INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

UMI®

BENZENE AND TOLUENE MIXING RATIOS IN INDOOR AIR OF HOMES WITH ATTACHED GARAGES AND MEASUREMENT OF RESPECTIVE BIOMARKERS OF EXPOSURE AND VENTILATION EFFECTS

A

THESIS

Presented to the Faculty

of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

By

Maggie A. Isbell, M.S.

Fairbanks, Alaska

December 2000

BENZENE AND TOLUENE MIXING RATIOS IN INDOOR AIR OF HOMES WITH ATTACHED GARAGES: MEASUREMENT OF RESPECTIVE BIOMARKERS OF EXPOSURE AND EFFECTS OF VENTILATION

By

Maggie A. Isbell

RECOMMENDED:

Advisory Committee Chair

Department Head

APPROVED:

Dean, College of Science, Engineering, and Mathematics

Dean of the Graduate School

12-1-00

Date

Abstract

Benzene and toluene mixing ratios were measured in the indoor air of homes with attached garages for several seasons using a thermal desorption GC-FID sampling and analysis protocol (EPA T0-17). Benzene in the living area of these homes ranged from 1-72 ppbv and toluene ranged 3 -111 ppbv. The garage levels of benzene ranged from 8-304 pbbv and the toluene levels ranged from 14-591ppbv. Numerous experiments and a model support the hypothesis of a single source of toluene and benzene.

Source strength estimate calculations supported the hypothesis that gasoline in the attached garage is the primary source of these compounds in living area air. They also showed that the home with the air-to-air heat exchangers and forced ventilation had less transport of aromatics than an unventilated home.

Perturbation experiments showed that a metal gas can filled with gasoline in the garage and an indoor window open were important factors for benzene and toluene levels in the living areas of the homes. For most experiments,weighted regression analyses of toluene and benzene mixing ratios were consistent with a sole source.

Finally, no correlation was observed between the levels of benzene and toluene measured in living areas and their respective urinary biomarkers: t,t-MA and hippuric acid.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

List of Appendices

ACKNOWLEDGEMENTS

I would like to start by expressing my gratitude to my committee members: Drs Larry Duffy, Tom Clausen, Bill Simpson, Richard Stolzberg and Robert Perkins, for all of their encouragement, mentorship and patience. I am especially grateful to Dr. Larry Duffy for giving me the opportunity to present this work at conferences and do a volume of writing long before this thesis.

I would like to especially thank Dr. Rich Benner and Dr. Russell Dietz for believing in me and permitting me to follow through on my ideas through the use of their laboratory instrumentation and tracer technology.

I would like to thank Sheila Chapin and Marlys Schneider for all of their support in and out of the realm of Chemistry. I would also like to thank my dear friend Ann Lefavor, who taught me to reduce the use of the words " I can't " in my vocabulary. I am grateful to the Duffy, Benner, Schneider and Covell families who tolerated my presence in their homes making this work possible.

Most of all, I'd like to thank my dear husband, Izzy, whose support and encouragement brought me here to attempt to achieve a lifelong dream and who bolstered me though the harder times while on that journey.

Chapter 1. INTRODUCTION

'All substances are poisons; there is none that is not a poison. The right dose differentiates a poison and a remedy'

Paracelsus (1493-1541).

We are exposed to benzene and toluene in our environment from many sources. Long-term low-level exposure to these compounds in workplace environments are defined as time weighted averages (TWA) over either a eight- hour work day or 40 hour work week. The Occupational Safety and Health Administration (OSHA) has established exposure limits of 1 ppmv benzene per eight-hour workday and 100 ppmv toluene per work day (1987a and OSHA 1989). There is no organization that sets threshhold regulatory limits for continuous low-level exposure to these compounds in residences. However, the potential for residential exposures raise some important questions about homes with attached garages since the garage is likely to contain benzene and toluene sources.

- **What is the level of benzene and toluene sources in a typical garage?**
- **What level of benzene and toluene are residents exposed to in their living area from these garage sources?**
- **How do factors such as home ventilation, temperature, small engine and other fuel storage affect the level of these compounds in indoor air?**
- **Is it possible to establish a dose-response relationship for residents by measuring the indoor air levels of these compounds and their respective urinary biomarker?**

1.1 Benzene

Benzene occurs in our environment from a variety of sources. Natural sources of benzene in the environment include crude oil seepage, plant volatiles, and forest fires (Brief et al., 1980). Benzene has been reported to occur in several foods such as fruits, dairy products and eggs (Grob et al., 1990). The anthropogenic sources of benzene include emissions from chemical plants, rubber producing plants, fuel emissions and tobacco smoke (IARC, 1982; Wallace, 1996).

Outdoor background levels of benzene in rural air are reported to range between 0.1 – 1.8 ppbv (IARC, 1982; Wallace, 1990). In interior Alaska, indoor air levels of benzene in residences with attached garages has been observed in the range of 0.2 – 72 ppbv (Isbell et al., 1999). A major source of benzene is gasoline from cars and small engines. The majority of gasoline in the United States are regulated to contain $\leq$1.0%(v/v) benzene, but European and Alaskan gasoline can contain as much as 3-4% benzene (v/v) (IARC, 1982; Williams MSDS, 1997; Sawyer, 1993). Benzene is reported to comprise approximately 4% of the hydrocarbon emission of automotive exhaust (US EPA, 1980). Another source is cigarette smoking which may be responsible for 50% of indoor exposures to benzene (Wallace, 1996).

Benzene may enter the body through several routes: inhalation, ingestion, and dermal exposure. The body dose is usually the highest through inhalatiou.

Inhalation exposure occurs through inhalation of ambient air containing benzene. Ingestion exposure can occur through eating or drinking substances contaminated with benzene or naturally containing benzene. Dermal absorption occurs through the skin and usually involves direct contact with a benzene containing liquid such as gasoline.

Approximately 50% of the inhaled concentration of benzene is exhaled depending on rates of metabolism and respiration of the individual (Pekari et al., 1992; Goldstein and Witz, 1992). The fraction of inhaled benzene that is absorbed reaches the blood stream through the alveoli in the lungs. Once in the blood stream, the benzene is distributed throughout the body. A significant portion of the absorbed benzene is stored in the fat and bone marrow. The metabolism of benzene to its metabolites is essential for subsequent toxic effects (Cooper et al., 1988; Snyder et al., 1993). The exact mechanism by which benzene exerts its toxicity is uncertain, but most hypotheses include the necessity of liver metabolism and bone marrow metabolism for a toxic suspect metabolite(s) to be formed (Goldstein and Witz, 1992).

Many metabolic pathways have evolved to eliminate toxic xenobiotic chemicals. Several pathways involve converting lipophilic xenobiotics to more hydrophilic compounds that can be excreted from the body. Often, there are several steps or phases in this elimination. The first step, called Phase I reaction, usually oxidizes

into the xenobiotic chemical creating a more hydrophilic product. The primary enzyme system that accomplishes this oxidation is the cytochrome P-450 system (Goldstein and Faletto, 1993). Phase I metabolism of benzene by cytochrome CYP-450 2E1 occurs primarily in the liver although bone marrow has also been found to contain low levels of this enzyme and these isoenzymes (Ross, 1996). In Phase II metabolism a larger water soluble molecule is attached to the oxidized site of a Phase I metabolite. The Phase II conjugation creates a compound that is water soluble and readily excretable via the bile or the urine (Ballytyne et al., 1993). The majority of Phase I benzene metabolites undergo Phase II conjugation reactions in the liver and are excreted into the urine. Although these classes of reactions eliminate many potentially non-toxic nonpolar molecules from the body, their results can also be the activation of compounds to more toxic metabolites.

Figure 1.1 gives a schematic diagram of the Phase I metabolism and the most common Phase II pathway of metabolism (Henderson et al., 1985). The compounds p-benzoquinone, trihydroxybenzene, and trans,-trans-muconic acid are examples of Phase I metabolites of benzene. The compound S-phenylmercapturic acid is an example of a Phase II metabolite of benzene. The initial step in Phase I metabolism is the oxidation of the benzene ring by the CYP-450 2E1 isoenzyme to form benzene oxide. Another enzyme, epoxide hydrolase, adds water to this oxide to form the dihydrol which oxidizes to form catechol and o-benzoquinone. The benzene oxide can also rearrange to form phenol. The phenol can then be hydroxylated to form

catechol or hydroquinone. Through the benzene dyhydrol path a ring opening can occur that leads to the formation of trans,trans-muconic acid. All of this constitutes the Phase I metabolism part of the diagram. The Phase II metabolism part of the diagram is represented by the reaction of the benzene oxide with the glutathione enzyme to form a conjugate that ultimately ends up excreted into the urine as S-phenylmercapturic acid.

Phase I metabolites formed in the liver that do not undergo Phase II metabolism are circulated in the body and can enter the bone marrow (Rangan and Snyder, 1998). The phenol, 1,2,4-trihydroxy benzene, muconic–dialdehyde, and hydroquinone metabolites are a few of the prime suspects of benzene toxicity to the bone marrow. Some of the individual metabolites are toxic, but some studies suggest a synergism might exist between metabolites and this synergism is responsible for benzene toxicity to the bone marrow. This bone marrow toxicity results in aplastic anemia. Another form of bone marrow toxicity induced by benzene results in leukemia. This toxic effect is believed to proceed by mutagenesis either caused by binding of reactive metabolites to DNA in the marrow or by oxidative damage to DNA caused by the reactive intermediates or metabolites. One reactive intermediate, trans,trans-muconaldehyde , is also an alkylating agent that is suspected to be a leukemogen (Dor et al. , 1999). The most toxic metabolite of benzene is considered to be benzoquinone which is a direct alkylating agent (Goldstein and Witz, 1992).

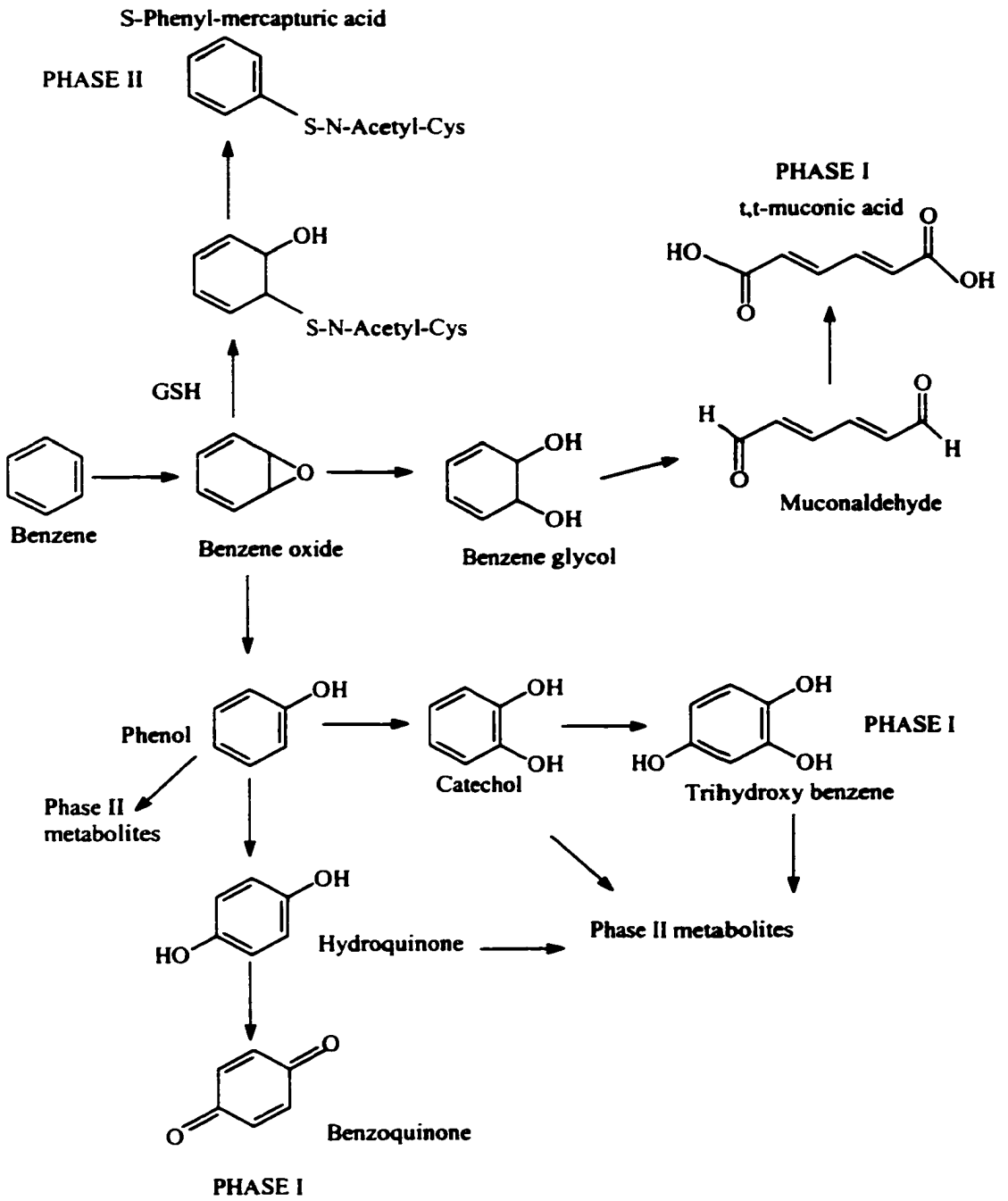


Figure 1.1: Benzene metabolism.

1.2 Toluene

Toluene occurs in the environment from a variety of sources. Toluene present in the environment is present as vapor. Almost all of the atmospheric toluene is thought to come from anthropogenic sources. A small amount also comes from natural sources, which include oil seepages and the tolu tree (U.S. Dept of Health and Human Services, 1995). The anthropogenic sources of toluene include paints, paint thinners, gasoline (15%v/v), kerosene, and tobacco smoke.

Outdoor background levels of toluene in lower U.S. rural air were found to be 0.35 ppbv (U.S. EPA, 1988). In Fairbanks, indoor air levels of toluene in residences with attached garages have been measured in the range of 0.1 – 111 ppbv (Isbell et al., 1999). Indoor air mixing ratios of toluene typically exceed outdoor air mixing ratios because of vaporization of toluene from household products and tobacco smoke (U.S. Dept of Health and Human Services, 1994; Lebret et al., 1986; Wallace et al, 1986). An individual smoking one pack of cigarettes a day would contribute an absorbed dose of toluene of 1000ug/day of toluene. Other sources of indoor exposures include glues, adhesives in carpets, and nail polish. Occupational indoor exposures are very high for people in the printing industry and average up to 250,000 ppbv per work shift.

Toluene can enter our bodies through several routes: inhalation, ingestion, and dermal absorption. Inhalation exposure occurs through inhaling ambient air

containing toluene and deliberate inhalation exposure occur through solvent abuse of gasoline, spray paints and smoking tobacco (Filley et al., 1990; Ashley et al., 1996). Toluene reaches the blood stream through the alveoli in the lungs following inhalation exposure. Toluene is rapidly absorbed in the lungs and preferentially stores in white matter in the brain stem. Ingestion exposure can occur through eating or drinking a substance contaminated with toluene. Dermal absorption occurs through the skin and involves contact with a toluene containing solvent such as gasoline or paint thinners.

Approximately 14% of an inhaled concentration of toluene is exhaled (Lof et al., 1993). About 75% of toluene absorbed into the body is eliminated within 12 hours to less harmful metabolites that are excreted in the urine (U.S. Dept of Health and Human Services, 1994).

Phase I metabolism of toluene by CYP-450 II C11and CYP -450 II E1 occurs primarily in the liver (U.S. Dept of Health and Human Services, 1994). Phase II metabolism in the liver converts the Phase I metabolites through a variety of reactions to compounds readily excreted in the urine. Figure 1.2 shows a diagram of toluene metabolism (U.S. EPA, 1985). Phase I metabolism involves the formation of either cresol or benzoic acid from toluene. The formation of glycine and glucuronide conjugates with cresol and benzoyl metabolites are all Phase II metabolic reactions. Approximately 60 - 80% of absorbed toluene will be ultimately

converted to hippuric acid and excreted in the urine (U.S. EPA, 1985; Lof et al., 1993).

The o-cresol and p-cresol intermediates are thought to be formed through a reactive arene oxide intermediate. Potentially the arene oxide would be capable of binding to macromolecules like RNA and DNA, but toluene is not a carcinogen like benzene. Rather, toluene depresses the central nervous system and cause tubular damage in the kidneys (Liu and Fetcher, 1997; Burbacher, 1993; Foo et al., 1988; Purcell et al., 1990). Other suggested mechanisms of toxicity of toluene involve alterations of nerve cell structure and function (U.S. Dept of Health and Human Services, 1994). The tubular damage in kidneys reverses itself when toluene exposure is eliminated, as do the neurological symptoms associated with short-term low-level exposure. Acute repeated high exposure to toluene can cause permanent brain damage caused by destruction of neuronal cells.

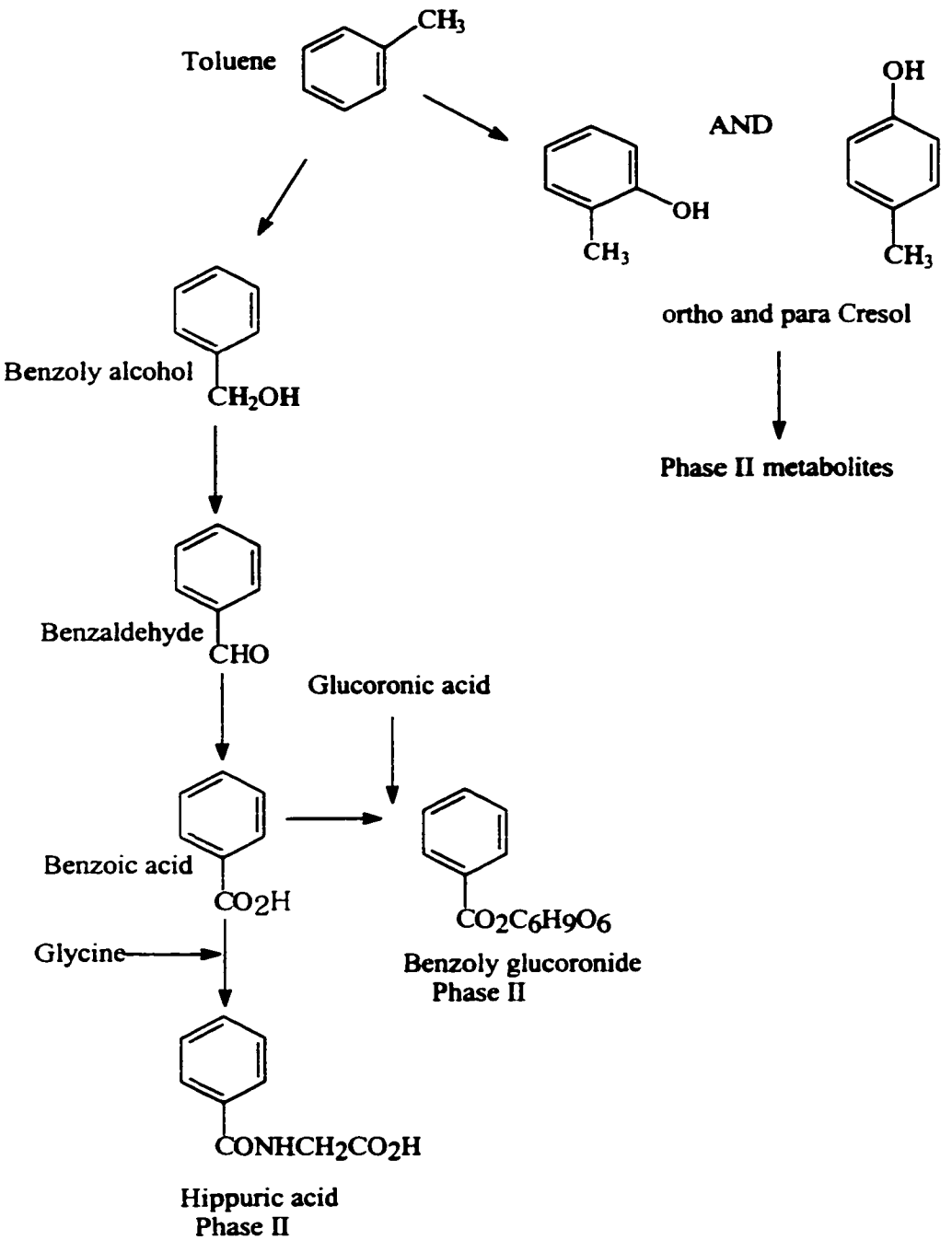


Figure 1.2: Toluene metabolism.

1.3 Biomarkers of Benzene and Toluene

In general, biomarkers can be divided into three distinct types: 1) biomarkers of exposure, 2) biomarkers of effect and 3) biomarkers of susceptibility (Dor et al., 1999; Holian, 1996). Biomarkers of exposure provide proof that an exposure has occurred to a compound. Biomarkers of effect require that a physiological disorder or biological change has occurred as a direct result of exposure to a particular compound. The effect should be directly related back to the exposure. Biomarkers of susceptibility are a sign of an individual's predisposition towards sensitivity to a compound and provide information about variability that is observed between individual responses to exposure. This study focused on biomarkers of exposure for benzene and toluene.

Biomarkers of exposure for a given compound are selected according to specific criteria (IPCS, 1993). They should be specific, sensitive, quantitative through dose-response relationships and understood in terms of general population background levels. Analytical measurement of the biomarker is best designed to be routine, noninvasive of the subject, reproducible and rapid. This type of biomarker includes:1) the parent compound;2) simple metabolites of the compound and 3) adducts formed by reactive metabolite binding to proteins such as DNA , serum or hemoglobin (Mediores et al., 1997).

Biomarkers of exposure to benzene can be found in the blood of an exposed individual, but a major disadvantage of using blood is that the sampling procedure is invasive. Benzene has been measured in the blood of an individual following an exposure, but one concern in practice is that there are no analytical checks that can indicate the loss of benzene from a blood sample prior to analysis (Ashley et al., 1996; Dor et al., 1999). Another biomarker of exposure found in the blood is the S-phenylcysteine adduct of hemoglobin. However, measurement of S-phenylcysteine adduct of hemoglobin is time consuming and is plagued with problems of inaccuracy even for higher exposure to air levels of benzene of up to 23 ppmv (Bechtold and Henderson, 1993).

Urinary metabolite biomarkers used for monitoring exposure to benzene include phenol, trans,trans-muconic acid (t,t-MA), and S-phenylmercapturic acid. Urinary benzene is not typically monitored since only about 0.1% of an inhaled dose can be found in the urine. Phenol is not useful for exposures less than 10 ppmv in an 8-hour day (Bechtold et al., 1991). Also, people who have never been exposed to benzene have been found to have relatively high phenol levels attributed either to the ingestion of vegetables and ethanol or exposure to other aromatic compounds. Cigarette smoke can complicate interpretation of results (NIOSH 1974). From a 1 ppmv exposure of benzene in air, 3.9% of the inhaled dose is excreted as urinary t,t-MA, while 0.11% is excreted as urinary S-phenylmercapturic acid (Boogard et al. ,1995). For lower doses, below 0.1 ppmv , t,t-MA is reported to represent between 7

and 58% of the inhaled benzene (Yu and Weisel, 1996). The half lives for t,t-MA and S-phenylmercapturic acid are 5 and 9 hours respectively (Dor et al., 1999). There is a dietary source of the t,t-MA metabolite, sorbic acid, which makes t,t-MA less specific as a marker of benzene exposure (Ducos et al., 1990).

No dietary sources of S-phenylmercapturic acid are known and S-PMA is regarded as being a highly specific marker of exposure to benzene (Stommel et al., 1989). This specificity makes the S-phenylmercapturic acid attractive to use in benzene studies. Unfortunately, this specificity is offset by a complex sample workup, and a difficult methodology for this assay (Ball et al., 1997;Einig and Dehnen, 1995; van Sittert et al., 1993).

Despite the dietary source, t,t-MA is the favored urinary biomarker for monitoring exposure to benzene. The wide use of this biomarker for monitoring exposure is based upon the simplicity of the t,t –MA HPLC assay as well as the importance of its metabolic route that proceeds through trans,trans-muconaldehyde, a toxic metabolite (Weaver et al, 1996; Dor et al., 1999; Schad et al., 1992; Lee et al. 1993).

Biomarkers of exposure for toluene can be determined through analysis of the breath or blood for toluene, and the urine for hippuric acid (Kawai et al., 1992a; Kawai et al., 1992b; Saker et al., 1991; Foo et al., 1991). Approximately 80% of a 0.2 ppmv dose of inhaled toluene is excreted as hippuric acid (Lof et al. , 1993). The

half-life for hippuric acid is 5 –6 hours (Lof et al., 1993). Sodium benzoate, which is a commonly found food preservative, is a dietary source of hippuric acid (Wilhelm, 1982). The invasiveness of blood sampling and practical concern of loss of toluene prior to analysis of the sample makes urinary hippuric acid the preferred biomarker of exposure for monitoring exposure to toluene. Also, the ease of HPLC analysis for hippuric acid in urine accounts for its use in occupational and environmental exposure studies (Foo et al., 1991; Kawai et al., 1992b, Tardif et al., 1989).

Chronic low dose exposure to benzene and toluene has not been extensively studied. Two studies found that there was a good correlation for atmospheric benzene and urinary t,t-MA for benzene mixing ratios of less than 1 ppmv (Ong et al., 1996; Johnson et al., 1999). This observation is somewhat surprising in light of the fact that occupational health studies have found high levels of t,t-MA in some non-exposed groups (Johnson and Lucier, 1992; Lauwerys and Buchet, 1994; Gobba et al., 1997). For toluene, studies suggest that hippuric acid is not a useful biomarker below occupational exposures, although these studies reported no biomarkers data at low levels of toluene exposure (Annit-Poika et al., 1989; Foo et al., 1991). Further investigation is needed to investigate the usefulness of hippuric acid and t,t-MA as biomarkers for assessing low level VOC exposures.

1.4 Comparison and History of Measurement Methods for Benzene and Toluene

Many methods exist for the determination of concentrations of benzene and toluene in air. In the standard method for active sampling of benzene and toluene in air, a known volume of air is drawn through a tube packed with activated charcoal sorbent, which traps the benzene and toluene vapors. The benzene and toluene are then extracted by transferring the charcoal to a small vial containing a known amount of carbon disulfide. The concentration of these compounds in the resulting solution is determined with a gas chromatograph equipped with a flame ionization detector or mass spectrometer (NIOSH, 1984; NIOSH, 1987). One major drawback associated with this approach is that carbon disulfide is both a toxic and flammable solvent. The waste generated from this method is hazardous, and the disposal is costly.

Benzene and toluene can also be collected from air into stainless steel canisters called SUMMA canisters (U.S. EPA T0-14, 1998) and analyzed directly or following a preconcentration technique using gas chromatography. Usually, preconcentration involves cryogenic trapping on a column prior to chromatographic separation (U.S. EPA T0-3, 1984). Benzene and toluene can also be passively collected from air by organic vapor diffusion monitors containing a pressed charcoal disk. Following collection, the disk is extracted with carbon disulfide (Fung et al., 1986). More recently, an active sampling method utilizing Carbotrap 300 (Supelco) sorbent has gained popularity (U.S. EPA T0-17, 1997). Organic vapors trapped on this sorbent

are then thermally desorbed into a gas chromatograph equipped with a FID detector. The advantage to this method is that no extraction solvents are utilized, thus, eliminating loss of sensitivity and minimizing quantification error through sample preparation and chromatographic interference (Baxter et al., 1980). A recent study showed that there was no significant statistical difference found between the results obtained using a charcoal tube extraction method and the thermal desorption method (Isbell et al., 1999).

At the heart of the thermal desorption T0-17 method is the selection of a tube and sorbent packing for the compounds of interest. Figure 1.4 shows a Carbotrap 300 tube commonly used for sampling benzene and toluene from air (Supelco, 1998; U.S. EPA, 1997). These tubes are preconditioned by heating in the lab while purging with ultra high purity helium before field use. Two parallel tubes are used for each sampling with either different or the same air flows through both tubes. Sample tubes can be stored in a freezer for a year or more before they are analyzed. In general, the analysis involves thermal desorption of the compounds absorbed onto the sorbent onto a room temperature focusing tube at room temperature filled with similar sorbent. Thermal desorption of the focusing tube then transfers analytes directly into the gas chromatograph column by means of a capillary transfer line. The gas chromatograph is usually either equipped with a flame ionization or mass selective detector.

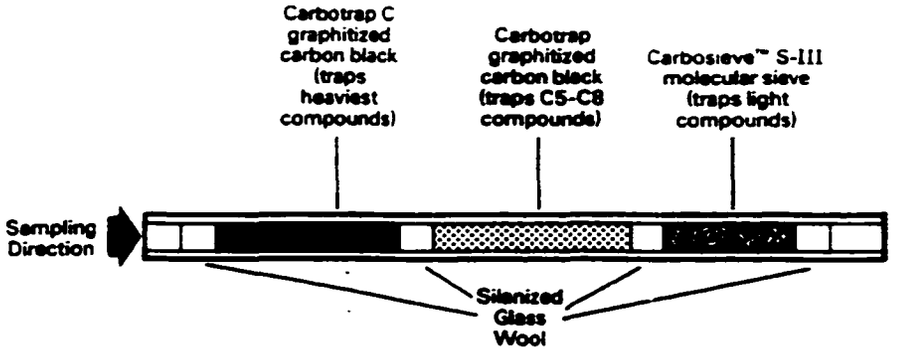


Figure 1.4: Diagram of a Carbotrap 300 thermal desorption tube.

1.5: Ventilation Measurements

Air infiltration and air movement within a home will affect the indoor air levels of benzene and toluene. In homes with attached garages, the air motion between the garage and home is particularly important as the garage is likely to contain benzene or toluene sources. Tracer techniques provide direct information about air infiltration rates in homes during actual living conditions. Several tracer techniques exist: 1) tracer decay, 2) steady-state mode, and 3) constant emission. A discussion follows of these three approaches with emphasis placed on the constant emission perfluorotracer technique employed in this research.

In the tracer decay method, an inert compound (typically SF_6) is released into the home and the decay of the concentration with time is measured (Harrje and Grot, 1978). For constant ventilation conditions, this decay is exponential in time and is characterized by a decay rate. The decay rate is directly proportional to the air changes per hour. These rates are typically determined within two to eight hours. In the steady-state tracer method, SF_6 is mechanically injected into the air space periodically to keep the SF_6 concentration constant (Harrje et al., 1975). The injection system is triggered by feedback from a real-time gas chromatograph (Condon et al., 1990). With this system, infiltration rates can be measured for long durations, and variations in infiltration rates caused by changing environmental conditions can be observed.

A constant-emission tracer technique was developed at Brookhaven National Laboratory (BNL). This method is called the Brookhaven National Laboratory Air Infiltration Measurement System (BNL/AIMS). This technique measures ventilation between different zones defined within a home (Thomas et al., 1993). The home is divided into zones in a way that treats the home as a multichamber system. Permeation tubes containing different perfluorotracer(PFT) compounds are placed in each zone, providing a known source of PFT compounds within the house. The gas-phase mixing ratios of these PFT compounds are allowed to stabilize for 24 hours before measurement.

Passive capillary adsorption tubes (CATS) filled with Ambersorb (Rohm and Haas Co.) are used to collect integrated tracer samples (Dietz and Cote, 1982). The tracer concentration for each CATS is determined in the laboratory by thermal desorption gas chromatography using an electron capture detector. The CATS are placed in and then removed from the home at predetermined time intervals. The PFT sources are left in the home until the measurement period is complete. Since both the PFT sources and the CATS are small (about 6mm by 30mm), they are noninvasive and easily transported.

The PFT sources are permeation devices that are made by filling a metal shell with liquid PFT and then crimping an elastomer plug on the end (Dietz and Cote, 1982).

This design reduces temperature and time dependence. The typical emission rates for these sources are between 5-20 nL/min. The typical tracers used are PMCP (perfluoromethylcyclopentane; PFT-8), PMCH perfluoromethylcyclohexane; PFT-2), and PDCH (perfluorodimethylcyclohexane; PFT-3). Different PFTs are placed into each zone within a home. These PFTs are nontoxic by inhalation or ingestion in the concentration range used in homes (Senum et al., 1980).

The ventilation characteristics of a home are described in terms of air flow rates from well mixed zones to all other zones including infiltration and exfiltration rates between zones and outside the home. The theory of a steady state ventilation model for a multi-tracer technique in multiple zones makes four important assumptions (D'Ottavio et al., 1988). First, the home can be divided into separate well-mixed zones defined as a zone having a well-mixed tracer concentration for the particular tracer used in that zone. Second, during the measurement period the system is at steady state. Sensitivity analyses indicate even the significant variations in ventilation that can occur over periods of a week or greater lead to an error of only 5-10% in the average flows calculated with the steady state assumption. Third, the tracer emission rate is considered constant at normal indoor temperature variations. Fourth, the outdoor contribution of these tracers is negligible, that is, three orders of magnitude less than the 1-10 pptv generated by a PFT.

A three-zone model is represented in Figure 1.5.1 for a three-zone home (D'Ottavio et al., 1980). One zone in this model can be viewed as a single compartment model that has an air flow into it, R_{01}, and an air flow out of it, R_{10}, both with units of m^3/h (Figure 1.5.2). In this zone is a concentration, C_T, of vapor phase tracer. This concentration has units of nL/m^3, which is equivalent to pL/L or pptv. Therefore, this concentration is actually a mixing ratio of the vapor phase of the tracer in the zone. In this zone there is one source of tracer, and S_T, is the constant emission rate of that tracer in that zone. The units of S_T are nL/h. The permeation rate of the tracer tube is known(S_T) and the vapor phase of the tracer is measured by adsorption onto the CAT in the zone , so the ventilation rate for that zone can be calculated from the quotient of S_T and C_T where $R_{01} = R_{10}$.

For the multicompartment model depicted in Figure 1.5.1 note that there is only one tracer source for each zone and each CAT picks up more than one tracer. A set of four equations can be generated for each zone and solved.

Let: R_{ij} = rate of air flow from zone i to zone j ($i \neq j$; zone 0=outdoors)

R_{ii} = sum of all air flows into or out of zone 1 ($I \geq 1$)

R_{oo} = sum of all infiltration flows = ΣR_{io}

C_{ij} = concentration of tracer i in zone j (C_{io}=0 for all i)

S_j - constant source emission rate of tracer in zone j

The four equations to calculate all ventilation flows for zone 1 are then:

Tracer 1: $R_{11}C_{11} - R_{21}C_{12} - R_{31}C_{13} = S_1$.

Tracer 2: $R_{11}C_{21} - R_{21}C_{22} - R_{31}C_{23} = 0$.

Tracer 3: $R_{11}C_{31} - R_{21}C_{32} - R_{31}C_{33} = 0$.

Air flow balance: $R_{01} = R_{11} - R_{21} - R_{31}$

The total infiltration rate and exfiltration rate for zone one can be calculated from these equations. The air change per hour (ACH h^{-1}) is calculated for each zone as the quotient of the total infiltration rates into that zone to the volume of that zone.

$R_{00} = R_{01} + R_{02} + R_{03}$ **and** $R_{10} = R_{11} - R_{12} - R_{13}$

Software, developed at Brookhaven National Laboratory, is used to calculate zone and overall air movements well as infiltration and exfiltration rates based on this multicompartment model (Dietz et al., 1985).

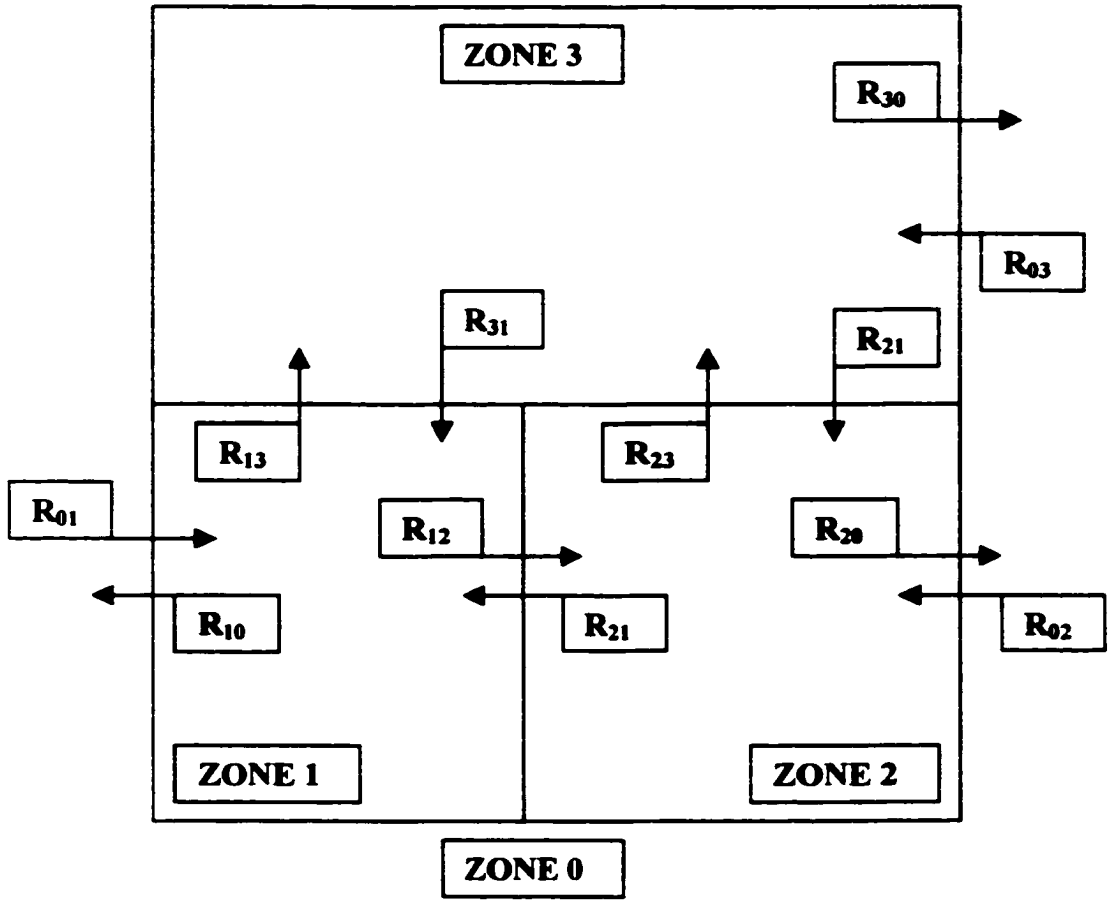


Figure 1.5.1: Air flow through a house that consists of three well-mixed zones. Zone 0 is outdoors.

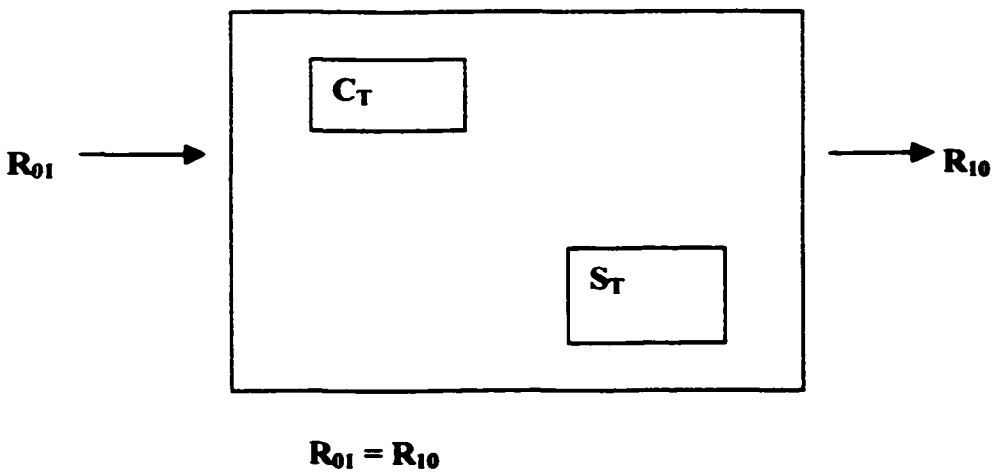


Figure 1.5.2: Simple one compartment model for ventilation calculations.

1.6. Simultaneous Measurement of Ventilation, Benzene, and Toluene.

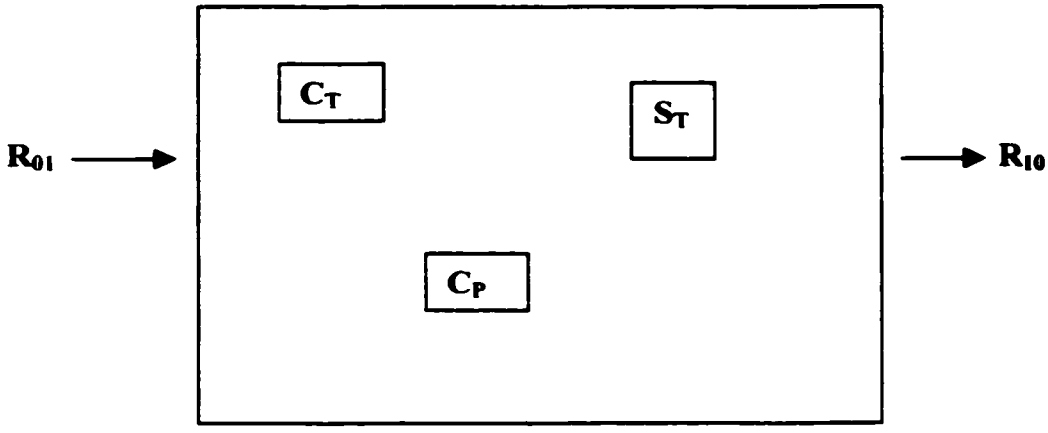
Ventilation measurements and air monitoring can be conducted simultaneously in homes as was done in this research. The three zones in the ventilation model are defined to be the garage, main living area and bedroom area. PFT tracers are placed in zones in the home 24 hours before the study begins, and not removed from the home until the study is complete. New CATS are placed into their respective zones at the same time the air monitoring begins each day.

The ventilation model for a one-zone single compartment model that includes air monitoring is given in Figure 1.6. In this situation a source strength estimate for the pollutant, S_P(mg/h), can be calculated using Equation 1.6. The rate of infiltration in and out of that zone, the concentration of the tracer (C_T), and the tracer source emission rate (S_T) are known from the tracer ventilation data. The mixing ratio of the pollutant in the zone , C_P, is measured.

Equation 1.6: $$S_P\ (ug/h) = C_P(ug/m^3) * S_T\ (nl/h)/\ C_T(nL/m^3)$$

In calculating pollutant source strengths an assumption is made that volatile organic compounds (VOCs) indoors do not undergo any chemical reactions (Thomas et al., 1993). Source strength estimates are useful for direct comparison between and

within zones in homes. They are important for locating the source(s) of the pollutant in the home and the pollutant's travel throughout the home. In this study, the garage of a home was hypothesized to be an emission source for VOCs in the home.



$$R_{01} = R_{10}$$

Figure 1.6: One compartment ventilation model that includes air monitoring.

1.7 Goals of this Thesis

I hypothesized that indoor air mixing ratios of benzene and toluene in houses with attached garages would be positively correlated with an individual's urinary level of t,t-MA or hippuric acid. I also hypothesized that indoor air mixing ratios of these compounds will increase during the winter months in Fairbanks, Alaska because heating considerations require the home to remain as airtight as possible. Finally, I hypothesized that small engine storage in the garage, additional fuel storage in the garage, and ventilation characteristics of the home will all play important roles in exposure an individual to these compounds inside of their homes.

As a test of these hypotheses, I report the results from measurement of indoor air mixing ratios of benzene and toluene in different homes with attached garages during different seasons. I also monitored one individual from each of three homes for urinary levels of t,t-MA and hippuric acid. Finally, the ventilation characteristics were determined for two homes while indoor air measurements were made simultaneously in the living area and garage for two seasons. Factorial experimental design was used to test several factors to discover the importance of fuel sources in the garage, house temperatures, and ventilation during winter and summer seasons.

CHAPTER 2 – MATERIALS AND METHODS

2.1 Reagents

Reagents used in these experiments were of reagent - grade quality. The solvents used included: methanol (EM Science, 99.8%); toluene (Fisher ACS,99.8%); ethyl benzene(Fisher ACS,99.8%); benzene(Fisher cert. ACS,99.8%-thiophene free); ethyl benzene(Fisher ACS,99.8%); acetonitrile (Fisher HPLC grade , 99.9%); ethylacetate (Baker HPLC grade, 99.6%). Solids used to prepare the biomarker standards included: hippuric acid (Aldrich, 98%); 3-methyl-hippuric acid (98%); 4-hydroxy-3-methoxybenzoic acid, vanillic acid (Aldrich 97%); trans-trans,muconic acid (t,t-MA)(Aldrich, 98%). Sodium chloride (Fisher cert ACS grade), Baker Analyzed HPLC water, and hydrochloric acid (Fisher Reagent grade, 35-38%) were also used.

2.2. Thermal Desorption

Analyses and sample collection were all performed in accordance with EPA Compendium Method TO – 17: Determination of Volatile Organic Compounds in Ambient Air, Using Active Sampling Onto Sorbent Tubes- Jan. 1997. Figure 2.1 shows the thermal desorption- GC-FID used for all experiments. Carbotrap 300 sorbent tubes, (Supelco # 20875), were used for standards and to collect samples, while a carbotrap 201 focusing tube, (Supelco 20865), was used to refocus inside the desorption unit. Samples and standards were analyzed using a Dynatherm ACEM 900 thermal desorption unit interfaced to a Hewlett Packard 5890 Gas

Figure 2.1: Tube conditioner, thermal desorption apparatus, and GC-FID (left to right).

Chromatograph equipped with a flame ionization detector. Data were collected from the GC using a Hewlett Packard 3396 B integrator. An EC carbowax capillary column, 30 x 0.45mm i.d. x 1.0um thickness, (Alltech,cat.19663), was used in the gas chromatograph. The hydrogen and nitrogen gases used for the detector were ultra high purity(>99.9%) and the air was " breathing" quality. Example GC-FID chromatograms of the standard mixture and an air sample can he found in Appendix A. Typical linear regression curve results for standards can be found in Appendix B.

Chromatography was performed with a programmed temperature cycle. The initial temperature was set at 32°C for 2 minutes followed by a 6.00 °C/min increase to 100°C and held for 6.00 minutes. A 2.3 mL/min flow of ultra high purity helium (99.9%) was maintained through the transfer line of the thermal desorption unit and the column to the detector. The split function was used so that there was a 1:10 split of all samples and standards between sample tube and sample saver tube. Temperature setpoints for the desorption unit were as follows: valve at 200°C; tube desorb at 330°C; transfer line at 225°C; trap desorb at 330°C. The time setpoints for the desorption unit was as follows: tube heat, trap heat both held for 5 min; tube dry and cool both set to a 1 minute duration.

The analytical performance was assessed daily using blanks and standards as reference material. A primary standard containing benzene, toluene and ethyl

benzene was prepared by delivering a volume of each using an Eppendorf mechanical pipette into a Class A volumetric flask and diluting to volume with methanol. The Eppendorf pipette was calibrated at selected volumes using analytical weights of multiple water deliveries into a beaker. Working standards were prepared from this standard stock batch when needed. All standards were stored in the refrigerator between uses.

Thermal desorption standards were prepared according to EPA TO-17 recommendation which instructs to inject a volume of a standard solution through a septum apparatus containing a thermal desorption tube maintained at 50 deg C with a helium flow of 60mL/min moving through the tube for 5 minutes after injection. Typical calibration curves were in the range of 125 ng to 850 ng analyte. Peak area response of the GC-FID was used to quantitate samples and standards. Calibration curves for benzene, toluene, and ethylbenzene were calculated by linear regression analysis of the standard data and these curves were then used to calculate concentrations of samples. Sample concentrations were then adjusted using sampling flow for that tube, time sampled, average temperature and pressure over the time sampled to give the mixing ratios in ppbv of benzene, toluene, and ethylbenzene in samples. The mixing ratio was calculated from the quotient of the number of mole analyte and the total number of moles of air sampled. The percent relative standard deviation (%RSD) was used to show the reproducibility of the method. The % RSD for this method is 1.9 for n = 6. The RSD was determined by

preparing multiple samples of benzene and toluene onto sorbent tubes and analyzing them individually.

A Dynatherm tube conditioner model 10 was used to precondition all tubes used for sample collection, blanks, and standards. The tubes were conditioned at 375°C for 15 minutes with ultrahigh purity helium (99.9%) flowing through them at 100mL/min. Tubes were allowed to cool to room temperature with helium still flowing through them. Once cooled, the tubes were stored in glass screw top vials and kept at -20°C until used.

2.3 Air Sampling

In the living area of homes, a Gilian III sampling pump connected to a Gemini Twin port sampler, (both from Lab Safety Supply), was used to pull simultaneous samples through sorbent tubes with flows on the parallel sampling ports typically set at 5mL/min and 20mL/min (Figure 2.2). Flows were measured accurately on each leg before and after sampling using a Gilibrator 2 (Sensidyne model 800287) flow measurement instrument. Samples were collected for approximately 12 hours at each location. Living area air samples were taken in all studies. In garages, an air cadet pump (Cole Parmer, model #7530-40) was used to collect samples onto thermal desorption tubes using the same sampling apparatus configuration as indoors. Garage sampling was only done in the winter 1999 and summer 2000 studies. Outdoor temperature, living area temperature, garage temperature were measured at night at the beginning of a sampling period and then again in the

morning at the end of the sampling period. The ambient barometric pressure was obtained at the same times by phone from the weather service bureau.

2.4 Furnace Duty Cycle Measurements

Campbell dataloggers CR10 and CR21X were used to continuously measure stack temperatures of furnaces in the two homes of the winter 1999 and summer 2000 study (Figure 2.3). A type K thermocouple was attached to the datalogger and then the thermocouple was inserted into the service hole of the furnace stack. The data stored in these loggers were downloaded and the duty cycle calculated as the quotient of the total time the furnace was on and the total time period monitored for each day.

2.5 Ventilation measurements

Brookhaven National Laboratory Air Infiltration Measurement System (BNL/AIMS) was used to measure the air exchange in the two homes studied in winter of 1999 and summer 2000. The air exchange measurements were used in combination with the benzene and toluene measurements to calculate source strengths in each ventilation zone.

Perfluorocarbon tracer (PFT) sources emit tracers at a known rate, and passive samplers (capillary absorption tube samplers, (CATS) absorb the tracers. The air concentration of the tracers is determined by thermal desorption of the CATS into a gas chromatograph, separation, and then quantification using an Electron Capture

detector. A time-averaged indoor tracer concentration is determined. The reciprocal of this concentration times the source rate is approximately equal to the average air infiltration rate. Brookhaven National Laboratory Tracer division supplied all PFTs, CATS, and performed all analyses and calculations on these samples (Dietz et al., 1985).

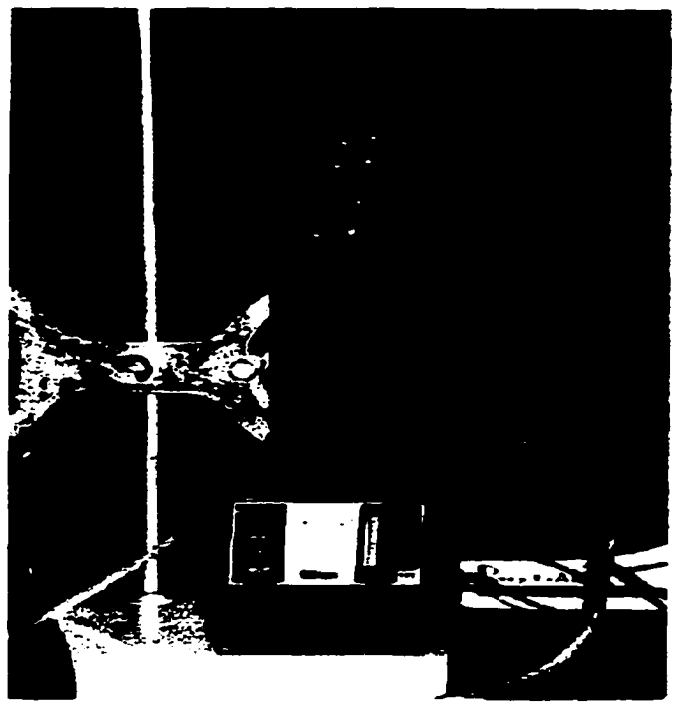


Figure 2.2: Air sampling equipment: Gilian pump and thermal desorption tubes.

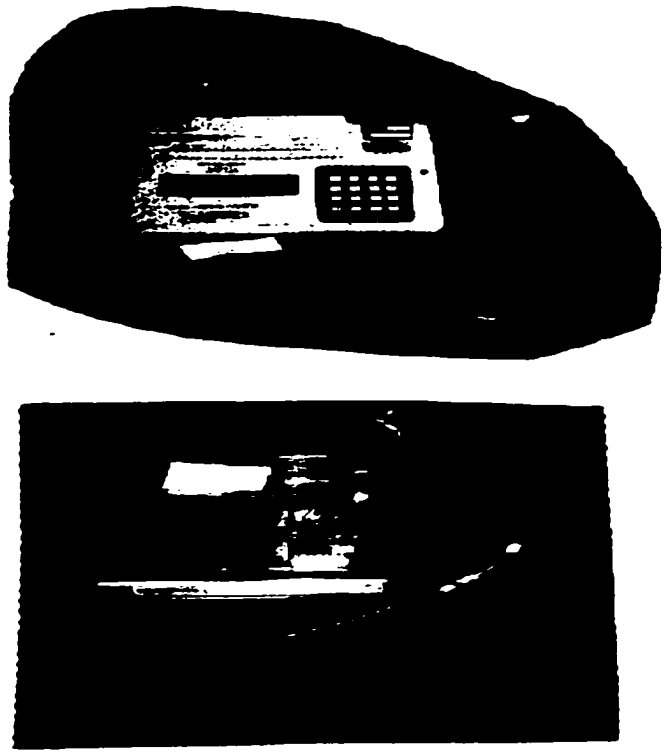


Figure 2.3: Microdataloggers and Type K thermocouples: CR21X (top) and CR10 (bottom).

Each of the two homes was divided into three zones: garage – zone 1, first floor- zone 2, and second floor – zone 3. Different PFTs were used in each zone. PMCP (perfluoromethylcyclopentane; PFT-8) was used in zone 1. PMCH (perfluoromethylcyclohexane; PFT-2) was used in zone 2. PDCH (perfluorodimethylcyclohexane; PFT-3) was used in zone 3. Figure 2.4 shows the PFTs used and a CATS. Figure 2.5 and Figure 2.6 show simple diagrams of the placement of PFTS, CATS and air sampling pumps in each of these homes. These diagrams are not intended to be floor plans for either home so doors are not illustrated in them. The straight line in both home's kitchens and garages indicate the window that was either opened or closed during the summer study. The garage door between the garage and living area of both homes was shut during each monitoring period. All windows were shut during the winter monitoring period.

The procedure used in both homes was to place the PFT sources in the homes 24 hours before the sampling period began. CATS were placed in the evening at beginning of each sampling period and then collected in the morning . Sampling with the CATS consisted of uncapping the end with the numbers engraved in the tube and then recapping when finished. The CATS were brought back to the laboratory and stored in a fume hood. PFT sources were collected after the entire sampling period was complete. PFT sources and CATS were mailed back to Brookhaven three days apart to prevent any contamination of the CATS.

Figure 2.4: PFT tracer sources (left) and CATS (right).

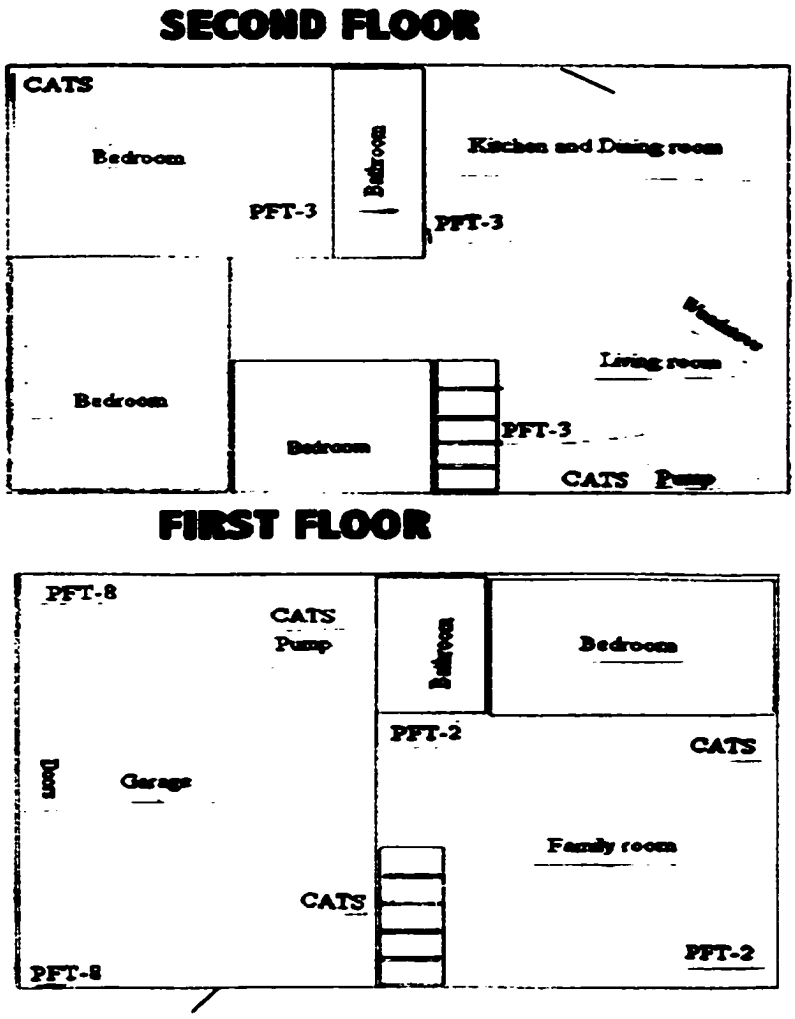


Figure 2.5 : Diagram of Home B: placement of PFTs, pumps and CATS.

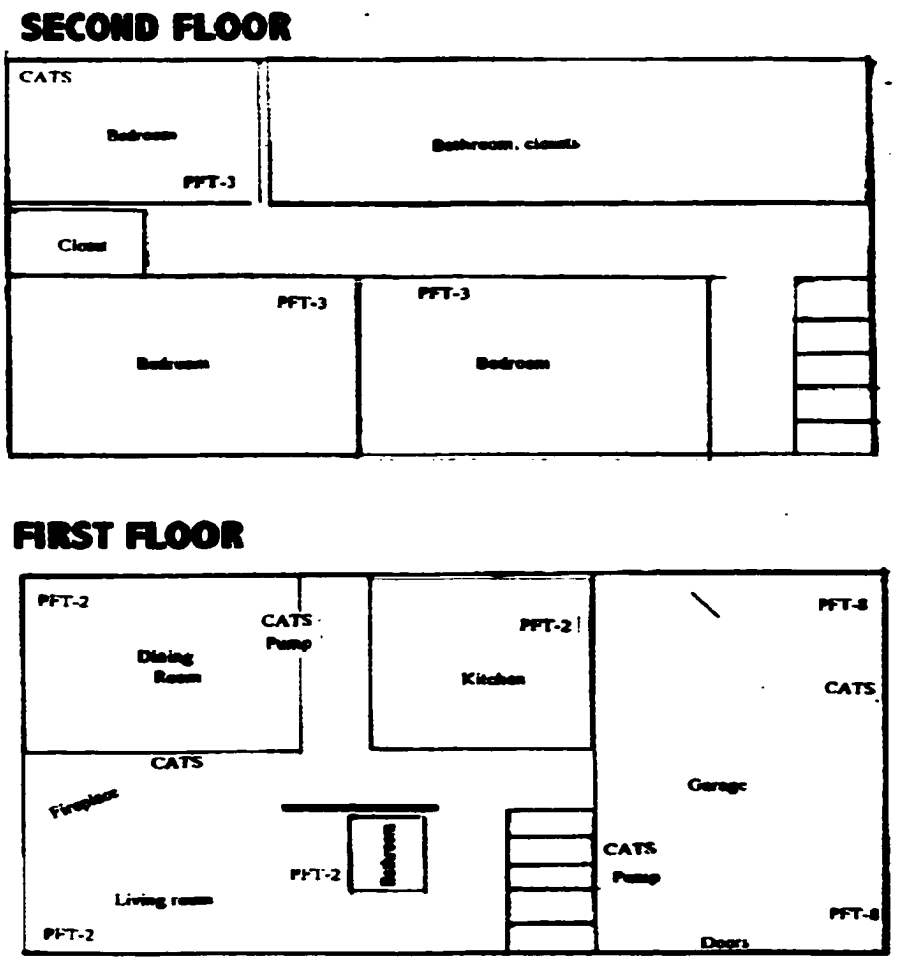


Figure 2.6: Diagram of Home M: placement of PFTs, pumps and CATS.

2.6 Two Level Factorial Experimental Designs

Two level factorial experimental designs provide a way of screening from many possible variables those that are actually important. The design permits detection of effects due to individual variables and interactions between variables. Evaluation of factorial design experiments can be done by using Design Expert (Design Expert, Stat Ease Inc., 1996) statistical software from which numerical values are obtained using an algorithm that calculates a numerical effect estimate for each combination of factors used in an experiment. Significant factors were identified as those with an effect estimate larger than twice the standard error. Normal plots of residuals, effect plots, Analysis of Variance (ANOVA), and Cook's distance plots for outliers were also used for experimental interpretation and were computed using the Design ease statistical software.

A 2^{6-2} fractional factorial experimental design was used for the winter 1999 and summer 2000 studies. The designs included 16 experiments divided into two blocks of four days. A general design matrix for a two level, 6 factor , 16 experimental design in standard order is summarized in Table 2.1. The factors used for the winter 1999 study are shown in Table 2.2. The factors used for the summer 2000 study are shown in Table 2.3. The conditions for each experiment in these studies were set up in the morning. Metal and plastic one-gallon gasoline cans were filled immediately before each block. Hand held chainsaws were filled with fresh fuel before each block.

Table 2.1: Design Matrix: Six Factor Two Level Experiments.

	Factor [a]					
BLOCK # 1 :						
Run	**A**	**B**	**C**	**D**	**E**	**F**
1	-	-	-	-	-	-
2	+	-	-	-	+	-
3	-	+	-	-	+	+
4	+	+	-	-	-	+
5	-	-	+	-	+	+
6	+	-	+	-	-	+
7	-	+	+	-	-	-
8	+	+	+	-	+	-
Block #2 :						
9	-	-	-	+	-	+
10	+	-	-	+	+	+
11	-	+	-	+	+	-
12	+	+	-	+	-	-
13	-	-	+	+	+	-
14	+	-	+	+	-	-
15	-	+	+	+	-	+
16	+	+	+	+	+	+

[a] **(-) = low level ; (+) = high level**

Table 2.2 : Winter 1999 Factorial Experimental Design.

FACTOR	(-)	(+)
A: Gas can outside home	NO	YES
B: Metal gas can in garage	NO	YES
C: Plastic gas can in garage	NO	YES
D: Living area temperature	normal	+ 5
E: Garage temperature	normal	-10
F: Type of home	home M	home B

Table 2.3: Summer 2000 Factorial Experimental Design.

FACTOR	(-)	(+)
A: Garage window open	NO	YES
B: Chainsaw in garage	NO	YES
C: Inside window open:	NO	YES
D: Fireplace/woodstove flue open	NO	YES
E: Plastic gas can in garage	NO	YES
F: Type of house	home M	home B

2.7 Biomarkers

Urine samples were collected as morning catches from one individual at each home. Biomarkers were determined in the summer 1998 and winter 1998-1999 studies. Biomarkers were not determined in the winter 1999 and summer 2000 studies. Urine samples were homogenized in the laboratory, split into two portions, and these were frozen until analysis. HPLC analyses were performed on a Beckman HPLC system, (Model 126 solvent pump), using a Model 168 diode array detector, a Model 126 solvent module, and a AS507E autosampler. System Gold software was used to collect and analyze data. The HPLC column was a Phenomenex hypersil C-18 (25cm x 4.6mm). A Buchler peristaltic pump was used to control flow rates of solutions through the PrepSep cartridges used in the t,t-MA method. Examples of typical linear regression curves for standards can be found in Appendix B for both biomarkers.

Human subjects were asked to complete voluntary participant consent forms as per the requirements of the University of Alaska Human Subjects Committee. They also completed a confidential questionnaire that surveyed perceived health, smoking status and exposure, time spent in the home, occupational exposures, and number of times they added gasoline to a vehicle. All participants were nonsmokers with little or no exposure to second-hand cigarette smoke. No occupational exposures to benzene or toluene were found for any of the participants in their work environment. All subjects perceived themselves to be in good health before and

during the time of participation in the study. All participants spent the majority of time inside of their homes during the time of air monitoring.

Peak area was used to quantitate samples and standards. Standards and blanks were used daily to assess analytical performance. A linear regression of the ratio of biomarker to internal standard response vs. biomarker standard concentration was used to calculate concentrations. Typical linear regression calibration curves can be found in Appendix B. These concentrations were divided by the concentration of creatinine in the urine sample to give the final results. Creatinine is a measure of renal clearance and is used to normalize urine volume over a sampling period. The hippuric acid RSD was 1.4% for n = 5. The t,t-MA method RSD was 6.3% for n = 5. These RSD were determined by extraction and analysis of multiple aqueous samples of each biomarker.

Urine samples were analyzed using a HPLC method for the t,t-MA and vanillic acid internal standard. A stock solution of t,t-MA was prepared in 80% acetonitrile in water. Before extraction, urine pH was measured and then adjusted to five using 6M hydrochloric acid when necessary. Vanillic acid was added to both standards and samples volumetrically.

First, a PrepSep SAX cartridge, (Fisher Scientific), was preconditioned using the following regime : 3mL of methanol followed by 3 mL of water using a flow rate of

2mL/min. Next, urine was applied to the cartridge using a flow of 1mL/min. The cartridge was then washed at 2ml/min with 1mL of water. The t,t-MA was eluted using a flow of 0.5mL/min and 5mL total of a 0.1 M hydrochloric acid solution. The mobile phase used for the HPLC analyses was an isocratic mixture: 4.5mL glacial acetic acid with 1.8 mL of 1.0M sodium acetate diluted to 1 liter with water set at 90% fraction; acetonitrile set at 10% fraction. A flow rate of 1.0mL/min was used for the mobile phase, and the detector was set to monitor for the t,t-MA and vanillic acid at a wavelength of 260nm.

NIOSH method 8301-1 was used to extract hippuric acid from urine. A 1mL aliquot of urine was delivered into a 25mL volumetric flask that also contained internal standard (3-methyl-hippuric acid), 40μL of concentrated hydrochloric acid and 0.35g of sodium chloride. Next, 4mL of ethyl acetate was added to the flask that was stoppered and then manually shaken for two minutes. The solution in the flask was then transferred to a test tube and centrifuged for 5 minutes. The ethyl acetate fraction was transferred into a vial and evaporated to dryness using a gentle stream of nitrogen. The contents of the vial were rehydrated with a volume of water, which was directly analyzed using HPLC. 3-Methyl hippuric acid was added to all standards and sample prior to extraction from a stock solution prepared in 80% acetonitrile. A flow rate of 1.5mL/min was used with an isocratic mixture of two solvents: 0.02% acetic acid set at 88%; acetonitrile set at 12%. The detector was set to monitor the hippuric acid and 3-methyl–hippuric acid at a wavelength of 254 nm.

A Sigma Diagnostics Creatinine Colorimetric kit (#555A) was used to determine the creatinine level in urine samples. Standards were prepared directly into cuvettes by adding volumes of the creatinine standard that corresponded to various concentrations of creatinine that define the linear range for analysis. A typical linear regression curve can be found in Appendix B. Next, 3mL of alkaline picrate solution was added to the cuvettes followed by a volume of deionized water that would bring the total volume of the solution to 3.3mL. Ten minutes after preparation the absorbance of the creatinine-picrate complex was measured before and after acidification at 500nm using a Hewlett Packard 8453 uv-visible spectrophotometer. Urine samples were prepared by adding 0.3mL of urine to a cuvet with 3.0mL of the alkaline picrate solution and the absorbance was measured after 10 minutes. Blank solutions containing no creatinine were also analyzed. Creatinine concentration was determined from regression lines of absorbance versus standard creatinine concentration and then corrected for dilution when necessary.

CHAPTER 3 – RESULTS

3.1 Air and Biomarkers

Summer 1998 Air and Biomarkers Results

The benzene and toluene mixing ratios in the living area air of 8 homes with attached garages were measured during July 1998 in Fairbanks, Alaska. A thermal desorption tube method and charcoal tube method were used in a collaborative study with Janet Ricker to compare the two methods. Urinary biomarkers for benzene and toluene were also measured for one adult member of each household.

The charcoal tube and thermal desorption tube methods are comparable (Table 3.1). A Wilcoxon t-test upholds the null hypothesis that there is no significant difference between benzene levels at each site using the two methods, (T_{calc}= 7; T_{crit} = 4, P = 0.05%)(Vankeerberghen et al., 1991).These data are the average values of duplicate tubes used to sample at each site and the average values of duplicate analyses for biomarkers. The data from both the thermal desorption method and the charcoal extract method follow a similar trend, (R^2 = 0.97), as seen in the correlation of benzene mixing ratios between the different methods when compared at each site (Figure 3.1).

A correlation (R^2 = 0.99) was found between benzene and toluene levels in air, suggesting they are from the same point source (Figure 3.2). A further correlation

(R^2 = 0.95) was found between benzene and toluene in the indoor air of these homes and the number of small engines stored in the attached garage (Figure 3.3).

No correlation was found between benzene and toluene levels in indoor air and their respective biomarkers, t,tMA and hippuric acid , in urine samples (Table 3.1).

TABLE 3.1: Summary of charcoal tube, thermal desorption , and biomarker result for summer 1998 study.

	Charcoal tube [a]	Thermal desorption)[a,b]		Δ[c]	Biomarkers (mg/g creat.)[d]	
Site no.	Benzene (ppbv)	Benzene(ppbv)	Toluene(ppbv)	Benzene	t,t-MA	Hippuric acid
1	0.2	1.2	3.4	1.0	0.12	0.59
2	39.1	72.0	111.2	31.9	0.23	0.22
3	0.2	0.4	0.1	0.2	0.40	0.64
4	26.4	34.2	48.7	7.5	0.33	0.64
5	6.5	5.1	3.9	-1.4	0.10	1.37
6	6.8	5.3	14.8	-1.5	0.61	0.37
7	3.9	11.2	15.4	7.3	0.16	0.55
8	4.6	8.8	16.4	4.2	0.86	0.21

[a] These are the average result for the low and high flow tubes at each site.

[b] $S.D._{pooled}$ = 3.4 ppbv benzene ; $S.D._{pooled}$ = 17 ppbv toluene for thermal desorption.

[c] Numerical difference in benzene levels between the two methods.

[d] $S.D._{pooled}$ t,t-MA/creatinine =0.24; $S.D._{pooled}$ HA = 0.25.

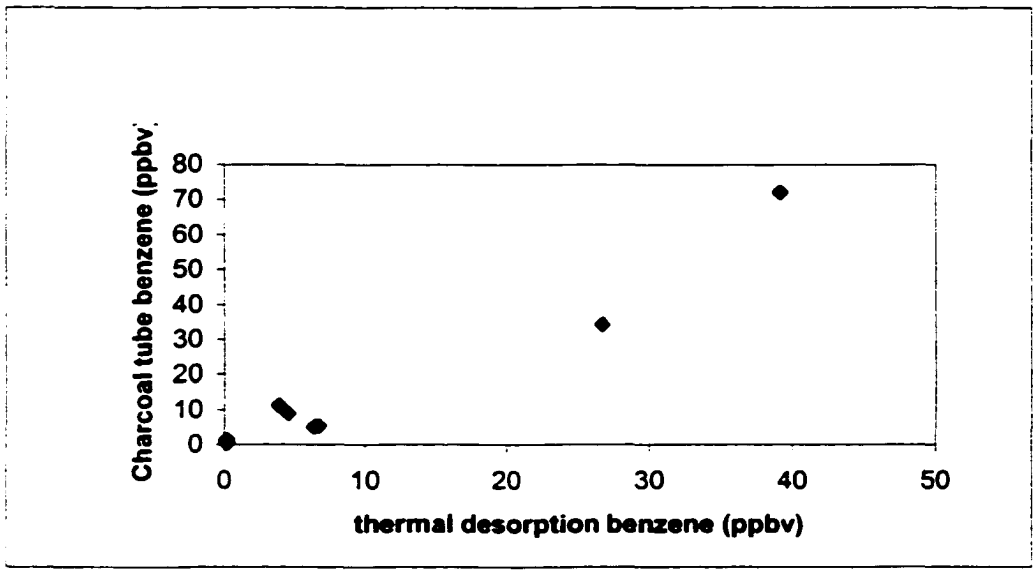


Figure 3.1: Comparison of living area benzene levels from charcoal tube and thermal desorption methods.

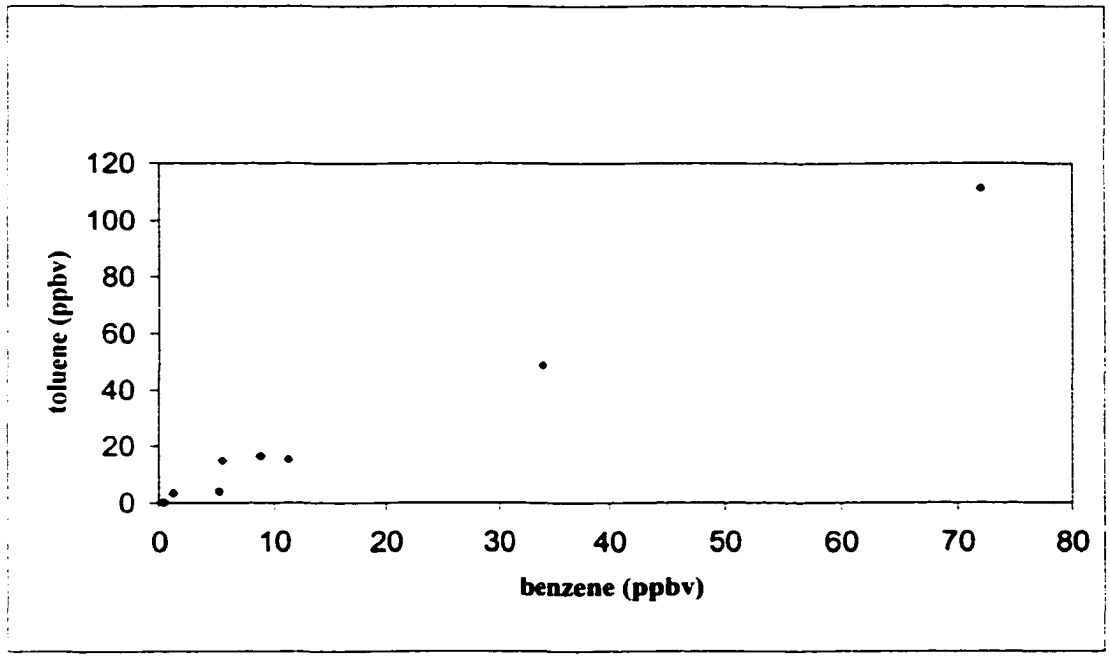


Figure 3.2: Living area benzene and toluene mixing ratios for thermal desorption.

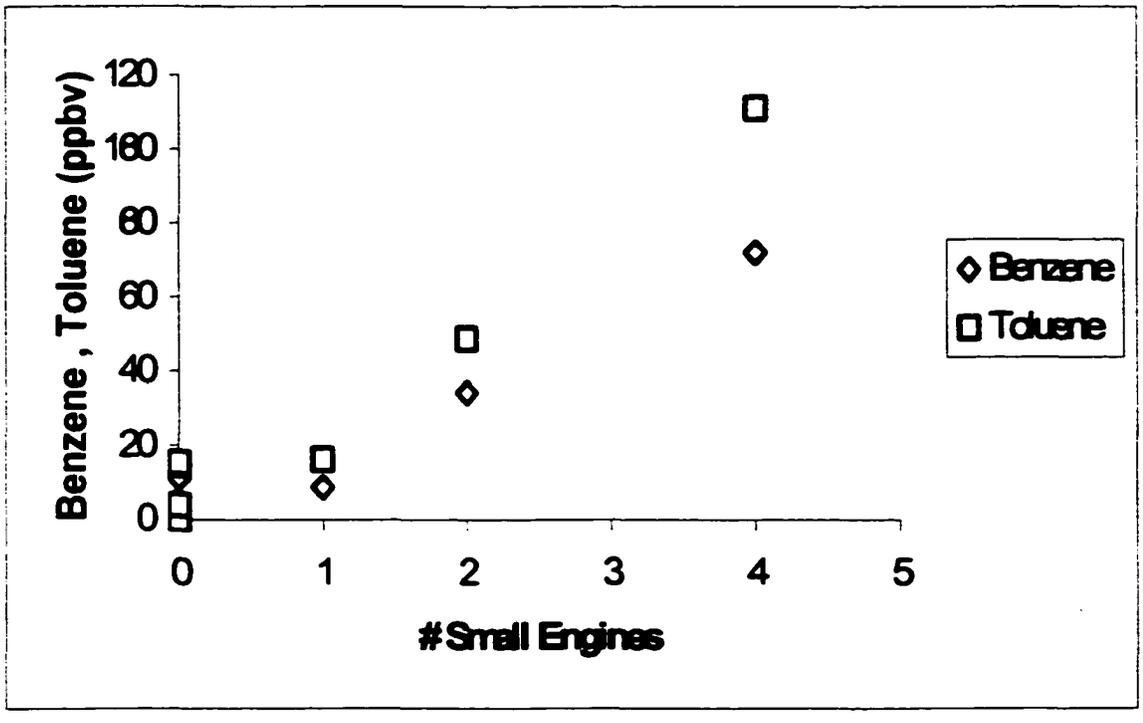


Figure 3.3: Living area benzene, toluene, and number of small engines.

Winter 1998 –1999 Air and Biomarkers Results

Benzene and toluene mixing ratios in the living area air of three homes (sites) with attached garages were measured once a week for 12 consecutive weeks from December 1998 through March 1999. The thermal desorption method was used for all samples. Benzene and toluene mixing ratio averages for duplicate tubes sampled, average results for biomarkers, and outdoor temperatures for the three sites are summarized in Table 3.2. Figure 3.4 shows the data for living area benzene and toluene mixing ratios for each site. A weighted linear regression least squares algorithm was used to fit the data for these plots (Ogren and Norton, 1992) No indoor sources of benzene or toluene were identified for any of the sites (cigarette smoke, solvents, hobby glues).

The mixing ratios of benzene and toluene are highly correlated at all sites. The similarity of slopes and the near zero y-intercepts at site 1 and site 2 suggests a common source. The lower slope (by a factor of 2) and non-zero y-intercept for site 3 suggests a different source or perhaps a removal mechanism for toluene. There is no obvious construction or use difference at site 3 to explain this difference. A trend was not observed between the day sampled and the benzene mixing ratio measured at each site, which suggests that the results were not effected by sampling the sites on different days of the week. (Figure 3.5).

A weak correlation, ($R^2 = 0.54$), between the benzene mixing ratios and the benzene urinary biomarker t,t-MA was only found for site #2 (Figure 3.6). There was no correlation observed between toluene air levels and the HA biomarker for any of the sites suggesting this biomarker is not suitable for monitoring exposure at these low levels of toluene.

Table 3.2: Summary of Benzene , Toluene, Biomarkers and Outdoor Temperatures[a,b].

Week	Benzene (ppbv)	t,t-MA/creat mg/g	Toluene (ppbv)	HA/creat g/g	Ave Outdoor Temp.(deg°C)
SITE #1					
1	4.8	1.7	13.4	3.3	-30
2	*	*	*	*	*
3	10.0	1.0	30.6	1.2	-6
4	8.6	1.5	21.6	1.8	-26
5	9.7	0.6	21.7	2.4	-30
6	5.6	0.2	11.7	1.4	-36
7	9.9	0.4	19.0	1.6	-31
8	3.0	1.0	7.3	2.8	-37
9	4.6	0.4	11.7	1.4	-38
10	16.2	0.4	41.6	2.5	-13
11	11.2	0.7	31.2	2.8	-23
12	6.6	0.8	18.6	1.2	-22
SITE #2					
1	2.8	3.8	14.0	1.6	-32
2	7.8	3.3	29.8	0.9	-10
3	6.9	3.2	19.5	0.9	-24
4	4.5	5.7	10.3	1.0	-28
5	8.0	7.3	16.2	2.3	- 8
6	6.5	8.0	14.1	0.7	-36
7	2.9	2.8	12.8	0.6	-38
8	1.6	1.5	9.3	0.8	-43
9	4.1	1.3	8.8	1.3	-31
10	10.4	13.0	32.1	1.3	-14
11	10.4	6.6	28.6	1.6	-21
12	7.6	7.0	24.1	1.4	-25
SITE # 3					
1	6.8	2.7	17.6	2.4	-26
2	14.1	5.8	31.8	1.3	- 4
3	8.8	2.8	20.5	2.8	-27
4	9.2	4.3	17.3	1.8	-35
5	8.8	5.7	19.3	1.6	-16
6	8.6	1.6	17.1	0.9	-36
7	8.8	1.1	17.8	1.4	-35
8	*	*	*	*	*
9	5.5	2.1	10.9	2.3	-43
10	6.1	5.2	10.0	2.4	-42
11	20.4	2.8	28.4	2.7	-13
12	12.4	5.3	21.9	1.2	-25

[a] S.D. $_{pooled}$ = 1.3 ppbv benzene; S.D. $_{pooled}$ = 2.9 ppbv toluene.
[b] S.D. site #1: 0.50mg/g t,t-MA, 0.70g/g HA; S.D. site #2: 3.3 mg/g t,t-MA, 0.40g/g HA; S.D. site #3: 1.7 mg/g t,t-MA; 0.60 g/g HA.
* No data obtained at these sites for this week because occupants were not at home.

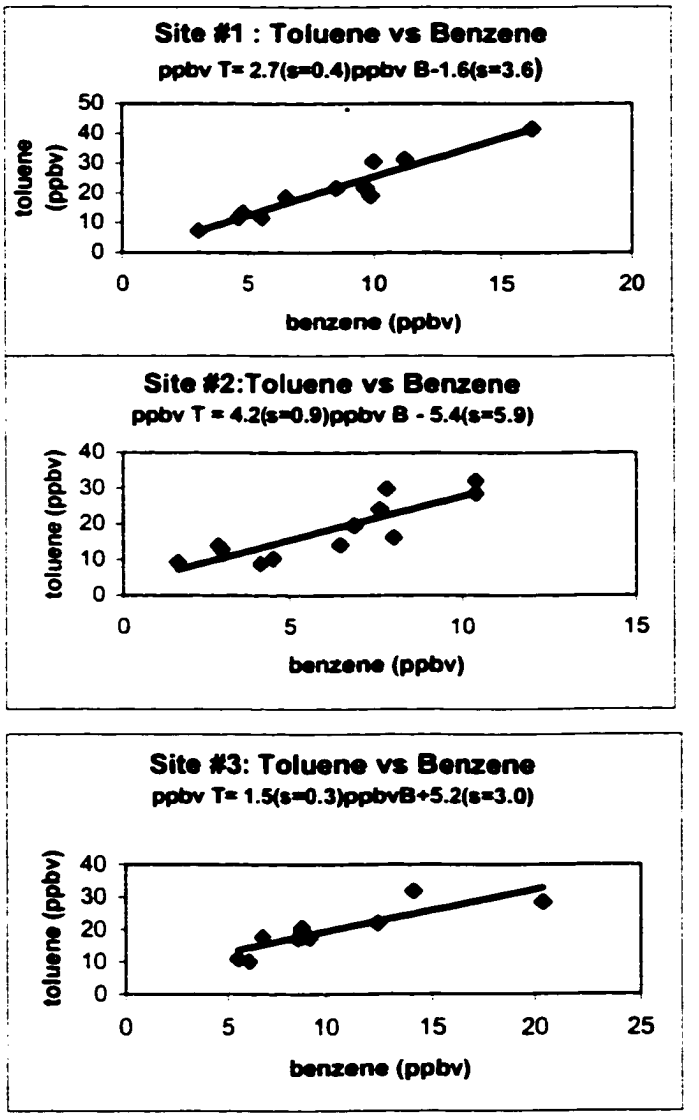


Figure 3.4: Living area toluene and benzene mixing ratios for all sites.

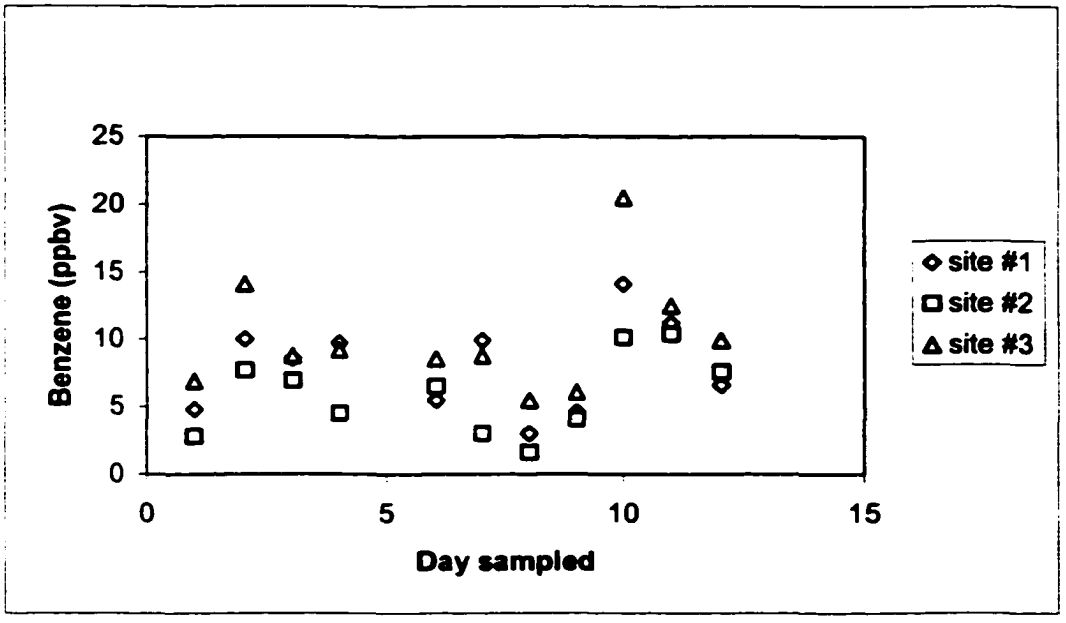


Figure 3.5: Living area benzene mixing ratios for all sites and the day sampled.

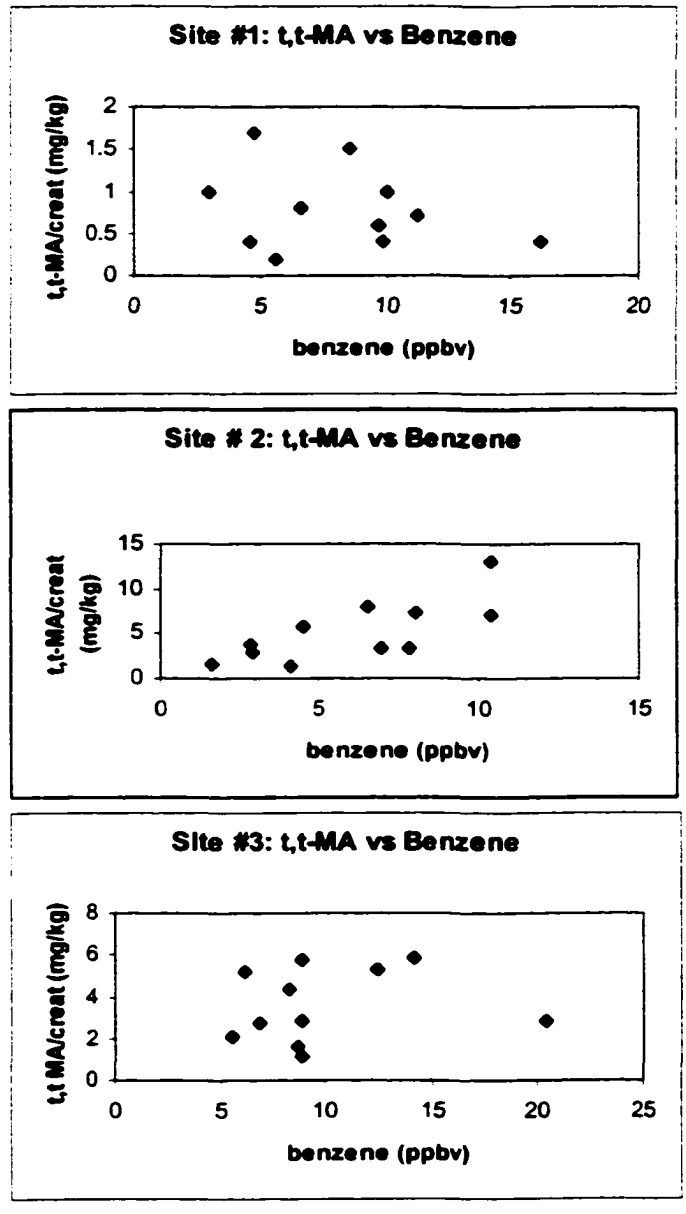


Figure 3.6 : t,t-MA vs benzene mixing ratio for each site. R^2= 0.076 for site #1; R^2 = 0.54 for site #2; R^2=0.022 for site #3.

There is a strong positive correlation between benzene mixing ratio and outdoor temperature for all three sites over a temperature range of – 40 to –10 ° C with a increase from 5 ppbv to 10 ppbv benzene (Figure 3.7). This trend was also observed for toluene mixing ratios at each site. Indoor temperatures and garage temperatures at each site were consistent. Indoor air temperatures ranged between 60 to 70 ° F and garage temperatures ranged between 55 to 70 ° F. This is a novel observation since indoor levels were predicted to increase during the winter months because homes were shut tight.

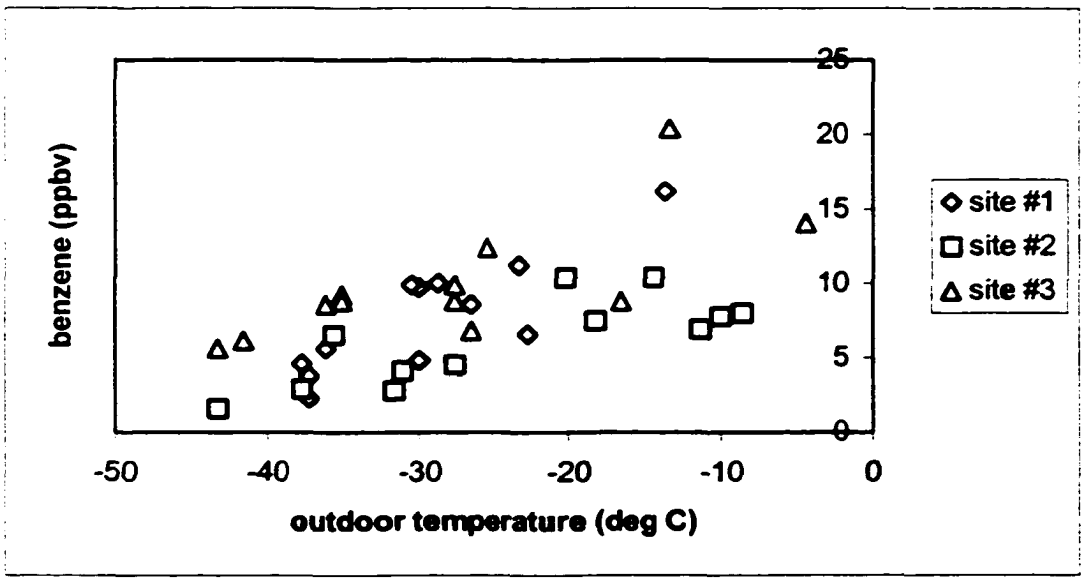


Figure 3.7: Living area benzene mixing ratios versus outdoor ambient temperatures.

Winter 1999 and Summer 2000 Air Measurements

Benzene and toluene mixing ratios were measured simultaneously in the living areas and garages of two homes for two sets of four days both in November of 1999 and May-June of 2000. The thermal desorption method was used to analyze all samples. No biomarker measurements were made during this period. The environments of both homes were systematically perturbed during this study. This is in contrast to the results reported for the prior seasons that are for the homes in their normal state.

A summary of the data for both seasons for House B and House M are given in Table 3.3 and Table 3.4. The design used for the systematic perturbations can be found in Tables 2.2 and 2.3. The data is reported as the average values for duplicate tubes sampled at the same time at each location and area of the home. The benzene and toluene levels were higher in the garage than the living area for both of the homes. The living area levels were higher in Home M than in Home B in both seasons. The summer levels were the highest in the garage of Home M.

Table 3.3: Summary of benzene and toluene mixing ratios for Home B, both seasons [a, b].

		Living Area		Garage	
		Benzene	Toluene	Benzene	Toluene
November 1999	Day	(ppbv)		(ppbv)	
	1	4.2	5.7	25.5	50.3
	2	5.0	5.8	32.5	56.8
	3	5.4	13.0	46.4	109.0
	4	6.7	11.4	67.0	142.0
	1	2.2	5.4	21.8	48.3
	2	7.3	14.3	64.9	182.0
	3	3.4	6.2	14.4	34.3
	4	4.7	8.9	25.2	62.7
May-June 2000					
	1	2.9	6.2	34.3	87.9
	2	2.7	5.8	59.6	177.5
	3	3.4	6.6	91.5	194.8
	4	1.1	4.4	69.4	167.8
	1	nd	nd	110.8	225.5
	2	1.2	3.7	54.3	122.2
	3	nd	1.2	7.5	14.4
	4	1.7	2.1	40.8	110.8

[a] Living area of both homes in winter S.D. $_{pooled}$ = 1.4 ppbv benzene; 2.6 pbbv toluene. Garage of both homes in winter S.D. $_{pooled}$ = 1.1 ppbv benzene; 2.4 ppbv toluene.

[b] Living area of both homes in summer S.D. $_{pooled}$ = 1.4 ppbv benzene; 1.6 ppbv toluene. Garage of both homes in summer S.D. $_{pooled}$ = 6.8 ppbv benzene; 20 ppbv toluene.

Table 3.4: Summary of benzene and toluene mixing ratios for Home M, both seasons[a,b].

		Living Area		Garage	
		Benzene	Toluene	Benzene	Toluene
November 1999	Day	(ppbv)		(ppbv)	
	1	15.7	37.0	32.8	75.3
	2	16.0	34.6	35.0	89.6
	3	25.2	61.4	26.3	68.9
	4	20.5	50.4	41.1	105.0
	1	12.4	37.0	35.1	91.2
	2	16.3	35.7	37.5	88.4
	3	17.2	33.3	24.4	62.1
	4	20.1	57.2	27.4	80.1
May-June 2000					
	1	6.8	16.4	145.6	412.8
	2	15.2	45.8	116.3	389.7
	3	6.7	19.8	36.0	97.3
	4	24.0	56.5	125.6	339.5
	1	2.2	7.4	206.2	591.4
	2	32.3	86.5	52.0	141.5
	3	3.9	18.3	304.2	638.5
	4	39.2	104.2	69.2	208.5

[a] Living area of both homes in winter S.D. $_{pooled}$ = 1.4 ppbv benzene; 2.6 pbbv. Garage of both homes in winter S.D. $_{pooled}$ = 1.1 ppbv benzene; 2.4 ppbv toluene.

[b] Living area of both homes in summer S.D. $_{pooled}$ = 1.4 ppbv benzene; 1.6 ppbv toluene. Garage of both homes in summer S.D. $_{pooled}$ = 6.8 ppbv benzene; 20 ppbv toluene.

The levels of benzene and toluene are highly correlated at both homes for both seasons (Figures 3.8, 3.9, 3.10 and 3.11). A weighted linear regression least squares algorithm was used to fit the data for these plots (Ogren and Norton, 1992). Home M is the same home as site #3 (Figure 3.4) in the 1998-1999 studies. The slopes of these curves are different but the sampling conditions (month, time duration, fuel sources in garage, outdoor temperatures) were also different. The non-zero y-intercepts for Home B garage in the winter, Home M living area in the winter, and Home M garage in the summer suggests an additional source or differential loss. No trends were observed in either home between the living area and garage benzene mixing ratios, garage or outdoor temperatures. The temperatures measured in the living area, in the garage, and outdoors for both homes and seasons are summarized in Table 3.5. The desired temperatures in the winter systematic design for the living area (normal and +5 deg) and the garage (normal and 10 deg lower) proved difficult to achieve in these homes during the monitoring periods.

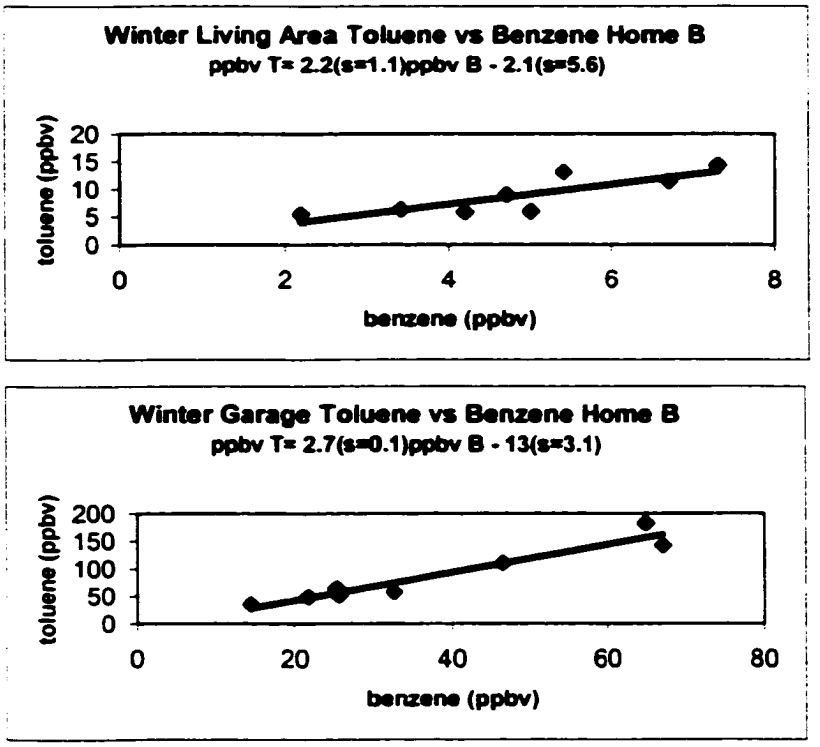


Figure 3.8: Winter toluene and benzene mixing ratios for Home B.

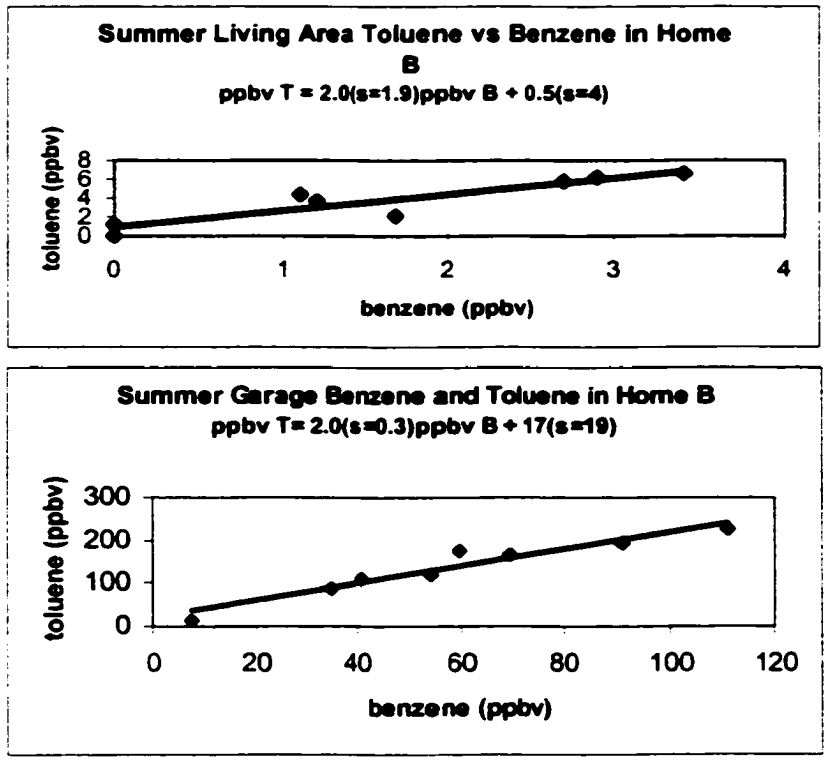


Figure 3.9: Summer toluene and benzene mixing ratios for Home B.

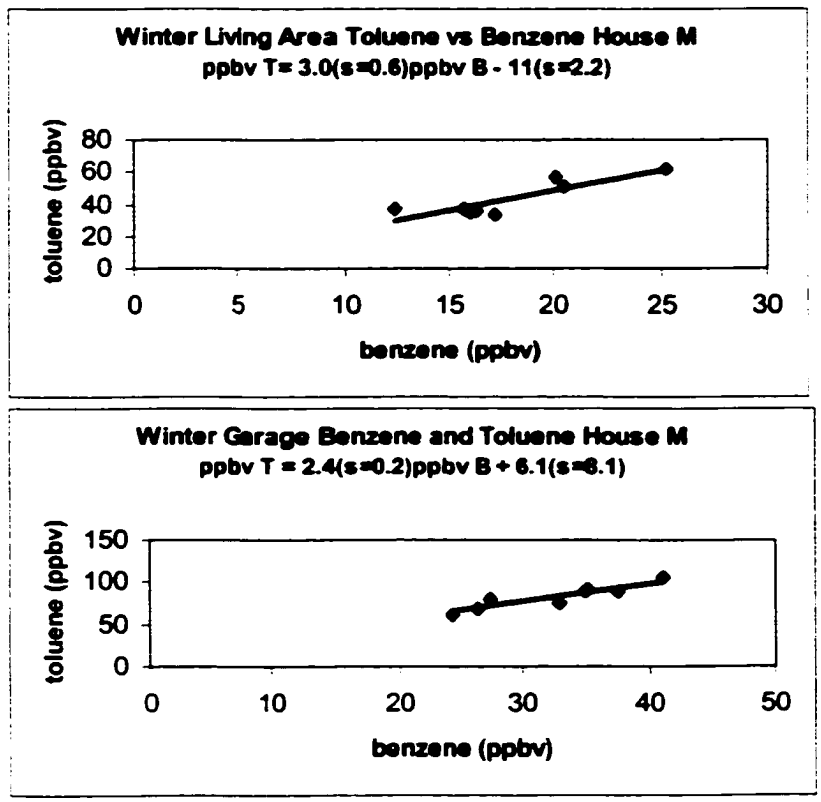


Figure 3.10: Winter toluene and benzene mixing ratios for Home M.

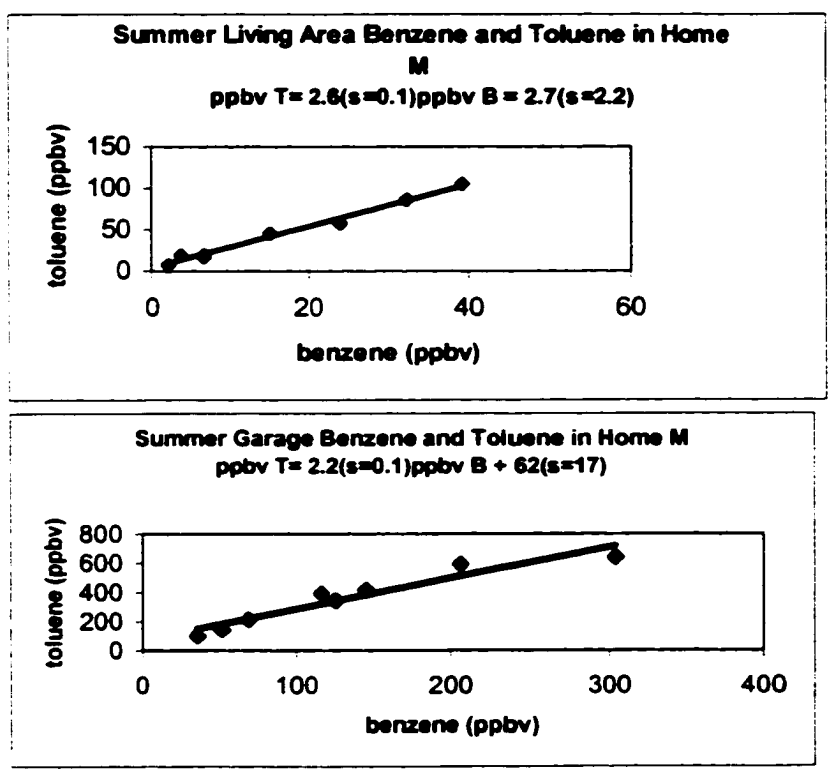


Figure 3.11: Summer toluene and benzene mixing ratios for Home M.

TABLE 3.5: Summary of Temperatures Measured at Home B and Home M, both Seasons.

	Temperatures (° C)		
November 1999	**Living Area**	**Garage**	**Outdoor**
Home B	15.0	12.2	-14.5
	17.7	12.7	-21.7
	15.5	15.0	-17.7
	18.3	15.0	-16.6
	18.3	14.4	-20.0
	18.3	8.3	-21.1
	15.5	9.4	-23.3
	15.0	12.2	-30.0
Home M	22.7	20.0	-25.5
	23.8	20.0	-21.1
	25.5	26.6	-21.1
	20.5	26.1	-20.0
	19.4	24.4	-21.7
	22.2	20.5	-22.2
	20.0	18.8	-24.4
	23.8	25.5	-33.3
May-June 2000			
Home B	16.1	16.1	8.6
	16.1	21.1	11.4
	15.0	20.5	7.8
	17.2	20.5	8.3
	24.4	25.0	19.4
	22.2	20.5	21.7
	24.4	20.0	18.8
	21.6	19.4	17.7
Home M	20.5	21.1	21.1
	21.1	20.5	22.8
	20.5	16.6	20.5
	20.5	20.5	20.5
	18.9	22.8	24.4
	20.5	22.8	22.8
	19.4	22.8	24.4
	19.4	22.8	21.6

3.2 Furnace Duty CycleMeasurements

Furnace duty cycle measurements were made during the November 1999 study using dataloggers for the first block of four days for both homes. The furnace duty cycle of both homes are consistent for each home, with Home M having the higher duty cycle (Table 3.6). Thermocouple temperatures of the furnace stack were taken every minute and stored in the datalogger. The duty cycle was calculated as the quotient of the total time the furnace was on to the total time of the monitoring period for each day. The furnace was defined to be "on" when the thermocouple reached 250 °C and "off when the thermocouple temperature cooled to 80 °C.

3.3 Ventilation Measurements

Ventilation measurements were made in the November 1999 study and the zonal air infiltration rates and exfiltration rates were calculated for each of homes using multicompartment model software program developed at Brookhaven National Laboratory (Dietz et al., 1985). Model – estimated source strengths, estimated benzene and toluene levels with their uncertainties were also calculated for each zone using an estimated surface deposition velocity. There were three zones designated to both homes: garage (zone 1); main level (zone 2); second floor (zone 3). In Home B the living area air measurements were made on the second floor. In Home M the living area air measurements were made on the same level as the garage.

Table 3.6: November 1999 calculated duty cycle for both homes.

			Temperatures (deg C)		
	Day	Duty Cycle	living area	garage	outside
Home B	1	0.5	15.0	12.2	-14.5
	2	0.5	17.7	12.7	-21.7
	3	0.5	15.5	15.0	-17.7
	4	0.5	18.3	15.0	-16.6
Home M	1	0.8	22.7	20.0	-25.5
	2	0.9	23.8	20.0	-21.1
	3	0.9	25.5	26.6	-20.1
	4	0.8	20.5	26.1	-21.7

The estimated benzene source strengths for the three zones in each home are summarized in Table 3.7 and Table 3.8. These values are the multicompartment model-estimated source strengths in each zone computed with an estimated surface deposition velocity. The largest source strengths measured in these homes was in the garage (zone 1) of Home M and ranged from 15 mg/h to 26 mg/h for benzene. Estimated source strengths measured in the garage (zone 1) for Home B ranged from 6.0mg/h to 28 mg/h for benzene. The estimated source strengths in the garage of Home B were similar to Home M on two days: Block one-day 3 and Block 2 – day 2. In Home B on day 3 of Block 1 there was a calculated toluene source strength in zone 3 the living area which was statistically significant. However this value is still much less than the calculated source strength for toluene in the garage on that day. In both homes, the magnitudes of the calculated source strengths show that the main source of benzene and toluene in other zones of these homes originates from the garage.

Diagrams of both homes and the ventilation summarize data for the same days (Figure 3.12 and Figure 3.13). Table 3.9 provides a legend to accompany these diagrams that indicates the calculated error of the values in the diagram.

Table 3.7: Estimated Benzene and Toluene Source Strengths (mg/h) In Home B [a, b].

Benzene	Day Sampled							
Location	1	2	3	4	1	2	3	4
ZONE 3 [c]	0.10	0.20	-.010	-0.20	-0.40	-0.2	0.3	-0.001
ZONE 2 [d]	-0.01	-0.10	-0.20	-0.40	-0.30	-.01	-0.01	-0.10
ZONE 1 [e]	9.0*	11*	17*	19*	11*	28*	6.0*	16*
Toluene								
Location	1	2	3	4	1	2	3	4
ZONE 3 [c]	0.50	0.10	3.0	1.0*	0.20	-0.40	1.0*	0.10
ZONE 2 [d]	-0.10	-0.30	0.80	-0.20	-0.90	-0.10	-0.20	-0.10
ZONE 1 [e]	25*	26*	56*	59*	32*	11*	19*	51*

[a] Median of calculated error for benzene: zone 1, ± 3.0 ; zone 2, ±0.30; zone 3, ±0.30.
[b] Median of calculated error for toluene: zone 1, ±7.0 ; zone 2, ±0.50; zone 3,± 0.50.
[c] Second floor and main living area
[d] First Floor
[e] Garage
* Values are significantly greater than zero based upon one standard deviation unit.

Table 3.8: Estimated Benzene and Toluene Source Strengths (mg/h) In Home M [a, b].

Benzene	Day Sampled							
Location	1	2	3	4	1	2	3	4
ZONE 3 [c]	0.10	0.10	1.0	0.40	-1.0	-2.0	-0.10	-2.0
ZONE 2 [d]	0.040	0.60	10	1.0	-1.0	2.0	3.0	8.0*
ZONE 1 [e]	20*	20*	15*	26*	23*	25*	16*	24*
Toluene								
Location	1	2	3	4	1	2	3	4
ZONE 3 [c]	6.0	6.0	13*	9.0*	3.0	1.0	4.0	1.0
ZONE 2 [d]	7.0	0.10	36*	10*	6.0	8.0	7.0	4.0
ZONE 1 [e]	61*	70*	53*	86*	79*	75*	52*	88*

[a] Median of calculated error for benzene: zone 1, ± 3.0 ; zone 2, ± 3.0 ; zone 3, ± 2.0.
[b] Median of calculated error for toluene: zone 1, ± 6.0 ; zone 2, ± 9.0; zone 3, ±7.0.
[c] Second floor
[d] First Floor and main living area
[e] Garage
* Values are significantly greater than zero based upon one standard deviation unit.

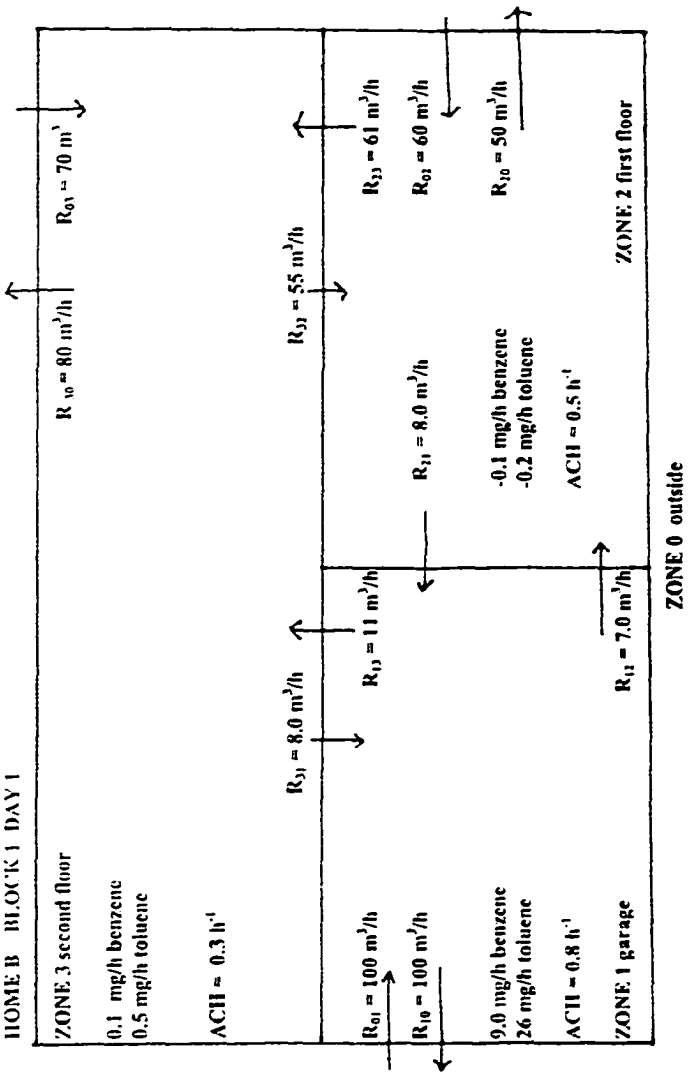


Figure 3.12: Ventilation diagram for Home B.

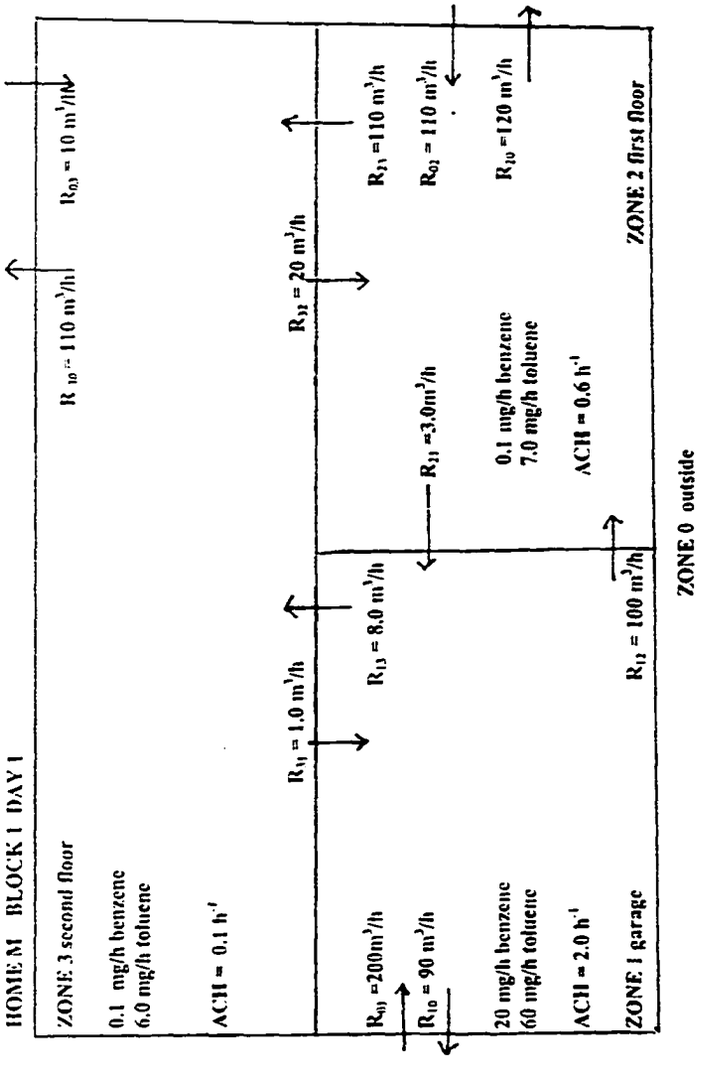


Figure 3.13: Ventilation diagram for Home M.

Table 3.9: Median calculated standard errors for values in ventilation diagrams of both homes.

HOME B

Value	R_{01}	R_{10}	R_{02}	R_{20}	R_{03}	R_{30}
±	13	17	10	18	15	23

Value	R_{12}	R_{21}	R_{13}	R_{31}	R_{23}	R_{32}
±	2.0	2.0	3.0	2.0	15	8.0

Value	ACH 1	ACH 2	ACH 3
±	0.10	0.10	0.10

HOME M

Value	R_{01}	R_{10}	R_{02}	R_{20}	R_{03}	R_{30}
±	21	26	29	38	16	19

Value	R_{12}	R_{21}	R_{13}	R_{31}	R_{23}	R_{32}
±	23	1.0	13	0.20	34	3.0

Value	ACH 1	ACH 2	ACH 3
±	0.20	0.20	0.10

The most notable aspect of these diagrams is that is that the air flow from the garage to the living area (zone 2) for Home M is consistently one order of magnitude larger than Home B suggesting the increased possibility of exposure to airborne pollutants from the garage for the occupants of Home M.

A leakiness factor, L, was calculated for both homes form the equation given below Table 3.10 and Table 3.11). A moderately sheltered terrain factor (descriptive of the surrounding topography), c , of 3 was used in the calculations. A wind speed, u, of of 0.36 m/s was assumed. The ACH excludes the garage zone and uses the air infiltration rates computed by the model for zones 2 and 3 divided by the total volume of those zones.

$$L = \frac{ACH}{0.006\Delta t + (0.03/c)u^{1.5}}$$ **(Dietz et al., 1986)**

A reasonably airtight home has an L between 1.0 and 1.5. A moderately airtight home has an L value between 1.6 and 2.4. A leaky home has an L value > 2.5 to 3.0. Home M can be considered a reasonably airtight home and Home B can be considered a moderately airtight home. Home B has an elaborate forced air exchange system and Home M has none. It is likely that Home B is tighter but ventilation puts it into the leaky class.

Table 3.10: House B Leakiness factor for November 1999.

Day	ACH (h^{-1})	Δt(deg C)	Leakiness factor , L
1	0.30	29	1.8
2	0.40	39	1.6
3	0.40	39	1.6
4	0.30	34	1.6
1	0.40	38	2.0
2	0.50	39	2.0
3	0.50	38	2.0
4	0.50	45	1.8

Table 3.11: House M Leakiness factor for November 1999.

Day	ACH (h^{-1})	Δt(deg C)	Leakiness factor , L
1	0.40	48	1.2
2	0.40	45	1.6
3	0.50	46	1.7
4	0.40	40	1.6
1	0.30	41	1.3
2	0.30	44	1.1
3	0.30	44	1.1
4	0.40	57	1.1

3.4 Winter 1999 and Summer 2000 Factorial Experimental Design

Pertubations of the environment during the November sampling included living area and garage temperature adjustment and addition/removal of fuel sources from the garage and the outside of the house. Pertubations of the environment during the May - June sampling were directed at ventilation and fuel source aspects which included opening/closing windows in the home, garage, flues in the woodstove or fireplace and addition /removal of fuel sources from the garage.

These results provided interesting insights into important factors that affect the levels of benzene and toluene in the living areas and garages of homes. Some, but not all, small engines contribute aromatics to the garage. None of the small engines contributed to the levels in the living area. A home with forced ventilation system has lower levels of these compounds in the living area than those with natural ventilation. The opening of a window inside a home has a more significant effect on living air levels than does opening a garage window or the flue to an indoor fireplace or woodstove.

The benzene and toluene air mixing ratios for the living area and garage of both homes were used as the responses in the factorial experimental design software, Design Expert (Stat-Ease Inc, 1996), for both seasons. The goal of this approach of analyzing the results for mixing ratios from the experiments where several factors were tested simultaneously is to create a predictive model in which significant factors are identified that affect benzene and toluene mixing ratios measured in

these homes. An Analysis of Variance (ANOVA) test is applied to the responses within the design matrix to test the null hypothesis that none of the variables included in the matrix result in an effect on the results obtained under the specific experimental conditions. The ANOVA provides an estimate of the pooled S.D. from the square root of the mean square term that is an estimate of the model's variance.

An effect size for each variable is calculated from the model and for this work, an effect size equal to or greater than twice the pooled S.D. was defined to be statistically significant. Normal probability plots are used to check the validity of use of an ANOVA, i.e., that the data is normally distributed. The data is also analyzed for residuals and outlier detection. Diagnostic plots of factors and effect size were also used to confirm the statistical results of the model.

Winter 1999 Factorial Experimental Design Results

The only significant factor found using the living area results for benzene and toluene was the house type (Figure 3.14 and Figure 3.15). Normal plots of effects are used to visualize those that are normally distributed (occur along a relatively straight line) and those true effects that cannot be attributed to chance occurrence (those that fall off the line to the right or left) (Box et al., 1978). Home M (average benzene: 18ppbv, toluene: 43.3ppbv) had consistently higher mixing ratios than Home B (average benzene:4.8 ppbv, toluene: 8.8ppbv). This makes sense because the ventilation characteristics of each home, discussed previously, do affect the level of these compounds inside each home.

The only significant factor found using the benzene and toluene results for the garages of both homes was the presence of a metal can containing gasoline inside the garage in Home B (Figure 3.16, Figure 3.17, Figure 3.18). The toluene mixing ratios were similar in both garages (Home B ave. toluene: 85.6ppbv; Home M ave. toluene: 82.3ppbv). This similarity supports the observation made previously that showed a strong correlation between mixing ratios of aromatics and number of small engines in the garage. The gasoline storage can that leaks could contribute to the level of those compounds inside a garage.

The living area and garage temperatures proposed in the systematic design were not the temperatures actually achieved in the homes during the study (Table 3.5).

Attempts to manipulate these temperatures with zone controllers in Home B and thermostat settings in Home M to the systematic design setpoints for each monitoring period were unsuccessful.

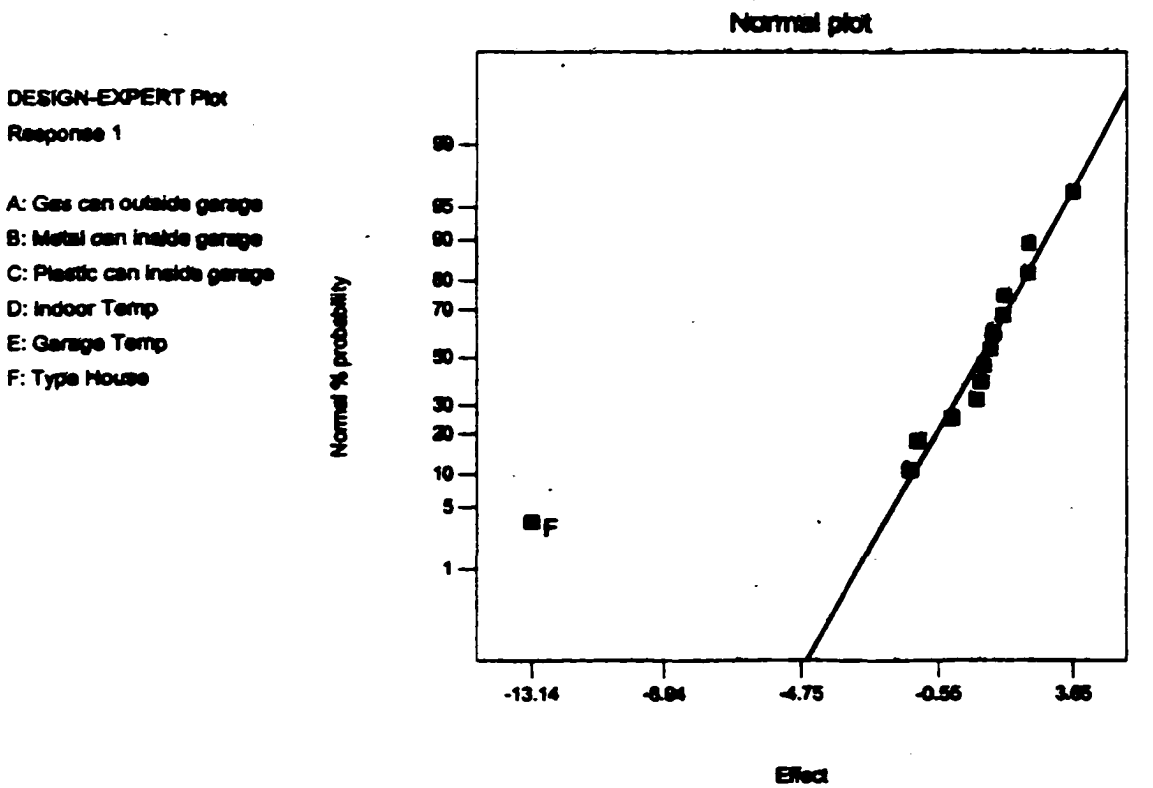


Figure 3.14: November 1999 Factorial design results for benzene in the living area of both homes.

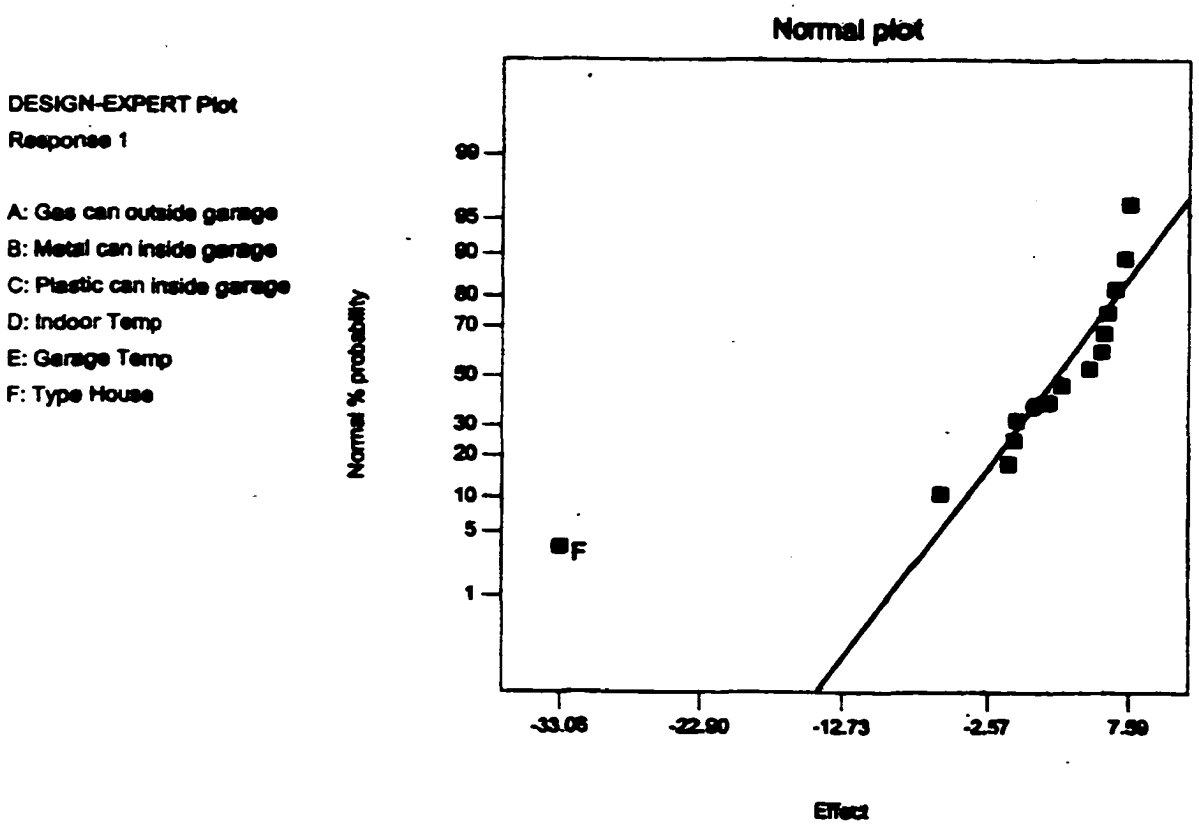


Figure 3.15: November 1999 Factorial design results for toluene in the living area of both homes.

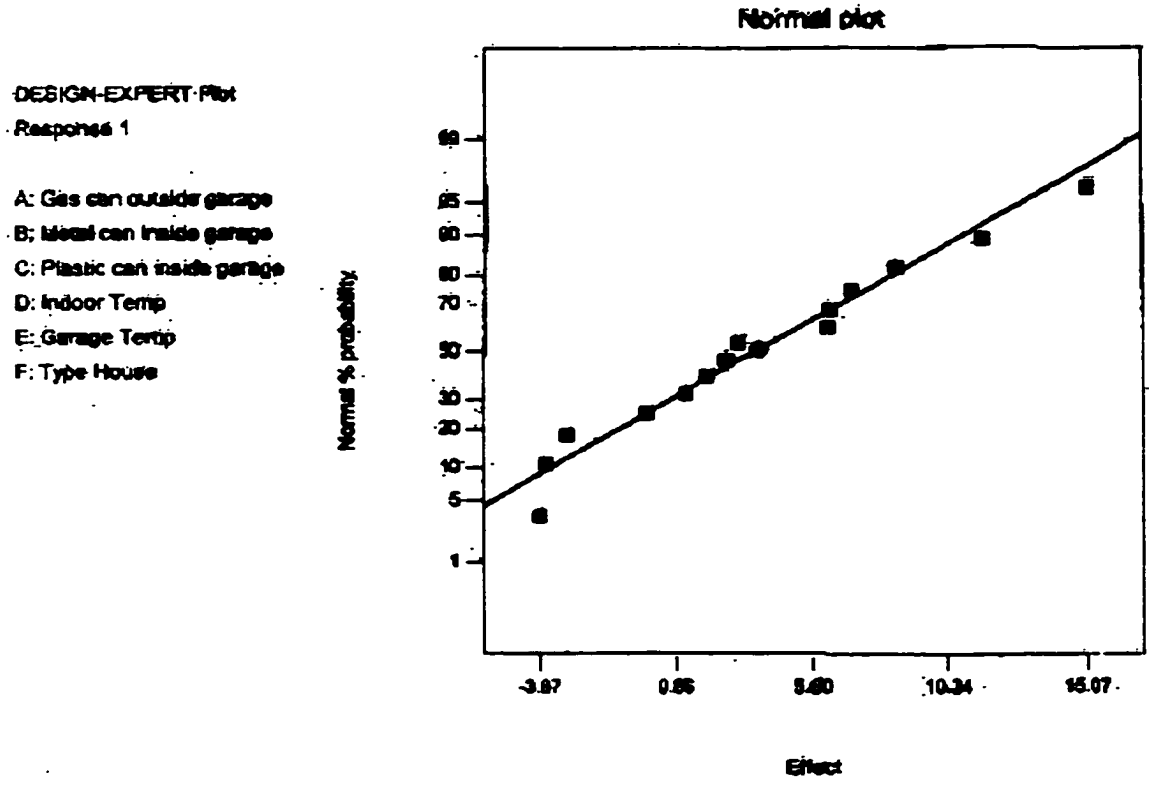


Figure 3.16: November 1999 Factorial design results for benzene in the garages of both homes

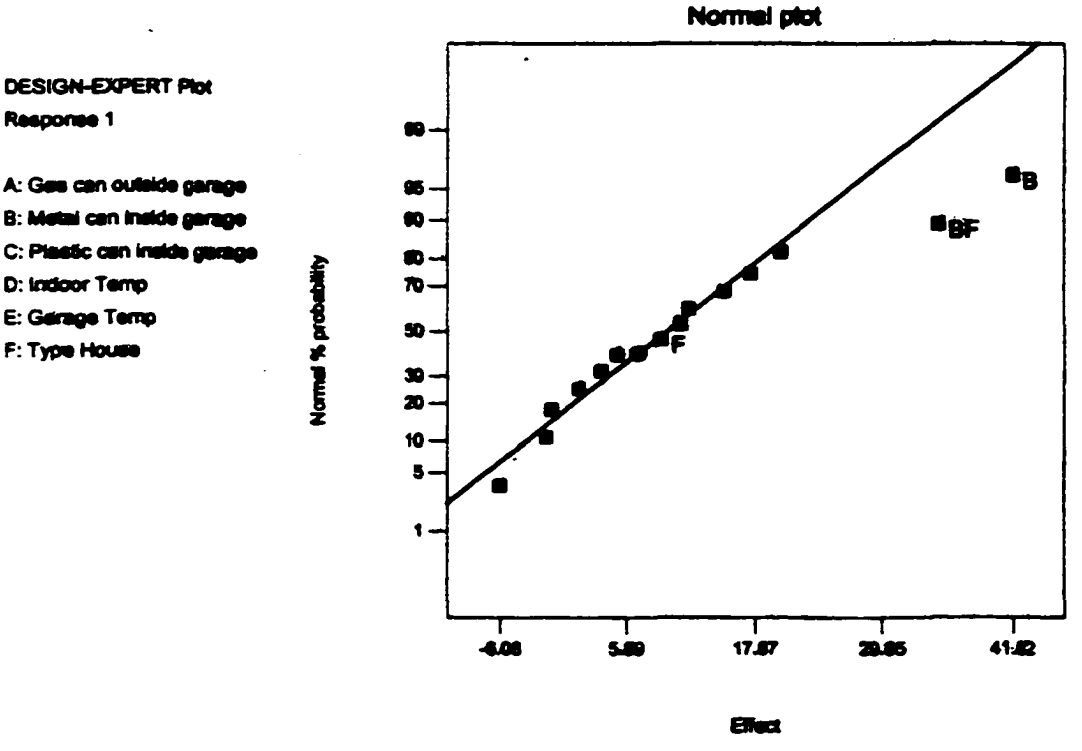


Figure 3.17: November 1999 Factorial design results for toluene in the garages of both homes.

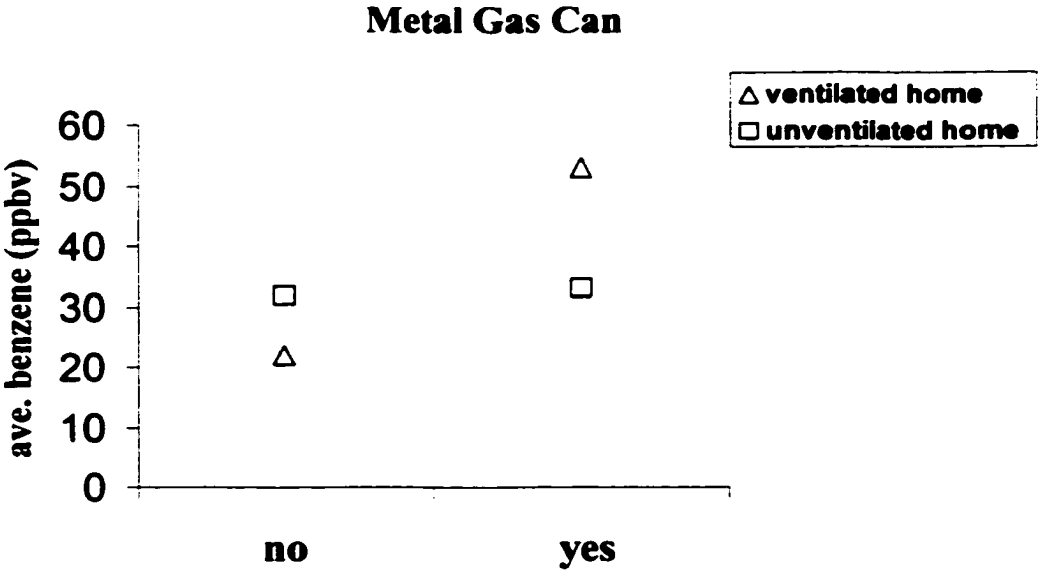


Figure 3.18:Metal gas can effect in garages of both homes.

Summer 2000 Factorial Experimental Design Results

The significant factor found for the living area of levels of benzene and toluene in the summer were the type of house and the opening of an inside window (Figure 3.19, Figure 3.20, Figure 3.21). Home M had consistently higher levels in the living area than Home B. (averages Home M; 16.2 ppbv benzene, 44.3 ppbv toluene; averages Home B: 2.2 ppbv benzene, 4.8 ppbv toluene). An outlier was detected in this data and so this model is based on five experiments.

No significant factors for summer were identified using the benzene and toluene mixing ratios from the garages (Figure 3.22 and Figure 3.23). Home M levels were over double that of Home B (averages Home M: 131.8 ppbv benzene, 352.4 ppbv toluene; averages Home B : 58.5 ppbv benzene, 137.6 ppbv toluene). Again, this supports the previous observation that the presence of small engines contributes to aromatics in the garage air. Home M garage also contained a snow blower and lawn mower besides the plastic can of gasoline and chain saw that were used in the experimental design. Home B garage only contained the plastic gasoline can and chain saw used in the study design.

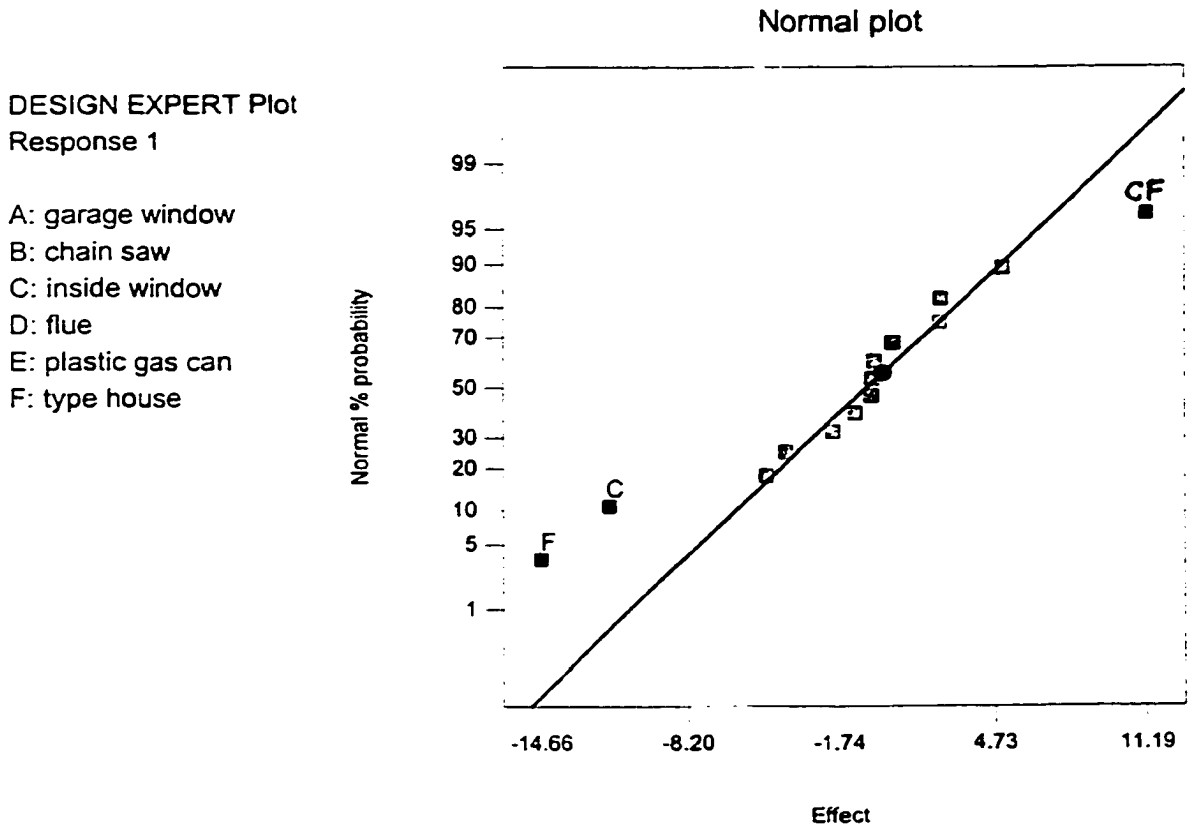


Figure 3.19: May-June 2000 Factorial design results for benzene in living area of both homes.

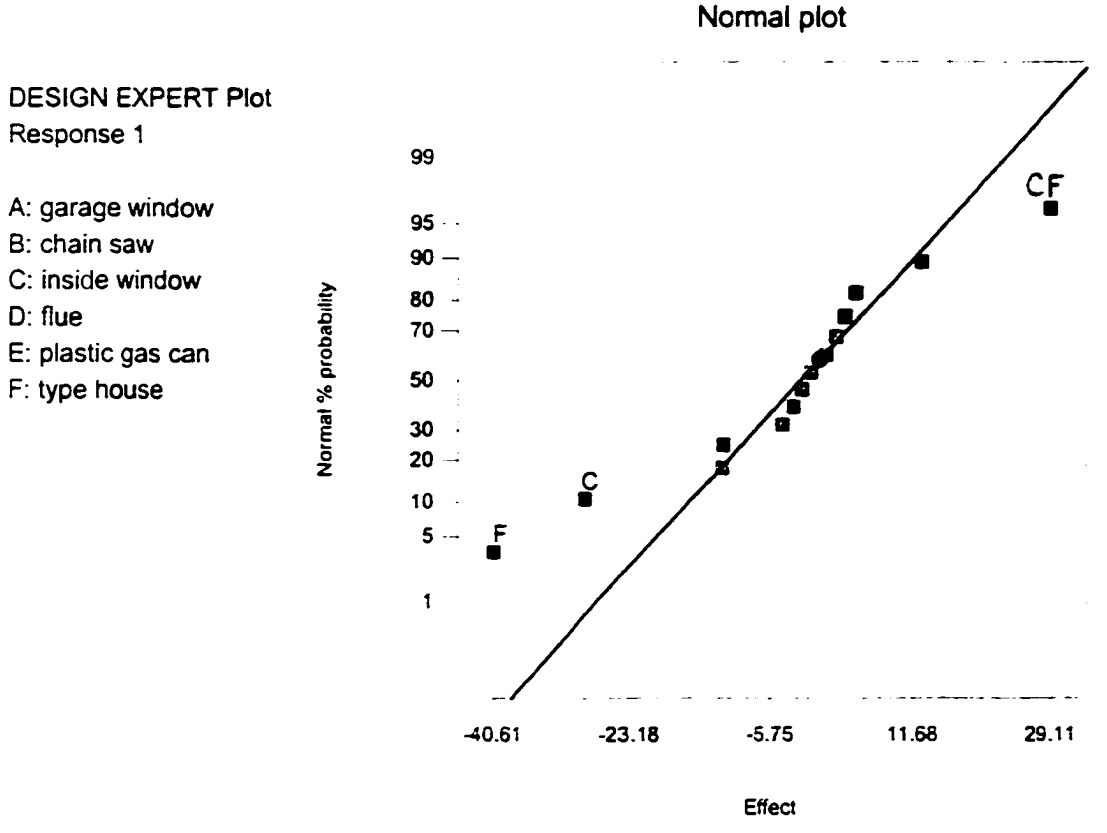


Figure 3.20: May-June 2000 Factorial design results for toluene in the living areas of both homes.

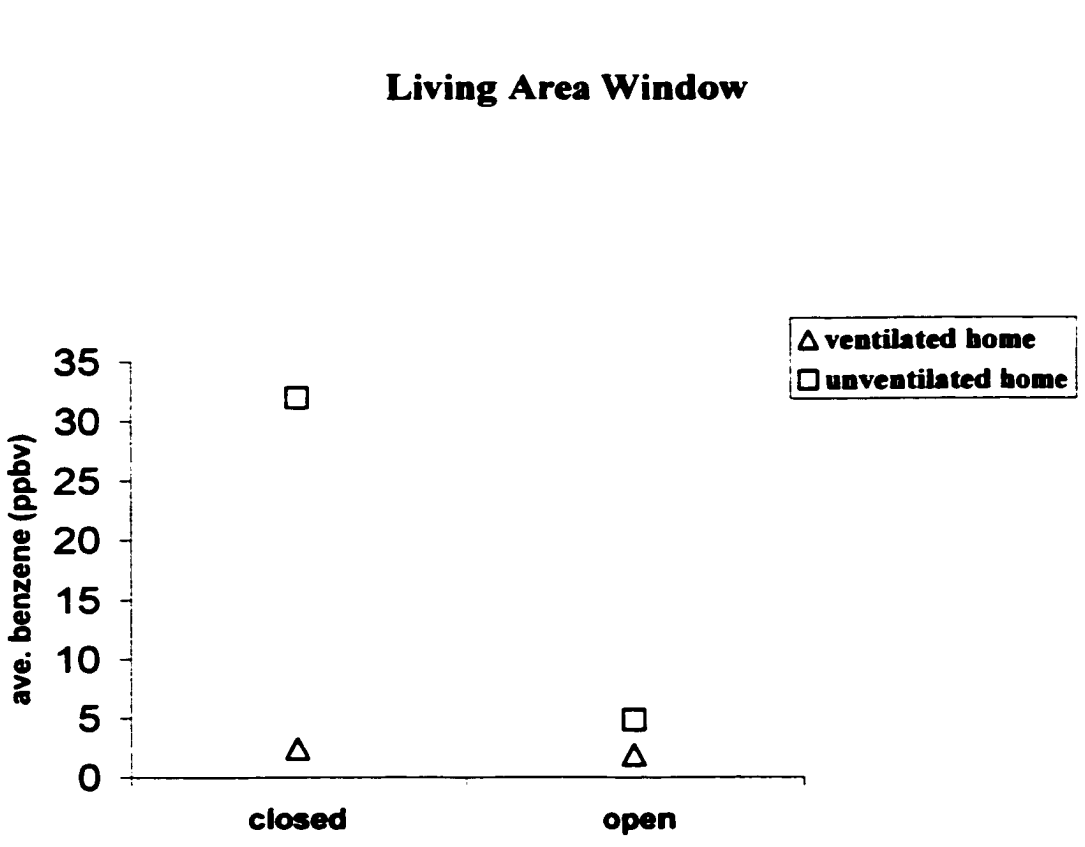


Figure 3.21: Inside window and home type effects.

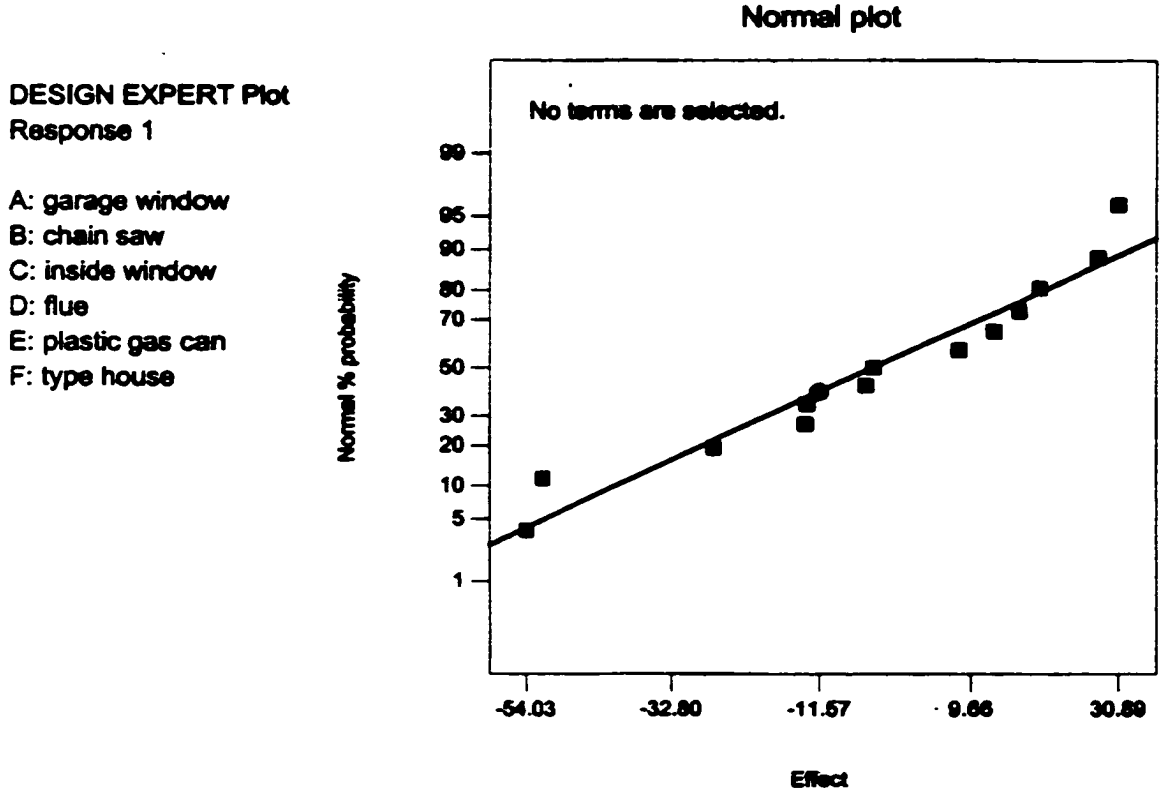


Figure 3.22: May – June 2000 Factorial design results for benzene in the garages of both homes.

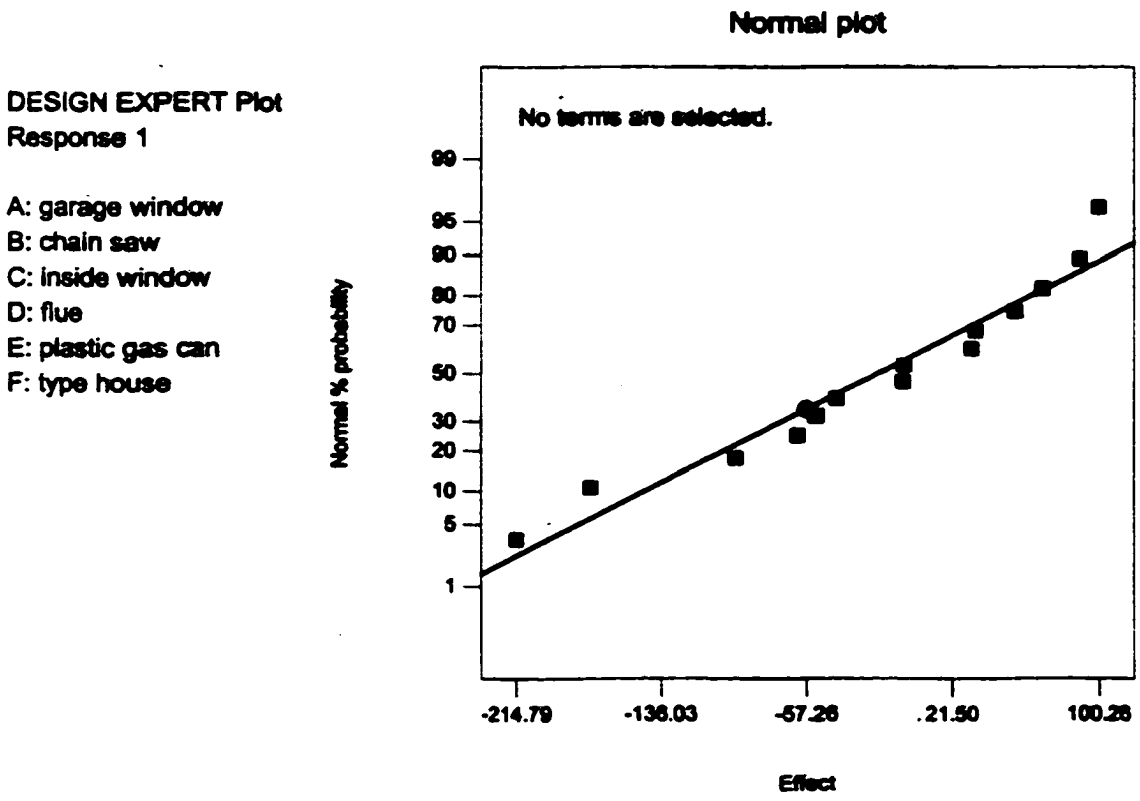


Figure 3.23: May-June 2000 Factorial design results for toluene in garages of both homes.

Chapter 4. Discussion

Two major anthropogenic sources of benzene and toluene in air are fuel emissions and tobacco smoke (U.S. Dept of Health and Human Services, 1994). Indoor exposure to benzene in residences without smokers appear to originate from gasoline storage in attached garages (Fellin and Otson, 1994; Weaver et al., 1996). Depending on the air flow from an attached garage and gasoline storage practices (Wallace, 1990; Thomas et al., 1993). Benzene has been found at levels 2 to 5 times higher in the indoor air of a home than outdoors.. Higher levels of toluene are almost always found indoors due mostly to infiltration of automobile emissions and the result of gasoline storage in attached garages (Chan et al., 1991; Hodgson et al., 1991; Kelly et al., 1993; Thomas et al., 1993).

Benzene and toluene are indoor air pollutants of concern in Alaskan homes with attached garages (Isbell et al., 1999). Many homes have attached garages containing large vehicles; fuel in containers; and small engines on snowmobiles, motorcycles, lawnmowers, and chainsaws. People spend a large amount of time at home indoors during approximately six months of the winter maximizing their exposure to these compounds. The homes in Fairbanks are generally designed and built with tight construction to minimize heat loss and associated cost during long and cold winter months.

Gasoline storage in attached garages may he more common in Alaska than in other states. This presents a higher exposure for Alaska residents to benzene and toluene by inhalation of indoor air. Alaskan gasoline is produced from Prudhoe Bay crude oil at two instate facilities. Data from the Williams facility, a topping plant, near Fairbanks, show that the gasoline contains about 38%(v/v) with the benzene composition about 3-4%(v/v). This is significantly higher than the standards set for reformulated gasoline (RFG) that permit a total aromatic content of 25%(v/v) and regulate the benzene content to ≤1% (v/v) (Sawyer, 1993). All gasolines, despite brand name, in the interior are based out of the William's stock. Consequently, monitoring for these compounds from Williams produced gasolines in indoor air is relevant to human health given the gasoline composition and storage practices in a typical interior Alaskan garage.

In this study, I found that the thermal desorption protocol (T0-17) provided a simple, solvent free, means of sampling and analyzing for benzene and toluene in air. The alternative charcoal tube method may give slightly lower results because the extraction efficiency may be less than 100%. The precision of the GC instrumental method for benzene and toluene was higher than that observed for the results of the actual samples. This is not a surprising outcome since the procedure for the actual samples also includes the variance from the sampling procedure and storage of samples.

Benzene in the living areas of homes monitored in this study ranged from 1 to 72 ppbv. The only exception was for Home B for two days in the second block of summer samples and this was because residents left different windows open inadvertently during the monitoring period (Table 3.3). A 1990-91 study conducted in Valdez, Alaska found mean indoor concentrations of benzene ranged form 2 to 6ppbv in the summer with an individual maximum of 66 ppbv benzene. In the winter, a mean range of 4 to 9ppbv was found with an individual maximum of 72 ppbv (Goldstein et al., 1992). Other studies found benzene indoor air concentrations to range between 2 to 9 ppbv (Thomas et al., 1993; Wallace et al., 1986; Tsai and Weisel, 2000). My results are in agreement with these studies and confirm the low levels in indoor air.

The garage was clearly shown to be the source of benzene and toluene in the indoor air of Home B and Home M (Table 3.17 and Table 3.18). Garage levels of benzene were found to be two to three times higher than indoors levels for Home M which had no elaborate ventilation system. The garage levels of benzene were 6 to 10 times higher than the indoor levels for Home B that did have an elaborate ventilation system. Winter levels of benzene in Home B and Home M ranged from 14 to 67 ppbv. Summer levels of benzene in these garages ranged from 7 to 304 ppbv. This is similar to findings of other studies that measured benzene levels of 3 to 360 ppbv in garage (Tsai and Weisel, 2000; Thomas et al., 1993).

The 1998 summer study of 8 different homes supported the hypothesis that the storage of small engines, whose tank seals are not regulated, may increase the risk of exposure to benzene and toluene in indoor air (Figure 3.3). It was also observed in the higher levels in the garage of Home M in the summer. This trend was not observed in the studies where the same home was sampled continuously throughout the winter of 1998-1999. A better estimate can be obtained in future studies by increasing the number of homes monitored as well as increasing the variety of small engine types stored in the garage. The inexpensive solution to indoor exposure to benzene and toluene form this source is to removes fuel form small engines when stored in the garage or to just store small engines in a shed outside the home and garage.

The results from the winter 1998-1999 study contradict the hypothesis that indoor levels of benzene and toluene would increase during the winter months when homes with attached garages are shut tight (Figure 3.7). A possible explanation might be that as the outdoor temperatures decrease the indoor/outdoor temperature differential increases and this is accompanied by an increase in ventilation in the sites studied. Although we did not make ventilation measurements during this monitoring period it has been reported that the flow of air in a home mimics a "stack". Warm air in the home rises and exfiltrates as it reaches the ceilings of rooms as cold air moves in to replace it at ground level (Dietz and Cote, 1982). Other studies have also demonstrated that outdoor temperatures are a factor

influencing ventilation and the subsequent dilution of VOCs in indoor air (Fellin and Otson, 1994). The results from the 1999 winter study of Home B and Home M do not show the same trend, but the outdoor temperature range during that monitoring period was much smaller. However, when the ventilation diagram of Home M is examined closely, an overall flow from the garage to the living areas is seen (Figure 3.13).

The monitoring results and ventilation measurements for Home B and Home M supported my hypotheses that home ventilation affects the levels of benzene and toluene in the indoor air of homes with attached garages. Two of the significant factors identified from that study was the type of house and whether an indoor window was open or closed. The type of house refers to one with natural ventilation and one with mechanical ventilation. The levels of benzene and toluene were consistently lower in the living area of Home B. Home B was the home that had an elaborate forced ventilation system in operation during the winter. The living area levels were even lower in the summer study when an indoor window was opened. The duty cycle of the furnaces in the garages of these homes did not appear to contribute to differences in garage levels of benzene or toluene.

The presence of a metal can filled with gasoline was found to be an important factor for the benzene and toluene levels measured in the garage in the winter. The presence of a plastic gas can filled with gasoline in the garage did not produce the

same effect. Since fuel storage containers may have a potential for leakage around the seals, another inexpensive remedy would be to store the fuel outside the garage. The presence of a metal gasoline filled can outside the garage door in the winter was not found to be a significant factor. This is a reasonable result since at the ambient outdoor temperatures during the monitoring period there would be little vaporization of the gasoline to enter the garage.

The type of home and an indoor window opened were found to be important factors for the benzene and toluene levels found in the living area of both homes in the summer. This is a reasonable result since the indoor window opened in both homes was on the same floor as the garage and was in the zone directly adjacent to the garage. The additional exfiltration of air created next to the source would naturally lower the pollutant levels measured in the nearby living areas. The opening of a garage window or the woodstove/fireplace flue did not produce the same effect. The presence of a chainsaw filed with fuel was not found to be an important variable.

Evaluation of the data in Table 4.1 suggests that using the ratio of toluene to benzene measured in air to interpret whether a single source (*i.e.* gasoline) or multiple sources contribute to the observed levels is credible. In this study, this ratio was obtained from the slope of the weighted linear regression analysis of toluene mixing ratio *vs.* benzene mixing ratios. The y- intercept from these weighted regression analyses were classified as either zero (within standard error of zero) or

non-zero. The zero y- intercepts can be interpreted as consistent with a single source of toluene and benzene. Variations in mixing ratio would be due to common source strength variation. The non-zero y- intercepts would be indicative of multiple sources. A regression analysis where the model is forced to a zero y-intercept should calculate a slope that represents that ratio of toluene to benzene in the single major source. This model also assumes that there is no differential loss of either compound in their transport from the source.

Table 4.1: Parameters of weighted regression analyses for mixing ratio of toluene vs. mixing ratios benzene[a].

Date	Home	Sample site	Slope	SD_s	y-intercept	SD_y	$Slope_z$	SD_{zs}
Winter	B	Living area	2.2	1.1	- 2.1	5.7	1.8	0.3
Winter	B	Garage	2.7	0.1	-13*	3.1	2.4	0.1
Winter	M	Living area	3.0	0.6	-11*	2.2	2.4	0.6
Winter	M	Garage	2.4	0.2	6.1	8.1	2.5	0.1
Summer	B	Living area	2.0	1.9	0.5	4.4	2.2	0.1
Summer	B	Garage	2.0	0.3	18	19	2.3	0.1
Summer	M	Living area	2.6	0.1	2.7	2.2	2.7	0.1
Summer	M	Garage	2.2	0.1	62*	17	2.6	0.1
		Median slopes	2.3				2.4	

[a] These parameters come from data in Figures: 3.18, 3.19, 3.20, and 3.21.

* non-zero y-intercepts

A thermodynamic model can be proposed to describe the toluene to benzene ratio. Raoult's Law that states "the ratio of the partial vapor pressure of each component to its vapor pressure as a pure liquid, p_A/p^*_A, is approximately equal to the mole fraction of A (X_A) in the liquid mixture"(Atkins, 1999). Raolut's Law equations can be written for toluene and benzene as a function of temperature (Eq.(1), (2),(3) and (4)). In these equations and those that follow, T°=298 °K, and T = the temperature of the fuel source assumed from the temperature of the garages.

$$p_{Tol} = p^*_{Tol} X_{Tol} \quad \text{(toluene)} \tag{1}$$

$$p_B = p^*_B X_B \quad \text{(benzene)} \tag{2}$$

$$p_{Tol} = p^*_{Tol}(T^\circ) X_{Tol} \exp[(-\Delta H_{vap\ Tol}/R)(1/T - 1/T^\circ)] \tag{3}$$

$$p_B = p^*_B(T^\circ)\, X_B \exp[(-\Delta H_{vap\ B}/R)(1/T - 1/T^\circ)] \tag{4}$$

The ratio of Equation (3) and Equation (4) gives Equation (5).

$$\frac{p_{Tol}(T)}{p_B(T)} = \left[\frac{p^*_{Tol}(T^0)}{p^*_B(T^0)}\frac{X_{Tol}}{X_B}\right] \times \left[\exp\left(\left(\Delta H_{vapB} - \Delta H_{vapTol}\right)/R\right)\left(\frac{1}{T} - \frac{1}{T^0}\right)\right] \tag{5}$$

$$\frac{p_{Tol}(T)}{p_B(T)} = CF(T^0) \times TF(T) \tag{6}$$

Equation (6) shows the two components that influence the toluene to benzene ratio. One is the composition factor, CF(T°) and the other is a temperature dependence factor, TF(T).

The compositional factor of Equation (6) is given in the first set of square brackets of Equation (5). To solve this equation, it is necessary to make an assumption about the mole fractions of benzene and toluene in gasoline we assume that the concentration of benzene and toluene is equal to the %(v/v) in gasoline reported from Williams refinery (3.5% for benzene, 15% for toluene) and we also consider the extreme cases of benzene and toluene concentrations. The mole fraction for each compound can be calculated using Equation (7). A predicted toluene to benzene ratio can be calculated by taking the ratio of the product of these mole fractions with the respective pure compound vapor pressures (Table 4.2).

$$\frac{X_{Tol}}{X_B} = \frac{(v\%_{Tol})(0.8660g/ml)/\ 92.13g/mole}{(v\%_B)(0.8765g/ml)/78.11g/mole} \qquad (7)$$

These predicted values based on Raoult's Law overlap with the lower experimental ratios we observed and the actual headspace measurements (Table 4.2).
An independent analysis of the headspace above gasoline using GC-MS gave results of toluene to benzene ratios in the vapor phase that ranged from 1.7 to 3.8 (Stolzberg, 1999).

We can also use Equation (6) to examine the temperature dependence of the toluene to benzene ratio. By focusing on the temperature factor in this equation , which is given in the second set of brackets in Equation (5), the effect of temperatures (cold garage to warm garage) on the vapor pressures of toluene and benzene can be estimated over a range of garage temperatures we can calculated a predicted ratio

of toluene to benzene (Table 4.3). The temperature effect on the ratio of toluene to benzene is small these temperatures. The temperature dependence of the vapor pressure of toluene and benzene is swamped out by the composition effect in these predicted ratios.

Table 4.2: Predicted ratios using the vapor pressure - mole fraction factor of Raoult's Law, CF(T°).

% toluene (v)	% benzene (v)	Ratio T/B[a]
16.0	2.0	2.9
15.0*	3.5*	1.0
13.5	5.0	1.6

*Average values reported by William's refinery for gasoline.

[a] calculated via Equation(5) and Equation (7) and using $p^*_B = 8.606$ kPa ; $p^*_{Tol} = 3.79$ kPa.

Table 4.3: Summary of values for temperature factor, TF(T) [a,b,c,d].

Temp.(°F)	Temp.(°C)	Temp.(K)	Temp.factor
40	4	278	0.8
60	16	289	0.9
77	25	298	1.0

[a] ΔH_{vap}= 38,000 (J/mol) toluene; ΔH_{vap}= 31,000(J/mol) benzene.
[b] T°(ref. Temp)= 298°K
[c] temperature factor = $X_{Tol}X_B \exp[(\Delta Hvap_B - \Delta Hvap_{Tol}/R)(1/T - 1/T^{\circ})]$
[d] These factors are to be multiplied by the CF(T) to predict a toluene to benzene ratio.

The y- intercept of a plot of p_T *v.s.* p_B would be zero, if dilution of a single source was responsible for the gas-phase benzene and toluene. All of the y-intercepts observed from the weighted regression analyses of the data from each monitoring period except four have zero y-intercepts within one standard deviation (Table 4.1). As previously suggested, the cases where the y-intercepts are zero within the standard deviation of the intercept can be viewed from this model as resulting from a single source, *i.e*, gasoline. A non-zero y-intercept would indicate multiple sources or differential losses of benzene and toluene present. All homes had underground fuel tanks using #2 diesel fuel oil. The benzene and toluene content of this fuel oil is negligible. An inventory of the homes did not reveal any other obvious sources of either compound.

Gasoline does not represent an "ideal" solution best described by Raoult's Law and so it is not surprising that it does not predict toluene to benzene ratios that describe what we see experimentally or actually measure in the headspace above gasoline. Henry's Law can be used to relate the partial pressure of a compound above a dilute non- aqueous solution to the concentration of that compound in the solution (Schwarzenbach et. al, 1993). Pertinent data such as Henry's Law constants for gasoline are not available. Gasoline does not represent a dilute solution of toluene and benzene. Also, gasoline represents a solution of benzene and toluene that is somewhere between that described by Henry's Law and Rauolt's Law.

My hypothesis that indoor mixing ratios of toluene and benzene in houses with attached garages would be positively correlated with an individual's urinary levels of t,t-MA or hippuric acid was not supported by the data in this research. A bimodal distribution of t,t-MA in a population has been previously reported citing efficient metabolizers and poor metabolizers (Gobba et al.,1997). However, the small number of subjects prevents the detection of such inter-individual variability so it would be difficult to use that as an explanation.

Additional factors affecting t,t-MA variation are diet , exercise, toluene levels, and time spent in side homes. The results for t,t-MA for site #2 could be attributed to the markedly different diet and exercise habits of the individual that provided urine samples , compared to the habits of the individuals at the other sites where no correlation was observed. This subject also spent at least 90% of their time at home. These results may indicate that these biomarkers are too variable at these non-occupational exposure levels where the contribution of dietary sources of sorbitol and sodium benzoate become significant sources of the metabolites in the urine.

CHAPTER 5 – FUTURE WORK

Future research should involve monitoring for benzene and toluene in the living area and attached garage of more homes simultaneously with ventilation measurements for period of at least 12 weeks in the winter and in the summer. The homes selected would represent a variety of home types with differing ventilation, heating, and design. It would be informative to include extra homes as controls in which there were no attached garages. The benefit of this would be that both seasons could be well characterized with respect to the behavior of these indoor air pollutants and potential for human exposure. Also, the effects of small engines, fuel storage, garage temperatures, living area temperatures could be statistically evaluated for a larger population than was evaluated in this work. It would be of upmost importance to actually measure the headspace and the liquid levels of toluene and benzene in the gasoline present in these homes during these studies.

Finally, a useful undertaking would be to interface the thermal desorption apparatus to a GC with a mass selective detector for the entire study. This would not only increase the sensitivity of the method but would also permit identification of other indoor air compounds. The change in levels these compounds in indoor air could also be tracked through both seasons and trends that affect their levels identified. Once compounds of interest other than benzene and toluene are identified in this study; perhaps biomarkers of exposure if they exist can be included in the next monitoring study.

References

Anit-Poika M., Ojala M., Matikanen E. 1989. Occupational exposures to solvents and cerebellar, brainstem, and vestibular functions. Int Arch Occup Environ Health 61, 397 - 401.

Ashley D., Bonin M., Cardinali F., McGraw J.,Wooten J. 1996. Measurement of volatile organic compounds in human blood. Environ Health Perpect. 104 (suppl 5) , 871 - 877.

Atkins P. 1999. Sixth Edition Physical Chemistry. W.H. Freeman and Company, New York, 171 -174.

Ball L., Wright A., Van Sittert N., Aston P. 1997. Immunoenrichment of urinary S-phenylmercapturic acid. Biomarkers 2, 29 - 33.

Ballantyne B., Marrs T., Turner P. 1993. General and Applied Toxicology, Stockton Press, 80 - 91.

Baxter H.G., Blakemoore R., Moore J.P. 1980. The measurement of airborne benzene vapour. Ann Occup Hyg 23, 132 - 177.

Bechtold W.E., Lucier G., Birnbaum L.S., Yin S. N., Li G.L., Henderson R.F. 1991. Muconic acid determinations in urine as a biological exposure index for workers occupationally exposed to benzene. American Industrial Hygiene Assoc. Journal, 52 (11), 473 - 478.

Bechtold W.E., Henderson R.F. 1993. Biomarkers of human exposure to benzene. Journal of Toxicology and Environ. Health 40, 377 - 386.

Boogard P.J., Sittert N.J. 1995. Biological monitoring of exposure to benzeue; a comparison between S-phenylmercapturic acid , trans,trans-muconic acid and phenol. Occup. Environ. Med. 52, 611 - 620.

Box G., Hunter W., Hunter J. 1978. Ch10: Factorial Design at Two Levels. In: Statistics for Experimenters. Wiley and Sons, NY: 307 - 345.

Brief, R.S., Lynch J., Bernath T., Scala R.A. 1980. Benzene in the workplace. American Industrial Hygiene Association Journal 41, 616 - 623.

Burbacher T. 1993. Neurotoxic effects of gasoline and gasoline constituents. Environmental Health Perspectives 101(suppl 6), 133 - 141.

Chan C.C., Ozkaynak H., Spengler J.D., Sheldon L. 1991. Driver exposure to volatile organic compounds, CO, ozone, and NO_2 under different driving conditions. Environmental Science and Technology 25, 964 - 972.

Condon P.E., Grimsrud D.T., Sherma M.H., Kamerud R.C. 1980. An automated controlled flow air infiltration measurement system. In: Building Air Change Rate and Infiltration Measurements, ed. Hunt C.M., King J.C, Treshel H.R. ASTM STP 719 , American Society for Testing and Materials, Phillidelphia,P.A., 60 - 72.

Cooper K.R., Syner R. 1988. Benzene metabolism. In:Alstoy M.,ed. Benzene Carcinogenicity. Boca Raton:CRC Press, 33 - 58.

Dietz R.N., Cote E. 1982. Air infiltration measurements in a home using a convenient perfluorocarbon tracer technique. Environ. International 8, 419 - 433.

Dietz R.N., Goodrich R.W., Cote E.A., Wieser R.F. 1985. Detailed description and performance of a passive perfluorocarbon tracer system for building ventilation and air exchange measurements. BNL Report 36327. Brookhaven National Laboratory, Upton, Long Island, N.Y.

Dor F., Dab W., Empereur-Bissonnet P., Zmirou D. 1999. Validity of biomarkers in environmental health studies: the case of PAHs and benzene. Critical Reviews in Toxicology 29(2), 129 - 168.

D'Ottavio T.W., Senum G., Dietz R.N. 1988. Error analysis techniques for perfluorocarbon tracer derived multizone ventilation rates. Building and Environment 23(3), 187 - 194.

Ducos P., Gaudin R., Francin J.M., Maire C. 1990. Improvement in the HPLC analysis of urinary trans,trans-muconic acid, a promising substitute for phenol assessment of benzene exposure. Int Arch Occup Environ Health 62, 529 - 534.

Einig T., Dehnen W. 1995. Sensitive determination of the benzene metabolite S-phenylmercapturic acid in urine by high-performance liquid chromatography with fluorescence detection. J. of Chroamtography 697, 371 - 375.

Fellin P., Otson R. 1994. Assessment of the influence of climatic factors on concentration levels of volatile organic compounds (VOCs) in Canadian homes. Atmospheric Environ. 28, 3581 - 3586.

Filley C., Heaton R., Rosenberg N. 1990. White matter dementia in chronic toluene abuse. Neurology 40, 532 - 534.

Foo S.C., Phoon W., Khoo N.Y. 1988. Toluene in blood after exposure to toluene. American Industrial Hygiene Association Journal 49(5), 255 - 258.

Foo S.C, Jeyaratnam J. ,Ong C.N., Khoo N.Y., Koh D., Chia S.E. 1991. Biological monitoring for occupational exposure to toluene. Am Ind Hyg Assoc J. 52, 212 - 217.

Fung K.K., Wright B.J. 1986. Monitoring of benzene in ambient air with organic vapor badges. Journal of Air Pollution Control Assoc. 36, 819 - 821.

Gobba F., Rovest S., Borella P., Vivolli R., Caselgrandi E., Vivolli G. 1997. Inter-individual variability of benzene metabolism to trans, trans- muconic acid and its implications in biological monitoring of occupational exposure. Sci Total Environ 199, 41 - 48.

Goldstein B.D., Tardiff R., Baker S, Hoffnagle G., Murray D., Catizone P., Kester R., Caniporoli D. 1992. Valdez Air Health Study. Technical Report prepared for Alyseska Pipeline Service Company, Anchorage, Alaska.

Goldstein B.D, Witz G. 1992. Benzene. In: Lippman, M. ed., Environmental Toxicants, Van Nostrand Reinhold, N.Y., N.Y.,76 – 99.

Goldstein J., Faletto M. 1993. Advances in mechanisms of activation and deactivation of environmental chemicals. Environ. Health Perspect. 100, 169 - 176.

Grob K., Frauenfelder C., Artho A. 1990. Uptake by foods of tetrachloroethylene, trichloroethylene, toluene, and benzene from air. Z. Lebensm Unters Forsh 191, 435 - 441.

Harrje D.T., Hunt C.M., Tresado S.J., Malik N.J. 1975. Automated instrumentation for air infiltration measurements in buildings. Report No. 13, Princeton University, Center for Environmental Studies, Princeton, N.J.

Harrje D.T., Dutt G.S., Beyaea J. 1979. Locating and eliminating obscure but major energy losses in residential housing. ASHRAE Trans. 85, 521 - 534.

Henderson R.F., Sabourin P.J., Bechtold W.E. 1989. The effect of dose, dose rate, route of administration , and species on tissue and blood levels of benzene metabolites. Environ Health Perspect 82, 9 - 17.

Hodgson A.T., Daisey J. M., Grot R.A. 1991. Sources and strengths of volatile organic compounds in new office buildings. J. of Air and Waste Management Assoc. 41, 1461 - 1468.

Holian A. 1996. Air toxics: biomarkers in environmental applications- overview and summary recommendations. Environ. Health Perspect. 104 (supplement 5) 851 - 900.

IARC (International Agency for Research on Cancer). 1982. Monographs on the evaluation of carcinogenic risk of chemicals to humans: Benzene 29, 99 - 148.

Ikeda M. 1992a. Comparative evaluation of urinalysis and blood analysis as means of detecting exposure to organic solvents at low concentrations. Arch Occup Environ. Health 64, 223 - 234.

IPCS 1993. Biomarkers and risk assessment: concepts and principles. Geneve.

Isbell, M.Ricker,J.,Duffy, L.K.,Gordian,M.E.,1999. Use of biomarkers in an indoor air study: lack of correlation between aromatic VOCs with respective urinary biomarkers. The Science of the Total Environment 241, 151 - 159.

Johnson E.S., Lucier G. 1992. Perspectives on risk assesment impact of recent reports on benzene. American Journal of Industrial Medicine 21, 749 – 757.

Johnson E.S., Halbi S., Netto G., Lucier G., Bechtold W., Henderson R. 1999. Detection of low level benzene exposure in supermarket wrappers by urinary muconic acid. Biomarkers 4(2), 106 - 117.

Kawai T., Yasugi T., Mizunuma K., Horiguchi S., Iguchi H., Uchida Y., Iwami O., Ikeda M. 1992b. Monitoring of workers exposed to a mixture of toluene, styrene, and methanol vapours by means of diffusive air sampling, blood analysis and urinalysis. Int Arch Occup Environ Health 63, 429 - 435.

Kelly T., Callahan G. 1993. Method development and field measurements for polar organic compounds in ambient air. Environmental Science and Technology 27, 1146 - 1153.

Lauwerys R.R., Buchet J.P 1994. Muconic acid in urine: a reliable indicator of occupational exposure to benzene. American Journal of Industrial Medicine 25, 297 - 300.

Lebret E., Van de Wiel H.J., Bos H.P. 1986. Volatile organic compounds in dutch homes. Environment International 12, 323 - 332.

Lee B.L., New A.L., Kok P.W., Ong H.Y., Shi C.Y., Ong C.N. 1993. Urinary trans,trans-mucinic acid determined by liquid chromatography; application in biological monitoring of benzene exposure. Clinical Chemistry 39, 1788 - 1792.

Liu Y.,Fetcher L. 1997. Toluene disrupts outer hair cell morphemetry and intracellular calcium homeostasis in cochlear of guinea pigs. Toxicology and Applied Pharmocology 142, 270 - 277.

Lof, A., Hjelm W., Colmsjo A., Lundmark B.O., Norstrom A. Sato A. 1993. Toxicokinectics of toluene and urinary excretion of hippuric acid after human exposure to $^{2}H_{8}$ – toluene. British Journal odf Industrial Medicine 50, 55 - 59.

Mediores A.M. , Bird M.G., Witz G. 1997. Potential biomarkers of benzene exposure. Journal of Toxicology and Environ. Health 51, 519 - 539.

NIOSH 1974. Criteria for a recommended standard. Occupational exposure to benzene. Washington ,DC; U.S. Dept of Health , Education, and Welfare, Public Health Service, National Institute for Occupational safety and Health. NIOSH-74-137.

NIOSH 1984. Benzene Method 1500 – 11501-7. In: NIOSH Manual of Analytical Methods, vol.2, 3rd ed , National Institute for Occupational Safety and Health.

NIOSH 1987. Toluene method 4000. In: NIOSH Manual of Analytical Methods, 3rd ed., National Institute for Occupational Safety and Health.

Ogren P., Norton R. 1992. Applying a simple linear least –sqnares algorithm to data with uncertainties in both variables. J. Chem Education 69, no. 4 A130 - A131.

Ong L.N., Kok P.N., Ong H.Y., Shi C.Y., Lee B.L., Phoon, N.H., Tan K.t. 1996. Biomarkers of exposure to low concentrations of benzene:a field assessment. Occup Environ Med 53: 328 - 333.

OSHA . 1987a Benzene. U.S. Department of Labor. Occupational Safety and Health Administration. Code of Federal Regulations. 29 CFR 1910.1028.

OSHA. 1989 Air contaminants. Final rule. Occupational Safety and Health Administration. Federal Register. 54: 2332-2960.

Pekari K., Vainiotalo S.,Hekkila O. 1992 Biological monitoring of occupational exposure to low level benzene. Scand. J Work Envrionmental Health 18, no.5 317 - 322.

Purcell K., Caso G.,Garga M. 1990. In vivo metabolic reactions of benzene and toluene. Toxicology Letters 52,141 - 152.

Rangan U.,Snyder R. 1998. Scientific update ou benzene. In: Preventative Strategies for Living in a Chemical World ,Bingham E., Rall D. ed. NY Academy of Sciences,106 - 113.

Ross, D. 1996. Metabolic basis of benzene toxicity. Eur J. Haematol. 57, 111 - 118.

Saker E.G, Eskew A. E., Panter J.W., 1991. Stability of tolnene in blood: its forensic relevance. J Anal Toxicol 15, 246 - 249.

Sawyer, R. 1993. Trends in auto emissious and gasoline composition. Environ. Health Perspec. ,101, supplement 6, 5 - 12

Schad H., Schafer F., Weber L., Seidel H. 1992. Determination of benzene metabolites in urine of mice by solid phase extraction and high performance liquid chromatography. J Chromatography 593, 147 - 151.

Schwarzenbach R., Gschwend P., Imboden, D. 1993. Environmental Organic Chemistry: Air-Water Partitioning: The Henry's Law Constant. John Wiley and Sons, New York, N.Y. 109-123.

Senum G.I., Gergley R.P., Ferreri E.M., Greene M.W.Dietz R.N. 1980. Final report of the evaluation of vapor taggants and substrate for tagging of blasting caps. BNL 51232, Brookhaven National Laboratory, Upton , N.Y.

Snyder R., Witz G., Goldstein B. 1993. The toxicology of benzene. Environ. Health Perspect. 100, 293 - 306.

Snyder R., Hedli,C. 1996. An overview of benzene metabolism. Environ Health Perspect. 104 (suppl 6) 1165-1188.

Stolzberg, R. 2000. Personal communication of results from Chemistry 413 laboratory class.

Stommel P., Muller G., Stucker W. Verkogen C., Schobel S. Norpoth K., 1989. Improvement in the biological monitoring of benzene exposure. Carcinogenesis 10, 279 - 282.

Supleco 1998. Efficiently monitor toxic airborne compounds by thermal desorption. GC Bulletin 849C, Supleco Inc.

Tardif R., Brodeur J. 1989. Simultaneous high-performance liquid chromatographic analysis of hippuric acid and ortho-, meta-, and para-methylhippuric acids in urine. J of Analytical Toxicology 13, 313 - 316.

Thomas K., Pellizzari C., Clayton A.,Perrit, R., Dietz, R., Goodrich R., Nelson W., Wallace L. 1993. Temporal variability of benzene exposures for residents in several New Jersey homes with attached garages or tobacco smoke. Journ. of Exposure Analysis and Environ. Epidemiology 3(1), 49 - 72.

Tsai P.Y, Weisel C. 2000. Penetration and evaporative emissions into a home from an M85-fueled vehicle parked in an attached garage. J. Air & Waste Management Assoc. 50, 371 - 377.

U.S. Department of Health and Human Services 1994. Draft: Toxicological profile for Toluene. Contract no. 205-88-0608.

U.S. Environmental Protection Agency Compendium Method T0-3, April 1984. Determination of volatile organic compounds in ambient air using cryogenic preconcentration techniques. EPA/14-89/018.

U.S. Environmental Protection Agency Compendium Method T0-14, June 1998. Determination of volatile organic compounds (VOCs) , in ambient air using SUMMA polished canister sampling and gas chromatographic analysis. EPA/600/4-89/018.

U.S. Environmental Protection Agency Compendium Method T0-17, January 1997. Determination of VOCs in ambient air using active sampling onto sorbent tubes.

EPA/625/R-96/010b.

U.S. Environmental Protection Agency (1980). Ambient Water Quality criteria for Benzene. (EPA-440/5-8018) Washington DC pp C1-C8, C16-C35,C67-C100.

U.S. Environmental Protection Agency (1985). Drinking water criteria document for toluene. Washington D.C.: U.S. Environmental Protection agency , Office of Drinking Water. EPA-CIN-408.

U.S. Environmental Protection Agency (1988), National Ambient Volatile Organic Compounds (VOCs) Data base update. Atmospheric Scieuces Research Laboratory. Research Triangle Park, NC:U.S. Environmental Protection Agency. EPA-600/3-88/010(a).

Van Sittert N.J., Boogaard P.J., Beulin G.D.J. 1993. Application of the urinary S-phenylmercapturic acid test as a biomarker for low levels of exposure to benzene in industry. Brit J of Industrial Med 50, 460 - 469.

Vankeerberghen, P., Vandenbosch C., Smeyers-Verbeke J., Massart D.L. 1991. Some robust statistical procedures applied to the analysis of chemical data. Chemomet Intell Lab Syst 12, 3-13.

Wallace, L.A., Pellizzari E.D., Hartwell T.D. 1986. Total exposure assessment methodology (TEAM) study: personal exposures, indoor – outdoor relationships, and breath levels of volatile organic compounds in New Jersey. Environment International 12, 369 - 387.

Wallace, L. 1990. Major sources of exposure to benzene and other volatile organic chemicals. Risk Analysis 10, no. 1, 59-64.

Wallace, L. 1996. Environmental exposure to benzene. Environ. Health Perspectives 104 (supp. 6) ,1129 - 1136.

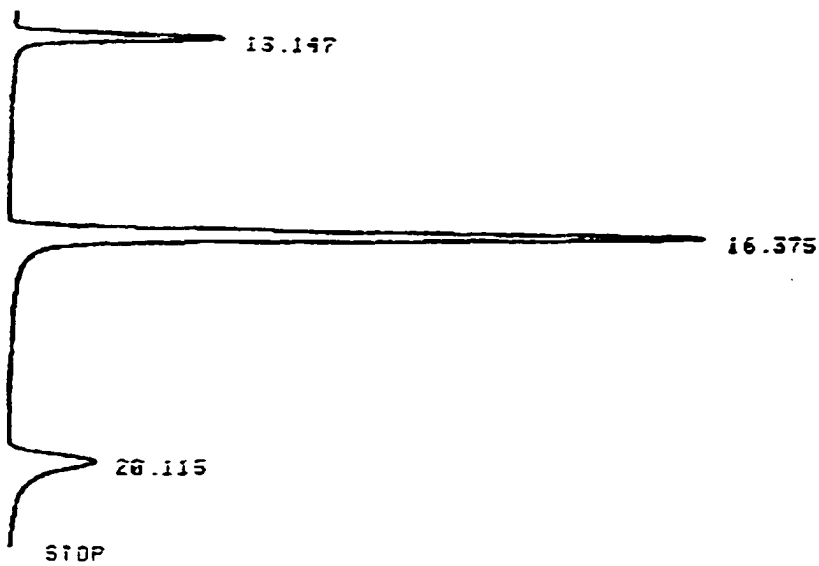
Weaver V., Davoli, C.T., Heller P.J., et al. 1996. Benzene exposure, assessed by Urinary trans, trans-muconic acid, in urban children with elevated blood lead levels. Environ Health Perspect 104: 318 - 323.

Wilhelm F. 1982. Human Biochemistry. Macmillion Publishing Company, 253.

Yu R. , Weisel C.P. 1996. Measurement of the urinary benzene metabolite trans, trans-muconic acid from benzene exposure in humans. J Toxicol Environ Health 48, 453 -459.

Appendix A

Standard chromatogram of benzene and toluene. Benzene retention time: 13.2 min., toluene retention time: 16.4 min.



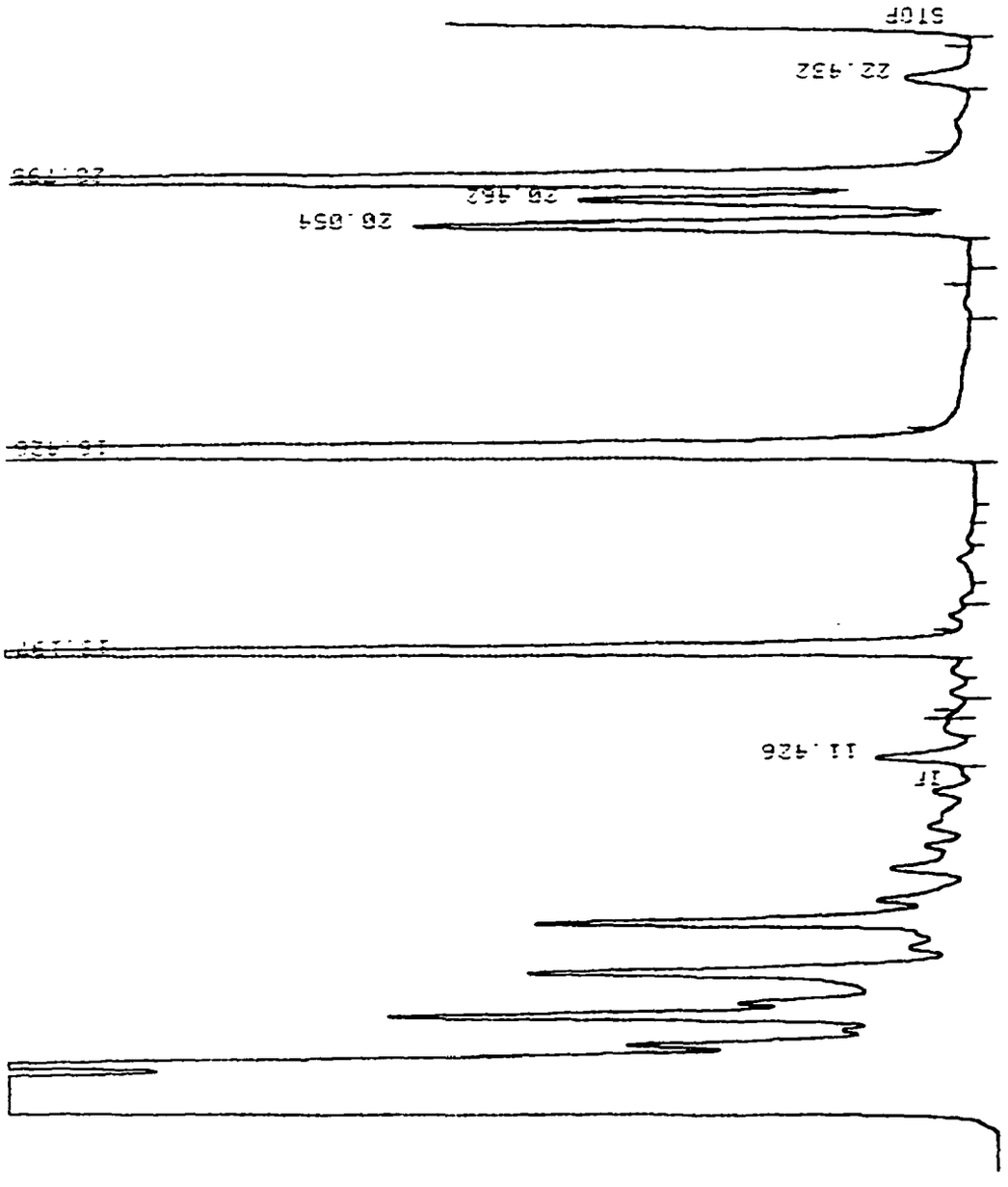
RUN# 206 JUL 26, 1981 02:01:34

AREA%

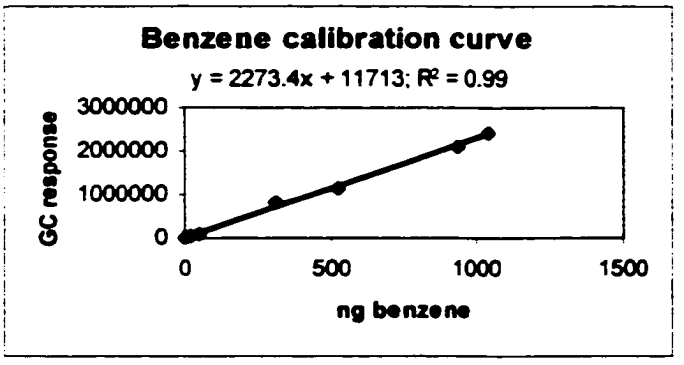
RT	AREA	TYPE	WIDTH	AREA%
13.147	19034	BV	.137	16.04035
16.375	60377	PB	.178	69.00861
20.115	13081	PV	.322	14.95100

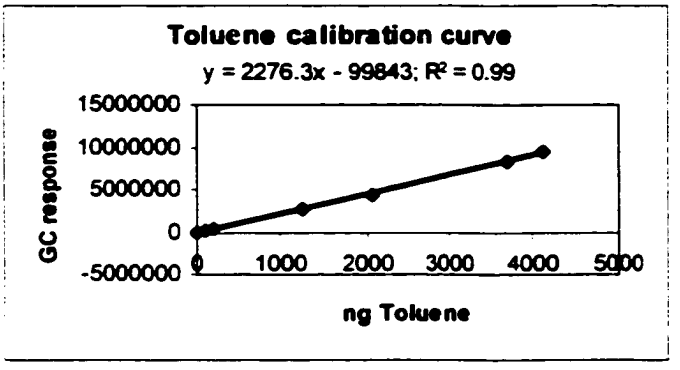
TOTAL AREA= 87492

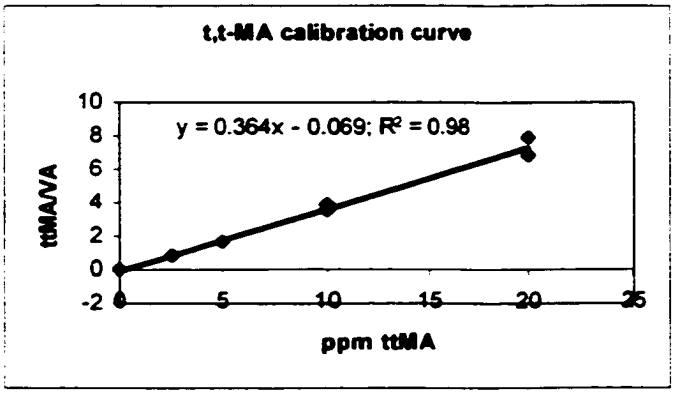
Appendix A: Typical air chromatogram. Benzene retention time: 13.2min;toluene retention time: 16.4min.



Appendix B: Typical linear regression calibration curves for benzene, toluene, biomarkers, and creatinine.







Appendix B: continued