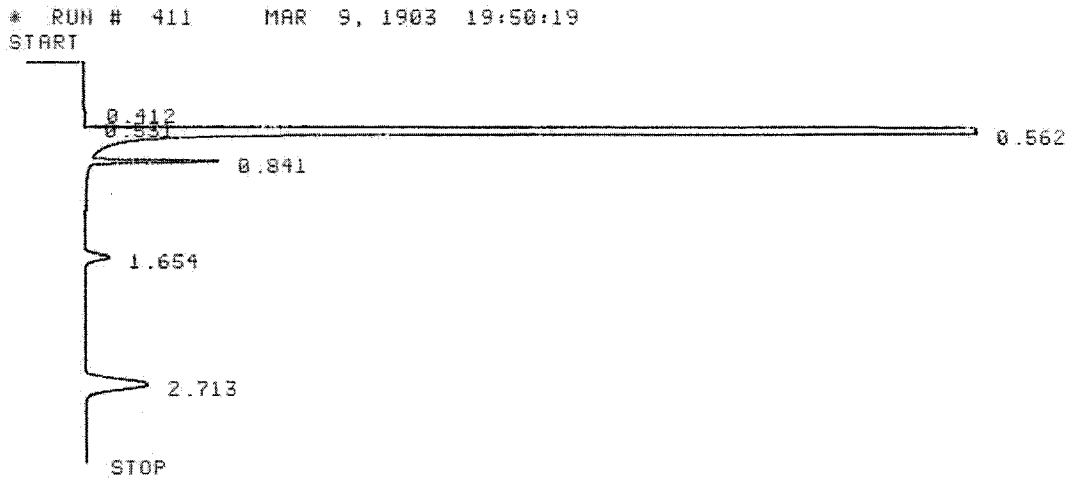


Figure 3: Isobutanol calibration curve

APPENDIX B



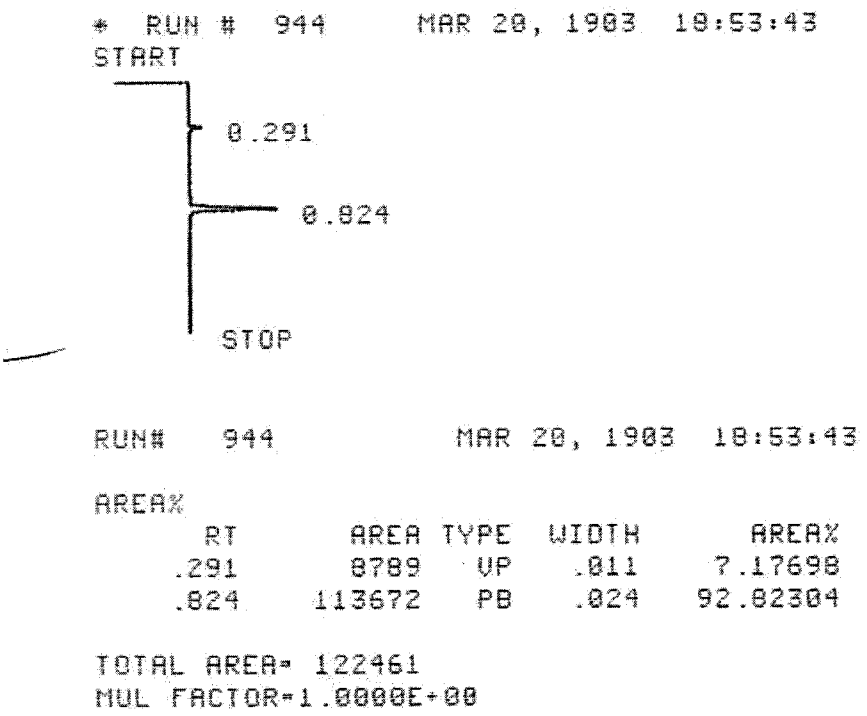
RUN# 411 MAR 9, 1903 19:50:19

AREA%

RT	AREA	TYPE	WIDTH	AREA%
.412	11808	VV	.013	.02772
.531	5891	PH	.018	.01383
.562	36382240	>SHB	.070	85.40080
.841	1733496	TBB	.025	4.06907
1.654	880730	PV	.064	2.06736
2.713	3587606	VV	.103	8.42127

TOTAL AREA=4.2602E+07
MUL FACTOR=1.0000E+00

Figure 10: Chromatograph of calibration standard



RUN# 944 MAR 20, 1903 10:53:43

AREA%

RT	AREA	TYPE	WIDTH	AREA%
.291	8789	VP	.011	7.17698
.824	113672	PB	.024	92.82304

TOTAL AREA= 122461
MUL FACTOR=1.0000E+00

Figure 11: Chromatograph of methanol headspace sample

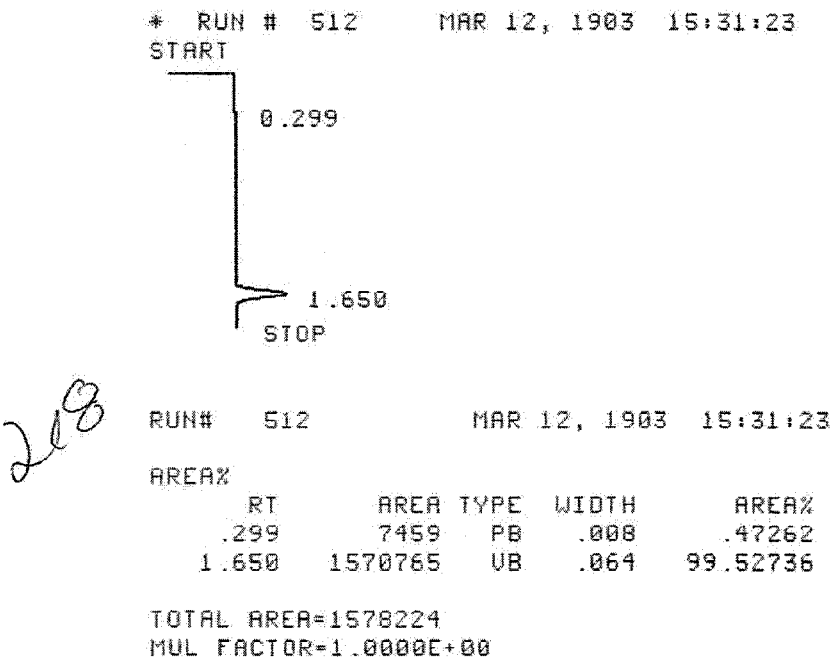
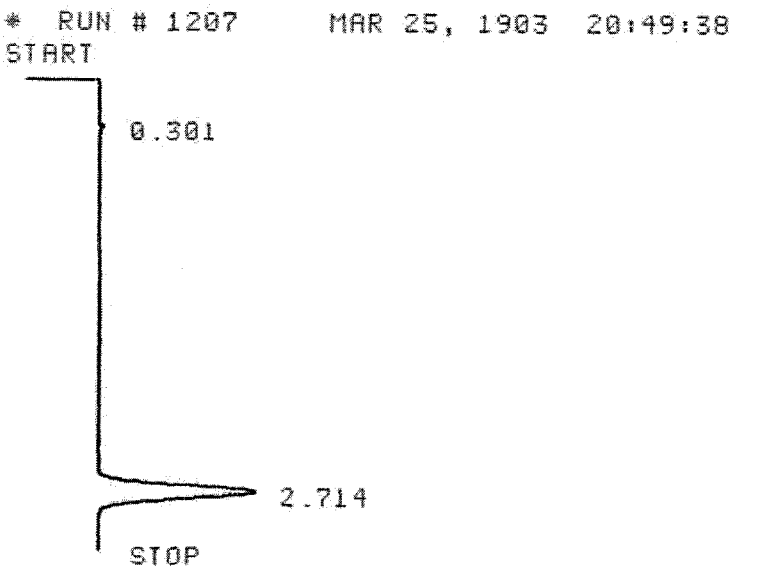


Figure 12: Chromatograph of chloroform headspace sample



RUN# 1207 MAR 25, 1903 20:49:38

AREA%

RT	AREA	TYPE	WIDTH	AREA%
.301	2781	PB	.009	.31684
2.714	874938	VP	.103	99.68317

TOTAL AREA= 877719
MUL FACTOR=1.0000E+00

Figure 13: Chromatograph of isobutanol headspace sample