2010

# Quantification and Reduction of Exposure to Residential Woodsmoke Particulate Matter

Megan Ann Bergauff
*The University of Montana*

# QUANTIFICATION AND REDUCTION OF EXPOSURE TO RESIDENTIAL WOODSMOKE PARTICULATE MATTER

By

MEGAN ANN BERGAUFF

B.S. Chemistry and Biology, Butler University, Indianapolis, IN, USA, 2005

Dissertation

Presented in partial fulfillment of the requirements
for the degree of

Doctor of Philosophy
in Chemistry

The University of Montana
Missoula, MT

May 2010

Approved by:

Perry Brown, Associate Provost for Graduate Education
Graduate School

Dr. Christopher P. Palmer, Chair
Chemistry

Dr. Tony J. Ward
Center for Environmental Health Sciences

Dr. Curtis W. Noonan
Center for Environmental Health Sciences

Dr. Garon Smith
Chemistry

Dr. Kent Sugden
Chemistry

Bergauff, Megan A., Ph.D., Spring 2010 Chemistry

Quantification and Reduction of Exposure to Residential Woodsmoke Particulate Matter

Chairperson: Dr. Christopher P. Palmer

Particulate matter is released during combustion reactions and can be harmful to human health. One common source for human particulate matter exposure is through biomass burning, primarily from wildfires or stoves used for heating or cooking in the home. A method was developed for the analysis of seven selected chemical tracers of woodsmoke (levoglucosan, dehydroabietic acid, abietic acid, vanillin, acetovanillone, guaiacol, and 4-ethylguaiacol) in particulate matter. This method was used to analyze particulate matter collected in Libby, MT, a community where woodsmoke is the predominant component of the particulate matter, before, during, and after a woodstove changeout program. Ambient levels of $PM_{2.5}$ and levoglucosan were found to decrease after the stove replacement, while the two resin acids remained the same or increased. The methoxyphenols measured showed no trend during the changeout, but were found to correlate to temperature on the day of sample collection. Samples collected inside individual homes in Libby before and after installation of a new woodstove showed similar results to the ambient samples. Initial attempts to replicate the real-world results in a laboratory setting were unsuccessful. Levoglucosan, dehydroabietic acid, and abietic acid were determined to be suitable tracers for woodsmoke in particulate matter, while vanillin, acetovanillone, guaiacol, and 4-ethylguaiacol were not.

Levoglucosan was investigated as a potential urinary biomarker for woodsmoke exposure. Preliminary studies using a mouse model were successful in demonstrating that levoglucosan can be detected in urine after exposure to both the pure compound and woodsmoke particulates. The method developed was shown to be specific for levoglucosan over other sugars and types of particulate matter. Inhalation of woodsmoke by mice resulted in an increase in urinary levoglucosan levels, however, similar results were not observed in human studies. Exposure to smoke from either a campfire or a woodstove did not result in a consistent increase in urinary levoglucosan in humans. Levoglucosan was found to be widely present in the human diet, resulting in fluctuating background levels that are higher than the effects of woodsmoke exposure on urinary levels.

**Acknowledgements**

Many people have helped me during my time as a graduate student, and without them completing this would not have been possible. First I would like to thank my advisor, Dr. Chris Palmer, for accepting me into his lab, getting me started on this project, and for all of the advice, assistance, and encouragement he has offered me in my time at UM.

Dr. Tony Ward and Dr. Curtis Noonan have also been very helpful to me during my graduate career, especially during the year that Chris was on sabbatical. Among many other things, Tony has provided me with valuable information about sample collection and Curtis has been of great assistance with statistical analysis and the biomarker studies. Dr. Chris Migliaccio also provided me with assistance in the biomarker studies, especially with all of the mouse exposures. I'd like to thank Dr. Earle Adams for the time spent teaching me how to use (and fix) the GC-MS, without which I could never have finished my project. Also, thank you to the other two members of my Ph.D. committee for their guidance and time; Dr. Kent Sugden and Dr. Garon Smith.

I'd like to thank my parents, Larry and Della Bergauff, my sister, Rachel Bergauff, and the rest of my family for their support and encouragement. I would never have made it this far without you.

I want to thank the members of the Palmer lab, both past and present, for their assistance and for making my days in the lab enjoyable even when things weren't working. I'd also like to thank everyone else who has worked on the woodsmoke project in the Ward, Noonan, and Migliaccio labs or through the REU program. Many people have given me assistance in learning new techniques, collecting and analyzing samples, or interpreting data and for this I am grateful. And to my friends, both in Missoula and across the country, thanks for providing me with a place to celebrate, complain about, and escape from everything that comes with graduate school.

This research was supported by funding from the Health Effects Institute (grant 4743-RFA04-4/06-4) and the National Institute of Environmental Health Sciences (grant 1R01ES016336-01). Additional support was provided by a fellowship from the Inland Northwest Research Alliance, a Bertha Morton scholarship from the University of Montana and a Lolo Walsh Anaker scholarship from the Department of Chemistry.

## List of Tables

## List of Figures

# Chapter 1: Introduction

## 1.1. Particulate matter

Airborne particulate matter, or PM, can be formed during combustion reactions and industrial processes. Its chemical composition can be a strong indicator of its origin, or source. Exposure to PM is known or suspected to have substantial adverse health consequences, and the United States Environmental Protection Agency (EPA) has set standards for maximum acceptable ambient PM levels.

PM with an aerodynamic diameter of 2.5 microns or less ($PM_{2.5}$) can travel farther into the lungs than larger particles and is thus believed to have a greater effect on human health. $PM_{2.5}$ has been tied to increases in the number of asthma attacks and has been indicated to increase the number of hospitalizations for upper respiratory effects.[1, 2] Long term exposure to $PM_{2.5}$ has been associated with increased acute and chronic mortality rates.[3] In situations such as chronic or occupational exposure, it is often difficult to measure the actual amount of smoke exposure. Personal $PM_{2.5}$ monitors are inconvenient and impractical in these settings. Estimating exposures can also be difficult because of variable $PM_{2.5}$ production depending on the fuel and burn conditions.[4]

One common source for human particulate matter exposure is through biomass burning, primarily from wildfires or stoves used for heating or cooking in the home. Exposure to wood smoke can occur outdoors through ambient air or indoors through cooking and heating devices, leakage from boilers and stoves, or from infiltration of outdoor sources.[5] High indoor levels of $PM_{2.5}$ from biomass burning are particularly a problem in developing countries where wood is the primary, and sometimes only, source of fuel.[6, 7] Some occupations, such as fire fighting or charcoal production, can result in high biomass $PM_{2.5}$ exposures as well.[5]

The EPA's National Ambient Air Quality Standards (NAAQS) include an annual standard (15 $\mu g/m^3$, based on a 3-year average) as well as a 24-hour standard (35 $\mu g/m^3$).[8] An area is designated as nonattainment for the fine fraction if the $98^{th}$ percentile values exceed either the daily standard or annual $PM_{2.5}$ standard, or if relevant information indicates that it contributes to violations in a nearby area.[8] No standards currently exist for indoor $PM_{2.5}$ levels.

Particulate matter is routinely sampled and collected in many communities[9], so the most useful tracer for wood smoke would likely be found in the PM. Air sampling can also be done with polyurethane foam (PUF) to capture volatile compounds in the environment. Sampling with a PUF cartridge is more specialized than PM sampling, but it is not routinely conducted due to the higher cost, and the amount of time that is required. Extensive characterization of the inorganic and organic composition permits apportionment of the total particulate matter in an air shed to various sources. However, complete characterization of the chemical composition is expensive, and is not necessary to monitor specific intervention programs. In such cases, it is more cost effective to determine the concentrations of selected specific chemical compounds that result from the specific source or sources of interest.

### 1.2. Woodstoves

Woodstoves are frequently used throughout the United States for heating and for heating and cooking throughout the world. The internal design of wood stoves in the US has changed entirely since 1990, as the result of the EPA's regulation established in the late 1980s.[10] The EPA's mandatory smoke emission limit for wood stoves is 7.5 grams of smoke per hour. With advances in technology and competition among manufacturers, the average emissions of certified stoves has declined steadily and today most current wood stove models emit only 2 to 4 g/h.[11] While the newer model stoves offer an improvement in particulate emissions, many homes still contain (and use) an older model stove that has higher emission levels. The changing $PM_{2.5}$ standards have provided incentive for communities to reduce particulate emissions and some communities are turning to more efficient woodstoves as a way to improve air quality.

The first example of a whole-town woodstove change out occurred in Crested Butte, CO in 1989-1990. In this change out, 281 old woodstoves were either retired or replaced with newer models.[12] A 40% reduction in ambient $PM_{10}$ levels was observed after change out was complete and individual stove emissions decreased by 67%. Visual air quality was also monitored in this study through light scattering, and a 59% improvement was seen after woodstove replacement.[13] Other woodstove change out programs are currently occurring in Dayton, OH, Southwest Pennsylvania, Yakima County, WA, numerous communities across the state of California, as well as Libby, MT.

The ambient effects of woodstove change out have been investigated; however, little information is available on indoor air quality before and after woodstove change out.

### 1.3. Libby, MT

Many rural communities have difficulty meeting the EPA's standards during the winter months due to $PM_{2.5}$ from residential wood stoves. One such $PM_{2.5}$ nonattainment area is the community of Libby, MT. Libby is a community of about 2700 people located in a valley in north-western Montana (elevation 628 m). There is no natural gas line in Libby, so homes are heated using electricity, propane, oil, or wood-burning stoves. In 2003, there were approximately 1500 registered wood stoves in Libby and the surrounding valley, with nearly 1300 of those considered to be outdated stoves that do not meet the current EPA guidelines.[10, 14, 15] In Libby, the primary species of wood burned are softwoods, particularly Douglass fir and larch. Temperature inversions in the winter trap pollution in the valley, resulting in high levels of ambient $PM_{2.5}$ during winter months. Throughout the winter of 2003/2004, $PM_{2.5}$ concentrations averaged 28.2 $\mu g /m^3$, with a high 24-hour concentration of 40.9 $\mu g/m^3$. Results from a Chemical Mass Balance source apportionment model identified woodstoves as the main source of the ambient $PM_{2.5}$.[16] With resources from private and public sources the community initiated a community-wide woodstove changeout program in 2005.

A partnership between the EPA, the Montana Department of Environmental Quality, and the Hearth, Patio, and Barbeque Association was responsible for changing out the old woodstoves in Libby, MT over the course of 3 years and replacing them with cleaner burning, more environmentally friendly EPA-certified models. EPA-certified stoves must meet the 1988 EPA certification emission standard of less than 7.5 g/h of particulate matter.[10] The conventional model EPA-certified woodstoves employ firebox installation and have a longer, hotter gas flow path with pre-heated combustion air to allow for more complete combustion. The changeout was completed in 2008, and nearly 1200 stoves in Libby were replaced or surrendered. In a study conducted in Libby, indoor levels of $PM_{2.5}$ were significantly reduced following the changeout of an old stove with an EPA-certified stove within the home.[17]

### 1.4. Chemical tracers for woodsmoke

Seven chemical tracers for woodsmoke were monitored in the Libby, MT ambient air throughout the duration of the woodstove changeout program.[18] The tracers that were chosen for this study are levoglucosan, dehydroabietic acid, abietic acid, vanillin, acetovanillone, guaiacol, and 4-ethylguaiacol (Figure 1.1). The specific markers were selected based on the results of a more complete analysis of particulate matter from a community where residential wood stoves are the dominant source of PM.[16] These compounds are commonly found in woodsmoke, represent three different classes of compounds, and are sourced from different wood fuel components.[16, 19] They have also been previously investigated as potential tracers for wood smoke contributions to $PM_{2.5}$.[18] The new method is a combination and adaptation of previously reported methods, optimized for the selected set of markers.[19-27]

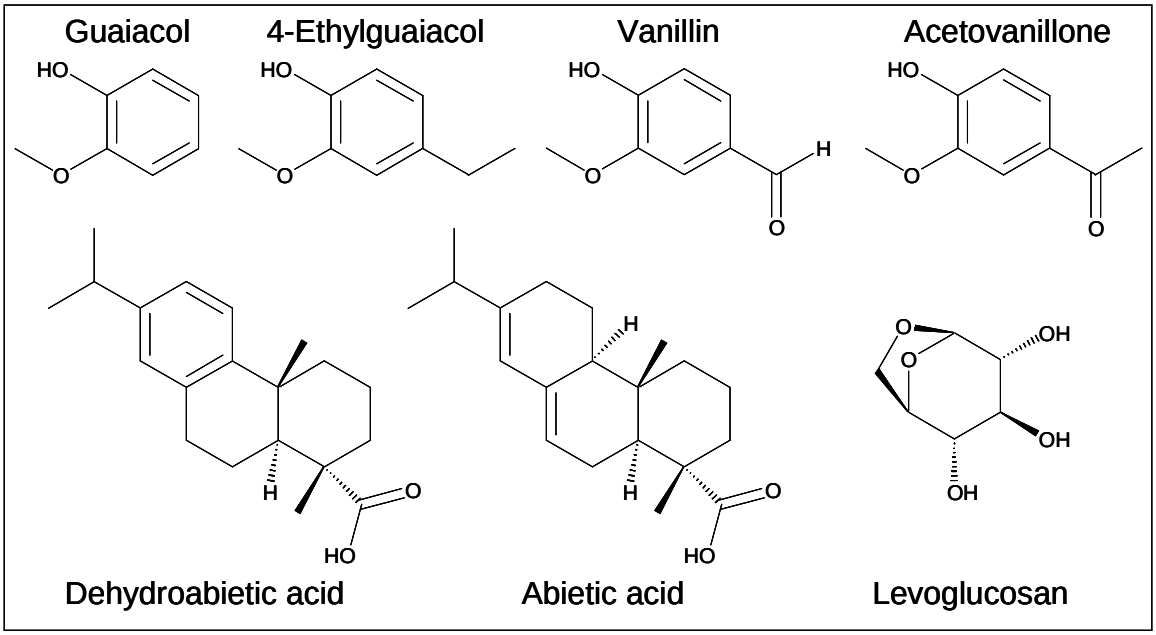


**Figure 1.1**
Structures of the selected chemical tracers for biomass burning.[18]

Levoglucosan is a sugar anhydride and is formed during the pyrolysis of cellulose. It is frequently used as a tracer for woodsmoke because it constitutes a high percentage of the organic component of the particulate phase, and is stable in the atmosphere.[23, 28-34] Before the changeout, levoglucosan was found to represent 11.8% of the total $PM_{2.5}$ mass and 13.9% of the total carbon in the particle phase in Libby.[16]

Dehydroabietic acid and abietic acid are both resin acids, which are present in softwood species.[16, 31] In Libby, the primary species of wood burned are Douglas fir, Ponderosa pine, and larch.[35] Both resin acids are released from wood during combustion and are non-volatile.[36] Resin acids have low solubility in water and can accumulate in tissues in fish or other organisms. They have been shown to have toxic effects on the liver and the potential to damage DNA.[37, 38] Abietic acid is released from wood unaltered, while dehydroabietic acid is formed from abietic acid and other resin acids during combustion.[24] The resin acids are resistant to chemical degradation in the atmosphere, however, dehydroabietic acid has been shown to undergo degradation in water when exposed to ultraviolet light.[36] Dehydroabietic acid was the most prevalent resin acid measured during the comprehensive chemical speciation study performed on the Libby particulate matter during the 2003-2004 study.[16] These resin acids represented a combined 1.5% of the total $PM_{2.5}$ mass in Libby before the changeout.

Vanillin, acetovanillone, guaiacol, and 4-ethylguaiacol are all methoxyphenols. Methoxyphenols result from pyrolysis of lignin, which is commonly found in cell walls of plants and is a waste product of the paper industry.[39] In addition to being a waste product of the paper industry, previous studies have shown that methoxyphenols can be present in winter urban air and have been suggested as potential tracers for woodsmoke in particulate matter.[25, 39-43] The type of wood burned, particularly hardwood versus softwood, can affect the ratio of different classes of methoxyphenols present.[39] The chosen methoxyphenols are semi-volatile compounds so their presence will not be limited to the particulate phase[44], but all were represented in Libby ambient air with a combined 0.093% of the total $PM_{2.5}$ mass before the changeout.[16] In the original study, vanillin and acetovanillone were the two most abundant methoxyphenols measured.[16]

### 1.5. Overview

Particulate matter composition was evaluated before, during, and after a woodstove replacement program where newer-model, lower emission EPA-certified stoves were installed. Air quality was evaluated both outdoors and inside homes during the changeout. Levoglucosan, a chemical tracer of woodsmoke, was evaluated as a potential urinary biomarker of woodsmoke exposure through both animal and human studies.

## Chapter 2: Air Studies

In western Montana (and other mountainous regions), valley communities that rely on woodstoves for home heating often have elevated ambient $PM_{2.5}$ concentrations throughout the winter months. This is due to particulate emissions from residential woodstoves coupled with temperature inversions that trap the pollutants in the valleys.[13, 16] These elevated concentrations often approach or exceed the 24-hr $PM_{2.5}$ NAAQS during the winter months.

A partnership between the EPA and the Hearth, Patio, and Barbeque Association was created to replace over 1000 old wood burning stoves in Libby, Montana with new EPA-certified stoves that are regulated to have lower PM emissions. This pre-existing changeout plan, coupled with the fact that woodsmoke emissions were found to make up 82% of the $PM_{2.5}$ in Libby[42], make Libby an excellent study site to evaluate the effects of woodstoves on $PM_{2.5}$.

Samples were collected in Libby outdoors over the course of four heating seasons during the woodstove changeout. Samples were also collected inside homes in Libby before and after the installation of a new, EPA-certified stove to evaluate the indoor air quality. In both the ambient and indoor environments, both $PM_{2.5}$ and the seven selected chemical tracers for woodsmoke were monitored. A laboratory study involving an older model and an EPA-certified stove was conducted in attempt to replicate the results observed in Libby.

### 2.1. Wood stove changeout

Between 2005 and 2008, nearly 1200 stoves were replaced in Libby with the majority occurring during 2006 and 2007 (Table 2.1). Families had the option to install an EPA-certified wood, pellet, propane, or oil burning stove during the changeout, however the majority of uncertified stoves removed were replaced with EPA-certified wood burning stoves. The catalytic unit in up to 100 existing EPA-certified stoves was also replaced in stoves that were useable but not in good condition.

**Table 2.1**

Proportion of woodstoves changed prior to each winter period and mean (standard deviation) winter period meteorological data across 4 years in Libby, MT (winter period is Nov. 1 to March 1).[45]

| | 2004/2005 | 2005/2006 | 2006/2007 | 2007/2008 |
|---|---|---|---|---|
| **Cumulative stoves changed[a]** | 0 | 9.3 | 58.7 | 96.2[b] |
| **Average Temperature (℃)** | -1.72 (4.50) | -2.11 (4.50) | -2.56 (4.84) | -2.56 (4.78) |
| **Average Relative Humidity (%)** | 85.5 (11.9) | 81.8 (16.5) | 87.0 (9.0) | 82.1 (9.5) |
| **Average Wind Speed (mph)** | 0.24 (0.28) | 0.17 (0.22) | 0.22 (0.35) | 0.32 (0.32) |
| **Average Precipitation (in.)** | 0.03 (0.11) | 0.07 (0.15) | 0.08 (0.16) | 0.07 (0.17) |

[a]Cumulative percentage of wood stoves replaced, rebuilt, or surrendered at start of each winter period, based on 1175 total stoves targeted.
[b]The majority (360/440) of the stove changeouts in this final period occurred during the winter of 2006/07.

## 2.2. Ambient air studies

Woodstoves have been identified as a major source of $PM_{2.5}$ in valley locations throughout the Northern Rocky Mountains where biomass combustion is the predominant source of home heating. Some of these communities, such as Libby, MT, have trouble meeting the EPA's annual and/or daily $PM_{2.5}$ standards. Since the EPA standards are for ambient air in a community, it is important to monitor and understand the effect of the changeout on the outdoor particulate matter levels. Ambient air issues can also affect the entire community and not just those residents with woodstoves in their homes.

### 2.2.1. Objective

The objective of this study was to measure and evaluate the changes in ambient $PM_{2.5}$ and seven selected tracers for woodsmoke throughout the woodstove changeout to evaluate the reduction in not only ambient $PM_{2.5}$, but those smoke particles generated from residential wood combustion. The seven selected chemical tracers for ambient woodsmoke were measured before, during, and after the stove changeout, along with $PM_{2.5}$ and several meteorological parameters.

### 2.2.2. Sample collection

Ambient particulate samples were collected on the roof of the Lincoln County Annex in Libby, MT throughout the last several winters as previously reported.[18] Samples were collected every 6 days during the winter months (November through February) following the EPA compliance schedule, starting in November 2004 and ending in February 2008. A BGI PQ200 $PM_{2.5}$ Federal Reference Method (FRM) $PM_{2.5}$

sampler (BGI, Inc., Waltham, MA) was fitted with a quartz filter for each sample day to collect the ambient $PM_{2.5}$. Pre-fired 47-mm quartz filters (fired at 500°C for 2.5 hours) were purchased from Chester LabNet (Tigard, OR), and delivered to Lincoln County personnel in a cooler. Clean quartz filters were stored in a refrigerator at approximately 2°C prior to sample collection. Following sample collection, the quartz filter samples were stored in a freezer at -20°C until analysis. Approximately 24 $m^3$ of air was sampled during each 24-hour episode. Quartz filter field blanks were also collected periodically throughout the program to address artifact contamination.

#### 2.2.3. Sample analysis

Samples collected between November 2004 and February 2008 were analyzed for the seven chosen chemical tracers for wood smoke.[18] Briefly, one half of each filter was spiked with the deuterated standards and then compounds were extracted with sonication into ethyl acetate containing 3.6 mM triethylamine. The volume of the solution was reduced and the samples were split into two equivalent portions. One portion was derivatized with a freshly prepared 2:3 mixture of acetic anhydride to triethylamine to be analyzed for the methoxyphenols. The other portion was evaporated to dryness, and then derivatized with a mixture of N-*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA), trimethylchlorosilane (TMCS), and trimethylsilylimidazole (TMSI) to be analyzed for levoglucosan and the resin acids. The portion for levoglucosan and the resin acids was diluted with ethyl acetate containing 3.6 mM triethylamine and both portions were analyzed by GC-MS. Analysis was performed on an Agilent 6890N Gas Chromatograph with an Agilent 5973 Mass Spectrometer.[18] For all compounds, highly selective quantitation was performed using the signal for representative ions for each compound extracted from the total ion chromatogram.

#### 2.2.4. Meteorological conditions

##### 2.2.4.1. Data collection

Meteorological data, including temperature, wind speed, relative humidity, and precipitation, was obtained from the database collected by the Western Regional Climate Center (WRCC), a division of the National Oceanic and Atmospheric Administration.[46] Data were recorded daily throughout the four winters of the changeout (Nov. 1 to March

1). The measurement station was located in Libby, at latitude 48° 23' 00" and longitude 115° 34' 00".

#### 2.2.4.2. Results

Several meteorological conditions, including temperature, relative humidity, maximum wind gust speed, and precipitation were monitored during the change out period (Table 2.1, p. 9). The only parameters that showed any statistically significant difference ($p<0.05$) from the pre-changeout year (2004/2005) were average precipitation for all three winters (2005/2006, 2006/2007, and 2007/2008) and relative average humidity during the winter of 2006/2007. At $p<0.01$, only average precipitation during the winter of 2006/2007 is statistically different than the pre-changeout year (2004/2005).

### 2.2.5. Ambient $PM_{2.5}$

#### 2.2.5.1. Data collection

$PM_{2.5}$ mass concentrations used for winter averages (Nov 1-March 1) were collected every 3 days by the Montana Department of Environmental Quality as part of the Libby $PM_{2.5}$ compliance sampling program.[47] The net mass on a Teflon filter was determined gravimetrically by weighing the Teflon filter before and after sampling with a microbalance in a temperature and relative humidity controlled laboratory environment. $PM_{2.5}$ reference methods require that filters be equilibrated for 24 hours at a constant (±5%) relatively humidity between 30% and 40% and at a constant (±2 °C) temperature between 20 °C and 23 °C to minimize particle volatilization and aerosol liquid water bias.[47]

#### 2.2.5.2. $PM_{2.5}$ Results

$PM_{2.5}$ levels in Libby decreased 20% during the course of our study, from 27.0 $\mu g/m^3$ (±2.0) in 2004/2005 to 21.8 $\mu g/m^3$ (±0.8) in 2007/2008 (Figure 2.1). The majority of the decrease occurred between 2005/2006 and 2006/2007, with the final year of sampling (2007/2008) showing no statistical difference ($p=0.81$) from the previous year (2006/2007).

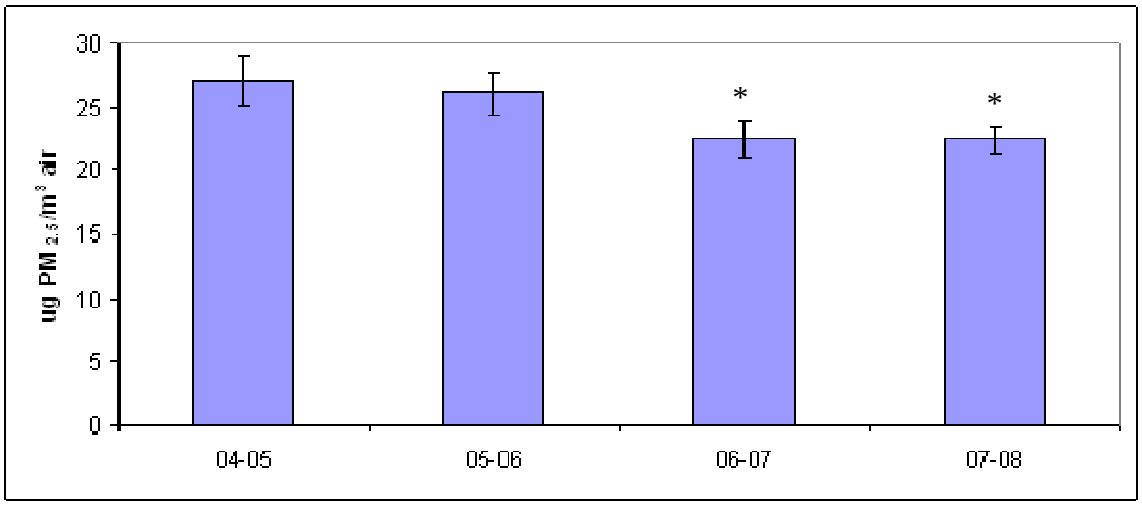


**Figure 2.1**
Average winter ambient $PM_{2.5}$ measured in Libby, MT.[45] (*difference from 2004/2005 is significant at $p<0.05$)

### 2.2.6. Initial heating season, 2004-2005

The method developed was initially applied to ambient samples collected in Libby, MT during the heating season of 2004-2005 to evaluate the composition of the $PM_{2.5}$ over the course of a heating season in Libby. The average concentration of each tracer over the season was determined (Table 2.2). A large range of day-to-day concentrations of all seven tracers was shown. The confidence intervals listed in Table 2.2 result from the day-to-day variability of the compound over the entire heating season and not necessarily variability in the analysis method. Using levoglucosan as an example, concentrations ranged 10-fold from 655 to 6807 ng/m$^3$ air (Figure 2.2).

**Table 2.2**
Average values of the selected tracers and $PM_{2.5}$ measured in Libby, MT during the winter of 2004-2005.[18]

| Compound | Average (ng/m$^3$ air) | 95% Confidence Interval | n detected/ n total | Correlation to $PM_{2.5}$ ($R^2$) | Slope of best fit line (ng analyte/ µg PM) | Error of the slope (ng analyte/µg PM) |
|---|---|---|---|---|---|---|
| Levoglucosan | 3040 | 675 | 18/18 | 0.7924 | 119 | 15 |
| Dehydroabietic acid | 364 | 70.6 | 18/18 | 0.7570 | 11.7 | 1.7 |
| Abietic acid | 30.3 | 9.45 | 18/18 | 0.8344 | 1.77 | 0.20 |
| Vanillin | 17.1 | 6.65 | 17/17 | 0.0023 | --- | --- |
| Acetovanillone | 3.14 | 2.18 | 9/17 | 0.0149 | --- | --- |
| Guaiacol | 4.27 | 3.35 | 18/18 | 0.1448 | --- | --- |
| 4-Ethylguaiacol | 0.67 | 0.46 | 18/18 | 0.0055 | --- | --- |
| $PM_{2.5}$ | 27.26 µg/m$^3$ | 4.61 µg/m$^3$ | 18 | --- | --- | --- |

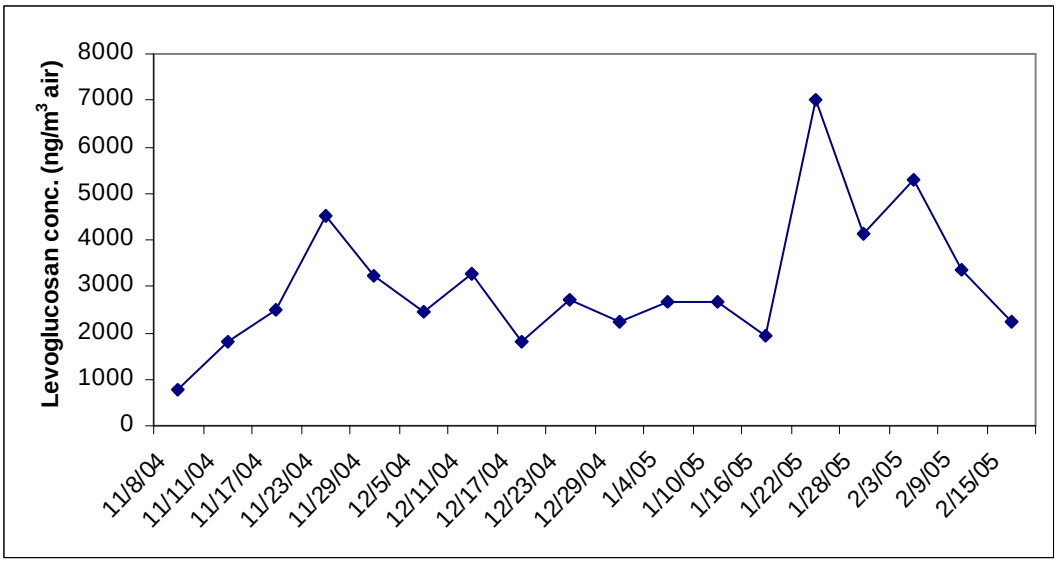


**Figure 2.2**
Variation in levoglucosan levels observed during the 2004-2005 heating season.

#### 2.2.6.1. Levoglucosan

The average concentration of levoglucosan measured in Libby was 3040 ng/m$^3$ air. The level of levoglucosan measured in Libby in this study was higher than levels measured in other US urban areas, such as Seattle, WA (13-760 ng/m$^3$)[26], Spokane, WA (2-327 ng/m$^3$)[48], and Fresno and Bakersfield, CA (23-7590 ng/m$^3$).[43] Levoglucosan levels measured during the winter of 2004-2005 were found to have a good correlation to $PM_{2.5}$ levels (Figure 2.3).

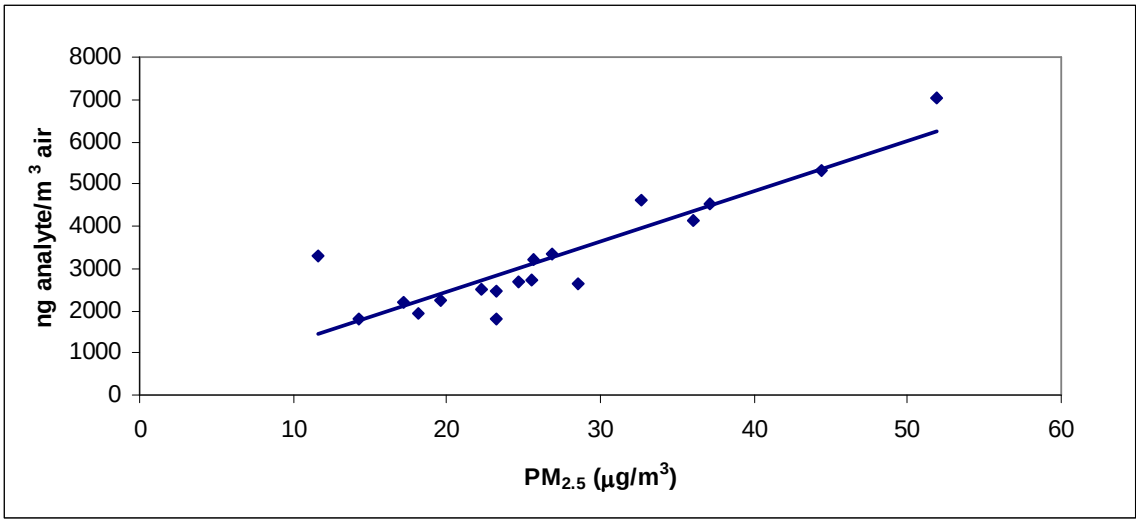


**Figure 2.3**
Correlation between levels of levoglucosan and $PM_{2.5}$ measured in Libby during the winter of 2004-2005.[18] ($R^2$=0.7924)

### 2.2.6.2. Resin acids

Dehydroabietic acid was measured at an average concentration of 364 ng/$m^3$ and abietic acid at 30.3 ng/$m^3$.The level of dehydroabietic acid measured in this study was significantly higher than level reported in forest fire smoke collected in an urban environment.[49] Dehydroabietic acid, and abietic acid were found to have a good correlation with $PM_{2.5}$ levels (Figure 2.4).

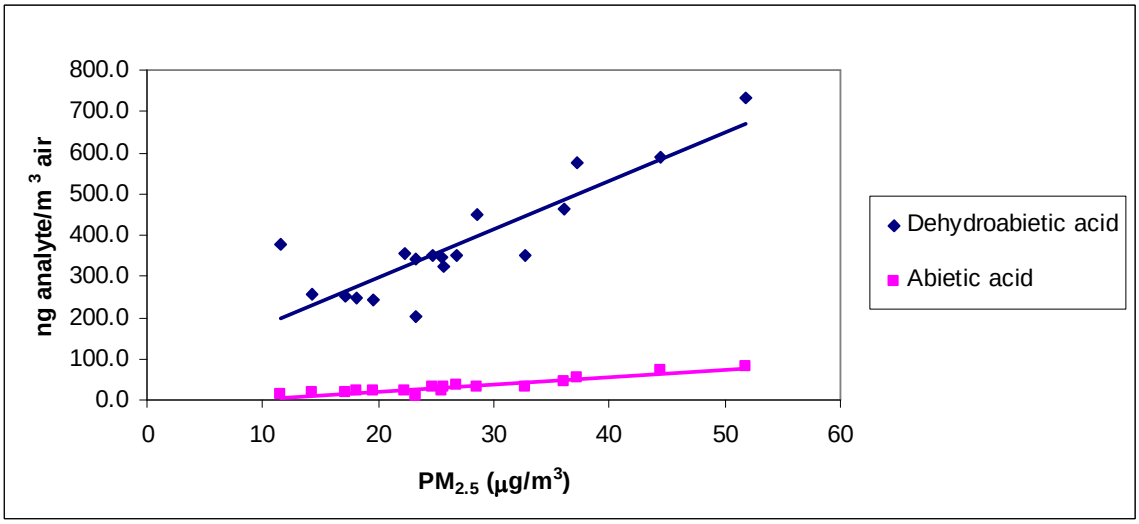


**Figure 2.4**
Correlation between levels of dehydroabietic acid or abietic acid and $PM_{2.5}$ measured in Libby during the winter of 2004-2005.[18] ($R^2$ for dehydroabietic=0.7570 and $R^2$ for abietic acid=0.8344)

### 2.2.6.3. Methoxyphenols

During the winter of 2004-2005, vanillin was found at an average concentration of 17.1 ng/m$^3$ and acetovanillone at 3.14 ng/m$^3$ in the PM. Guaiacol averaged 4.27 ng/m$^3$ and 4-ethylguaiacol averaged 0.67 ng/m$^3$. Levels of vanillin and other methoxyphenols were difficult to compare to previously reported values due to differences in sample collection methods and the semi-volatile nature of these compounds.[42] In this study, the levels of all four methoxyphenols measured showed poor correlation with $PM_{2.5}$, suggesting that these four compounds are not suitable tracers for wood smoke in PM sampling. (Figures 2.5a and 2.5b)

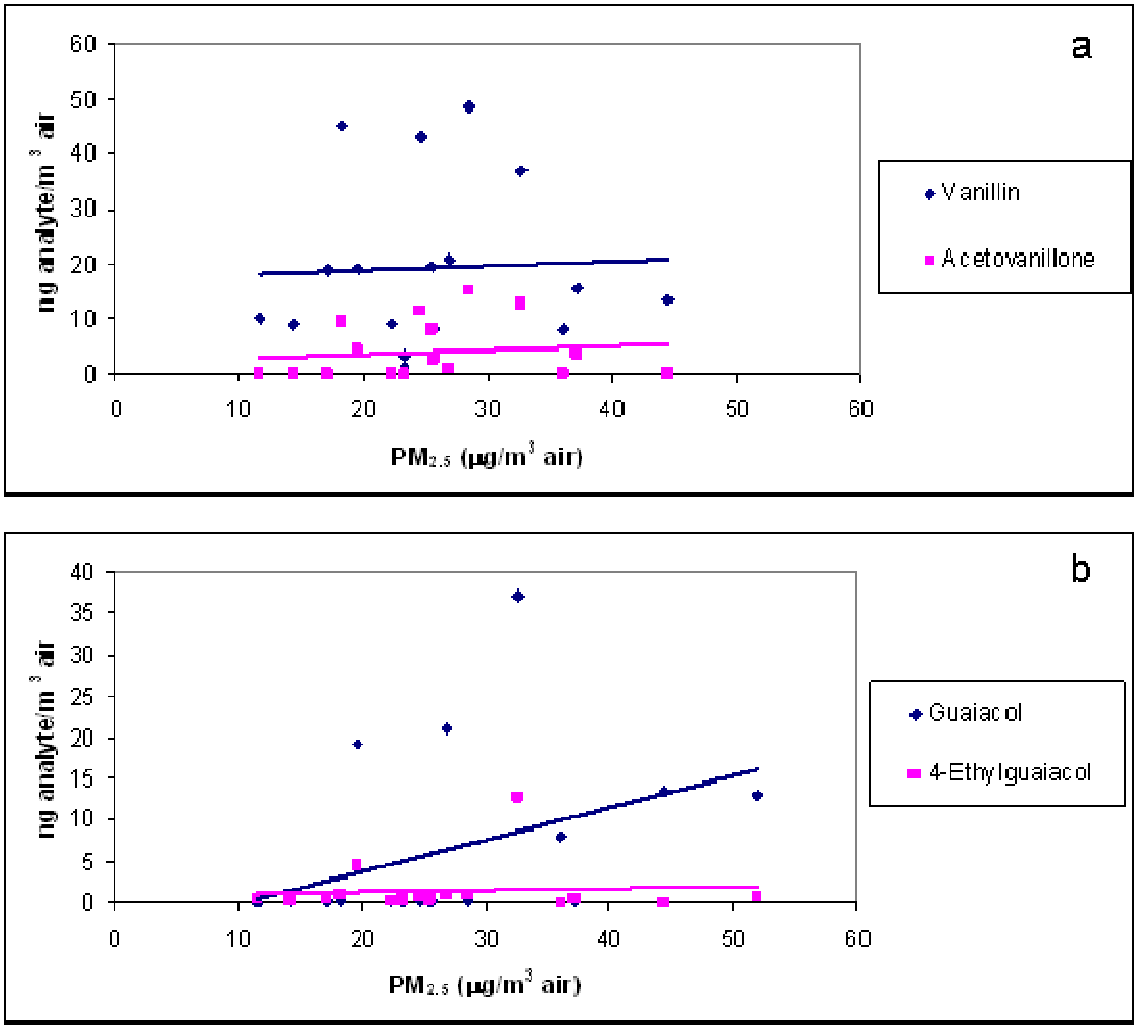


**Figure 2.5**
Correlation between levels of the selected tracers and $PM_{2.5}$ measured in Libby during the winter of 2004-2005.[18]
a.) vanillin ($R^2$=0.0023) and acetovanillone ($R^2$=0.0149)
b.) guaiacol ($R^2$=0.1448) and 4-ethylguaiacol ($R^2$=0.0055)

#### 2.2.6.4. Discussion

Since the majority of the $PM_{2.5}$ present in Libby was due to wood smoke, the observed correlation between levoglucosan or the two resin acids and $PM_{2.5}$ levels was expected. This suggests that these compounds are useful tracers for wood smoke in particulate matter. The slope of the association between $PM_{2.5}$ mass and each of these three compounds is reported in Table 2.2 (p. 10). These slopes indicate that levoglucosan represents about 11.9% of the $PM_{2.5}$ mass, while dehydroabietic acid accounts for about 1.2% and abietic acid is about 0.2%. Previously reported values of the ratio of levoglucosan to particulate matter in fireplace emissions are 0.8-26%.[20, 50] Reported values for dehydroabietic acid range from 0.3-8.7% and value for abietic acid range from 0.1-1.6%.[23, 24, 51, 52] Most of the reported values are from the burning of specific species of wood, such as Ponderosa or loblolly pine, which can produce different ratios of the resin acids than the larch and Douglas fir burned in Libby.

Because of their lack of correlation to $PM_{2.5}$, the four methoxyphenols are not useful tracers for wood smoke in particulate matter. Other methoxyphenols, such as propionylsyringol and butyrylsyringol, have been successfully used in a source apportionment model for particulate matter.[43] However, neither of these compounds were measured in the original chemical profile of the Libby particulate matter.[16]

During a typical Libby winter, temperature inversions frequently occur for extended periods of time, allowing for $PM_{2.5}$ emitted from valley sources to build up in the airshed. These temperature inversions are characterized by cold temperatures, low winds, and high humidity (fog). In an effort to investigate whether the measured concentrations of the analytes of interest were dependent on such meteorological conditions, the measured levels of all seven compounds were compared to meteorological parameters (including temperature, wind speed and direction, barometric pressure, daily precipitation (snowfall), and snowfall accumulation) for each of the sample days. Due to the size of the community and the limited resources dedicated to the existing meteorological monitoring station, the number of parameters that are continuously measured in Libby is limited. For example, relative humidity, which is one indicator of temperature inversions, was not measured in Libby during the winter of 2004-2005. In investigating the relationships between meteorological conditions and the concentrations

of analytes measured in this study, the only correlation found was an inverse relationship between temperature and the levels of vanillin and acetovanillone (Figure 2.6). This result is further evidence that the varied levels of methoxyphenols associated with PM are due to the semivolatile nature of these compounds. $PM_{2.5}$ levels, levoglucosan, dehydroabietic acid, and abietic acid did not show a direct relationship to any of the measured meteorological parameters.

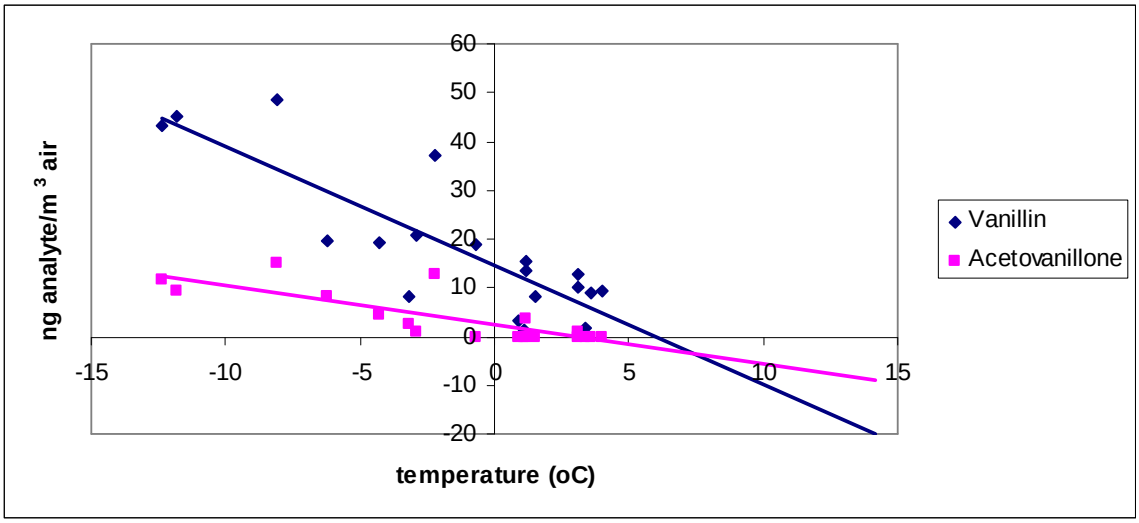


**Figure 2.6**
Correlation between levels of vanillin and acetovanillone and ambient air temperature on the day of sample collection measured in Libby during the winter of 2004-2005.[18] ($R^2$=0.7009 for vanillin and 0.6447 for acetovanillone)

### 2.2.7. Tracer results over entire changeout period

#### 2.2.7.1. Levoglucosan

Over the course of the changeout period, levoglucosan showed an overall decrease of 50% from 3036 ± 344 ng/m$^3$ to 1537 ± 117 ng/m$^3$, but little change between the last 2 years of the program (2006/2007 and 2007/2008) (Figure 2.7). The majority of the decrease occurred during the first year of the changeout (2004/2005 to 2005/2006), likely due to location of the stoves changed in this time period. Stoves in low-income neighborhoods were given priority in the changeout and were replaced first, which included the area immediately around the sampling site.[53] The fraction of $PM_{2.5}$ represented by levoglucosan also decreased during the changeout, following the same trend as the levoglucosan measurements (Figure 2.7). The decrease occurred during the first year of stove replacement (between 2004/2005 and 2005/2006) and then the

composition remained relatively constant. Levoglucosan has been found in lower concentrations in the particulate matter when a woodstove is operated with open airflow conditions, which allows for better combustion.[54]

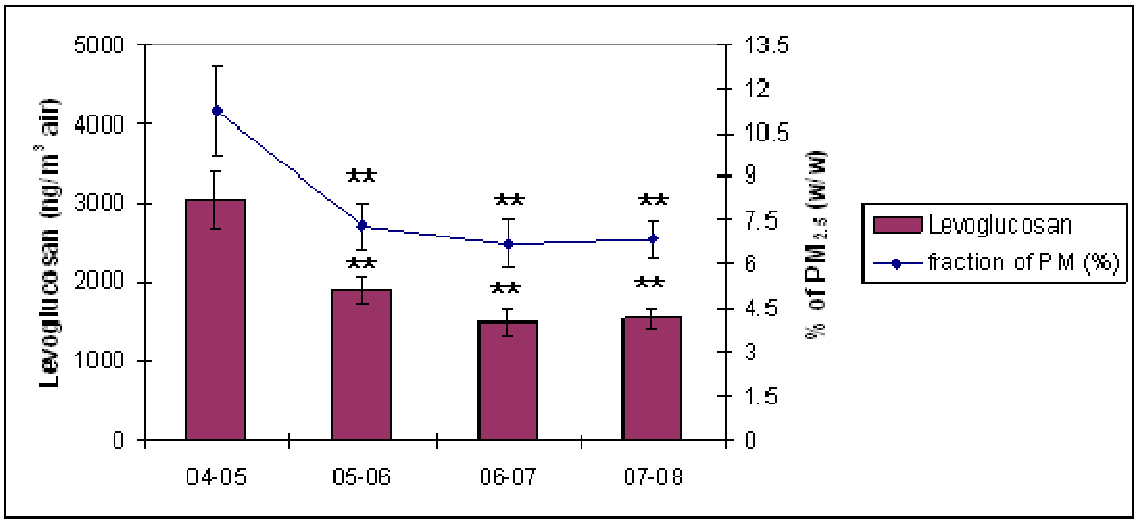


**Figure 2.7**
Average winter ambient concentration (±standard error) in Libby and fraction of $PM_{2.5}$ (±standard error) of levoglucosan.[45] (**difference from 2004/2005 is significant at $p<0.01$)

Data from the initial heating season of 2004-2005 in Libby show that levoglucosan has a good correlation to $PM_{2.5}$ in Libby, so it is likely that the observed decrease in both levoglucosan and $PM_{2.5}$ are a result of the woodstove replacement program.[18] Levoglucosan showed a correlation to $PM_{2.5}$ measurements over the 4 years of the program, with a combined $R^2$ value of 0.66 ($p<0.001$).

#### 2.2.7.2. Resin acids

Dehydroabietic acid showed a mixed response to the changeout. The levels increased by 36% during the first year of the changeout (2004/2005 to 2005/2006), and then showed a 40% decrease between the pre-changeout level and second year of the changeout (2004/2005 to 2006/2007) (Figure 2.8). The third year (2007/2008) showed levels comparable to the pre-changeout levels. The fraction of $PM_{2.5}$ made up of dehydroabietic acid followed a similar trend as the dehydroabietic acid measurements, increasing during the first year of the changeout (2005/2006), decreasing during the second (2006/2007), and then returning to levels comparable to the pre-changeout period in the final year (2007/2008).

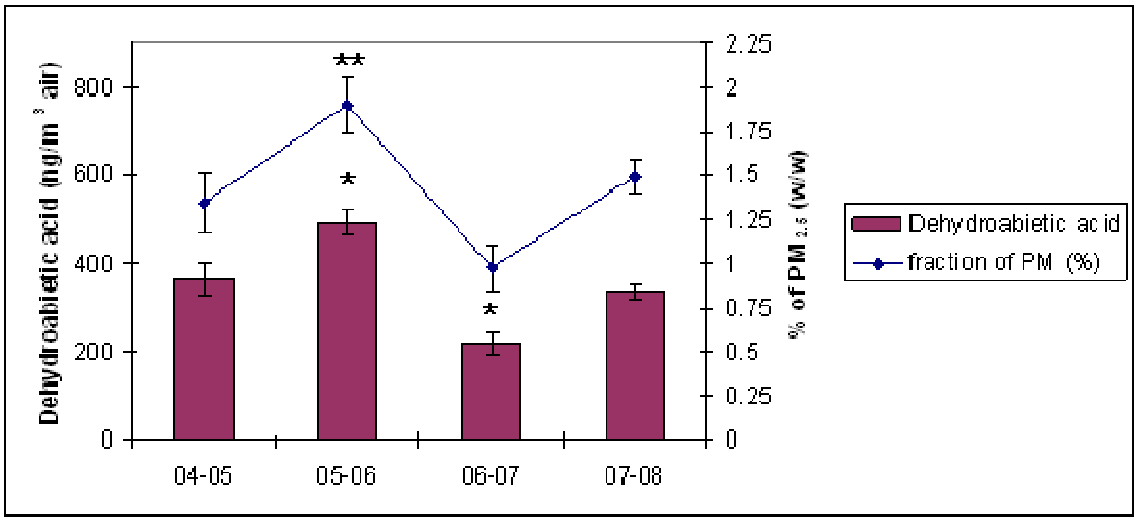


**Figure 2.8**
Average winter ambient concentration (±standard error) in Libby and fraction of $PM_{2.5}$ (±standard error) of dehydroabietic acid.[45] (*difference from 2004/2005 is significant at $p<0.05$, **difference from 2004/2005 is significant at $p<0.01$)

Abietic acid showed a clearer trend, with an increase of 120% over the entire study period (2004/2005 to 2007/2008) (Figure 2.9). The increase was not constant, with the second changeout year (2006/2007) showing levels comparable to the pre-changeout (2004/2005) and the first (2005/2006) and third (2007/2008) year showing higher levels. The fraction of $PM_{2.5}$ made up of abietic acid also followed the same trend as the abietic acid measurements. The final year of the changeout (2007/2008) showed the greatest increase, with an increase of over 2.5 times the pre-changeout values.

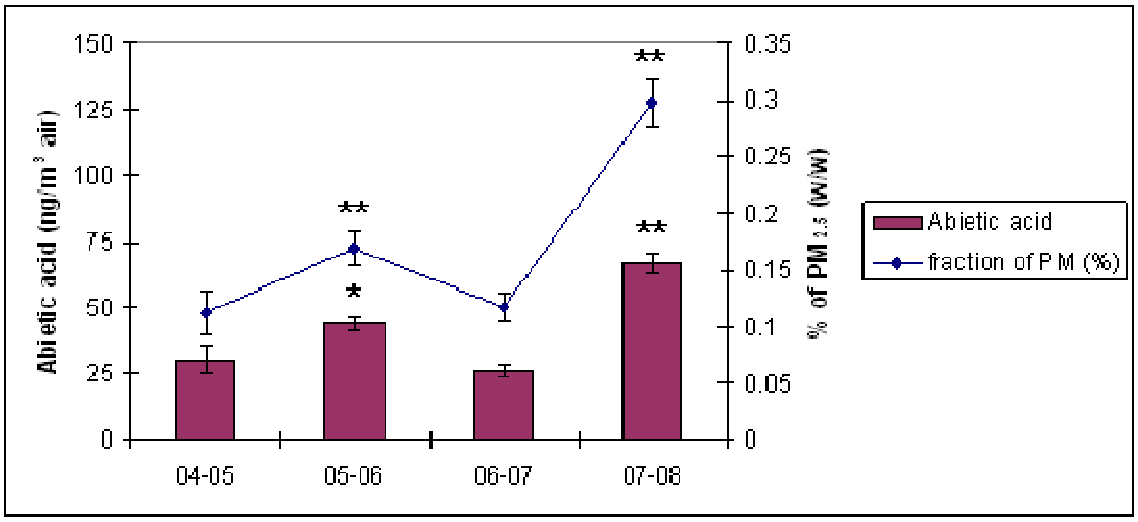


**Figure 2.9**
Average winter ambient concentration (±standard error) in Libby and fraction of $PM_{2.5}$ (±standard error) of abietic acid.[45] (*difference from 2004/2005 is significant at $p<0.05$, **difference from 2004/2005 is significant at $p<0.01$)

#### 2.2.7.3. Methoxyphenols

The methoxyphenols as a group showed no consistent response to the changeout. The measured levels of vanillin showed no statistical difference from the pre-changeout year (2004/2005) (Figure 2.10). Acetovanillone levels varied from year-to-year, but were only significantly different from pre-changeout (2004/2005) values during the last winter of the changeout (2007/2008) (Figure 2.10). Acetovanillone was detected in only about half of the samples analyzed (38/82), making it more difficult to detect any trends during the changeout. The fraction of $PM_{2.5}$ represented by vanillin showed no change during the entire changeout, while the fraction represented by acetovanillone was significantly different ($p<0.01$) only for the winter of 2007/2008, following the same trend as the acetovanillone measurements (Figure 2.10).

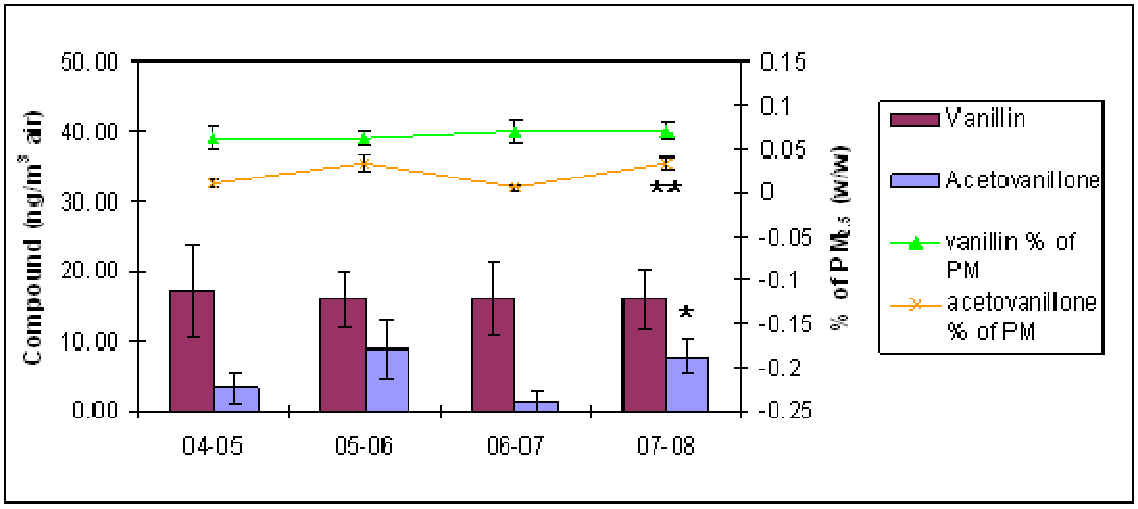


**Figure 2.10**
Average winter ambient concentrations (±standard error) in Libby and fraction of $PM_{2.5}$ (±standard error) of vanillin and acetovanillone.[45] (*difference from 2004/2005 is significant at $p<0.05$, **difference from 2004/2005 is significant at $p<0.01$)

Guaiacol and 4-ethylguaiacol also showed no clear trend during the changeout period (Figure 2.11). In the 2 years before the changeout, guaiacol showed a wide range of concentrations making it difficult to discern any decrease during the changeout. 4-Ethylguaiacol showed a decrease during the last year of the changeout (2006/2007 to 2007/2008), but showed no significant change during any of the other years. The portion of $PM_{2.5}$ made up by 4-ethylguaiacol showed no change during the changeout (Figure 2.11). The portion of $PM_{2.5}$ represented by guaiacol in 2006/2007 was the only winter to show statistically significant ($p<0.05$) decreases from the pre-changeout measurement (Figure 2.11).

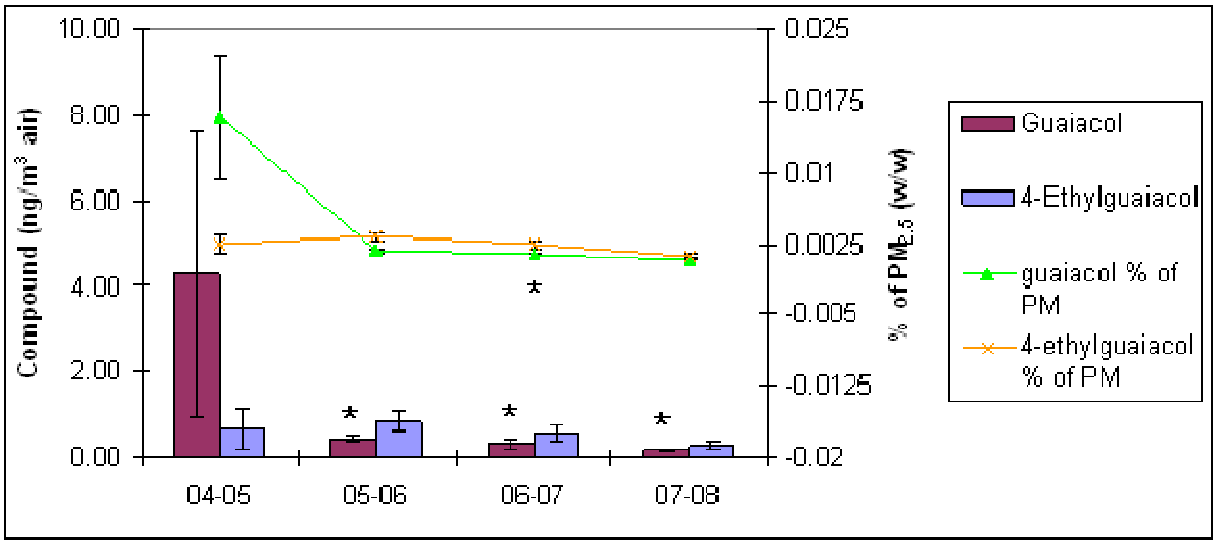


**Figure 2.11**
Average winter ambient concentrations (±standard error) in Libby and fraction of $PM_{2.5}$ (±standard error) of guaiacol and 4-ethylguaiacol.[45] (*difference from 2004/2005 is significant at $p<0.05$)

#### 2.2.7.4. Methoxyphenol correlation to temperature

The measured levels of vanillin and acetovanillone showed a correlation to ambient temperature on the day of sample collection (Figures 2.12 and 2.13). Methoxyphenol concentration increases with decreasing temperature, consistent with observations during the initial study period in 2004-2005.[18] Vanillin showed a correlation coefficient to ambient temperature of 0.68 ($p<0.001$) for all 4 years combined, with a range of 0.66 to 0.79 for the individual years (median= 0.70) (Figure 2.12). Acetovanillone showed a correlation coefficient to ambient temperature of 0.41 ($p<0.001$) for all 4 years combined and a range of 0.24 to 0.67 for the individual years (median=0.58) (Figure 2.12). Neither guaiacol nor 4-ethylguaiacol showed a correlation to ambient temperature (Figure 2.13). Both guaiacol and 4-ethylguaiacol are found predominantly in the vapor phase of wood smoke, so measurement and interpretation of their concentrations in the particulate phase is difficult.

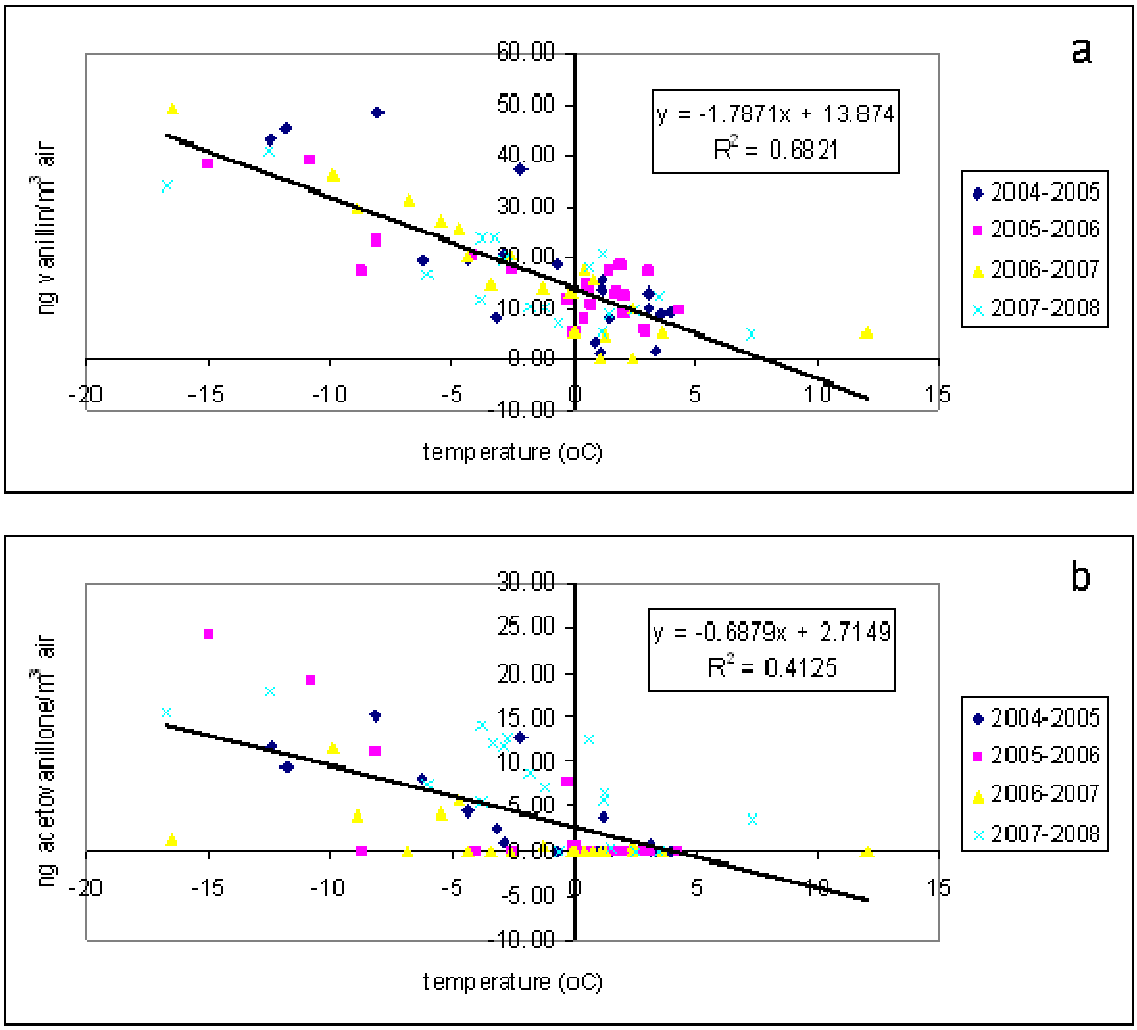


**Figure 2.12**

Correlation between levels of methoxyphenols and ambient air temperature on the day of sample collection measured in Libby of (a) vanillin (b) acetovanillone.

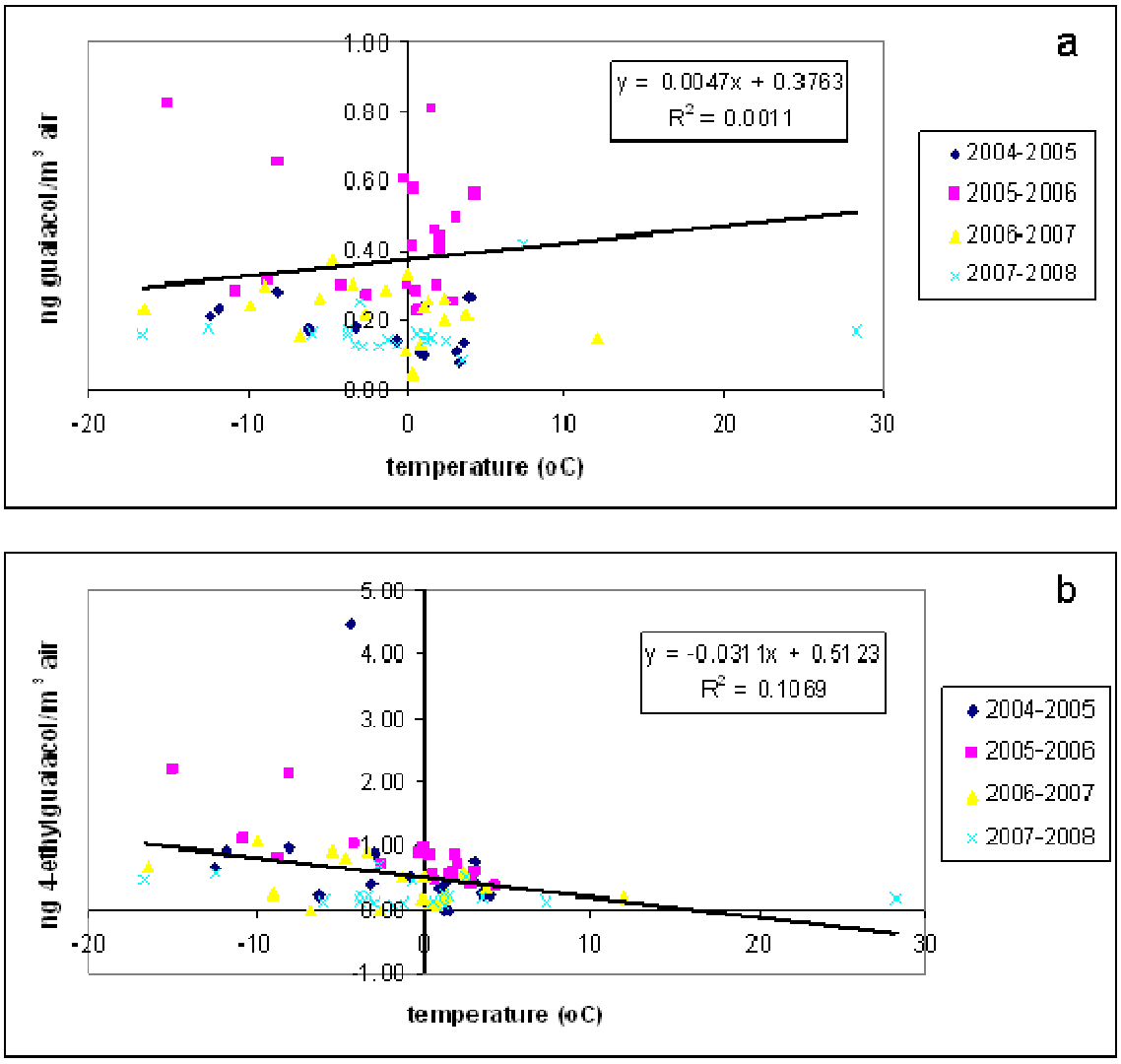


**Figure 2.13**
Correlation between levels of methoxyphenols and ambient air temperature on the day of sample collection measured in Libby of (a) guaiacol and (b) 4-ethylguaiacol.

### 2.2.8. Discussion

The new EPA-certified stoves installed in Libby allow for more complete combustion, so the observed 50% decrease in levoglucosan levels is similar to previously reported results obtained in an experimental setting of a 60-88% decrease in particulate phase levoglucosan when changing from a closed or partially closed airflow setting to an open one.[54] The level of levoglucosan measured in Libby even after the woodstove changeout was generally higher than levels measured in other US urban areas, such as Seattle, WA (13-760 ng/m$^3$)[26], Spokane, WA (2-327 ng/m$^3$)[48] and Fresno and Bakersfield, CA (23-7590 ng/m$^3$).[43]

We speculate that the increase or lack of decrease in resin acid levels in the $PM_{2.5}$ is due to the higher combustion temperatures in the new, EPA-certified stoves. Emission profiles of resin acids can vary depending on the stove and operating conditions used.[54] The new EPA-certified stoves allow for more complete combustion than the older model stoves. Unlike levoglucosan, which is produced during combustion, resin acids such as abietic acid are released in their unaltered form when the wood is burned.[24, 36] These resin acids are released due to volatilization by steam, and more complete combustion of the wood or higher combustion temperatures might be expected to result in greater release and incorporation into particulate.[24] Dehydroabietic acid in the air can also be affected by relative humidity as it can be degraded under UV light when dissolved in water.[36] However, relative humidity measured in Libby was only statistically different during 1 year of the study (2006/2007). Also, this increase in resin acid levels after the stove changeout observed in Libby is consistent with observations inside individual homes in Libby after installation of an EPA-certified stove[17], where measurements are less likely to be influenced by variations in meteorological conditions.

Two of the four measured methoxyphenols showed a correlation to ambient temperature on the day of sample collection, but not to daily $PM_{2.5}$ concentration. This is likely due to the semi-volatile nature of the methoxyphenols. It could also be possible that these compounds are correlated to temperature because people tend to burn less wood when the ambient temperature is higher, leading to lower levels of the compounds. If this was the case, levoglucosan and the resin acids should also show a correlation to ambient temperature as their levels are dependent on the amount of wood burned. Correlation coefficients for levoglucosan, dehydroabietic acid, and abietic acid to ambient temperature were 0.005, 0.0089, and 0.0181, respectively. Also, due to the winter inversions in the airshed and low average wind speed in Libby (Table 2.1, p. 7), it seems unlikely that variations due to day-to-day differences in woodstove use could account for the correlation observed between vanillin or acetovanillone and temperature. The low levels of guaiacol and 4-ethylguaiacol observed and temperature dependence of vanillin and acetovanillone make all four of the measured methoxyphenols unusable as tracers for woodsmoke in particulate matter.

## 2.3. Residential study

The EPA has set standards for outdoor $PM_{2.5}$ levels, however no comparable standard exists for indoor environments.[8] Indoor $PM_{2.5}$ from woodstoves and other sources can be a significant source of exposure as people spend the majority of their time indoors, up to 95% in some areas[55, 56]. While an outdoor reduction in $PM_{2.5}$ will allow the community to meet the EPA's standards, a reduction in indoor $PM_{2.5}$ can have a greater impact on individual exposure and health effects.

### 2.3.1. Objective

In this study, we measured the change in indoor air quality within homes that received a new EPA-certified woodstove. This was accomplished through measuring the changes in $PM_{2.5}$ and seven selected tracers for woodsmoke in homes before and after a woodstove replacement.

### 2.3.2. Sample collection

Samples were collected initially inside 21 homes in Libby from October 2006 through March 2007 to evaluate the indoor air quality resulting from a non-EPA certified wood stove. The homes selected had a planned woodstove change out during the winter of 2006/2007 and had no residents that smoked inside the house. Five of the original 21 homes were eliminated from the study for various reasons (see Table 4.4, p. 72), leaving a sample size of 16 homes.

One 24-hour sample was collected inside each home prior to the woodstove changeout and a second 24-hour sample was collected two to three weeks after the installation of a new EPA-certified woodstove. Two types of air samplers were deployed, including a portable TSI, Inc. DustTrak (Model 8520) that continuously measured $PM_{2.5}$ mass, and one Leland Legacy pump/Personal Environmental Monitor (PEM) sampler fitted with a 37-mm $PM_{2.5}$ quartz filter to collect $PM_{2.5}$ for chemical analysis.

### 2.3.3. Sample analysis

Samples were analyzed for the seven selected chemical tracers using the GC-MS method discussed previously in Section 2.2.3. The remaining half of the filter was used for analysis of organic carbon and elemental carbon (Section 4.5.1.)

### 2.3.4. Ambient measurements

#### 2.3.4.1. $PM_{2.5}$ Collection

Ambient $PM_{2.5}$ mass concentrations on the days of indoor sampling were taken from the Montana Department of Environmental Quality's Libby $PM_{2.5}$ compliance monitoring site.[47] The site was located within 2 miles of each home that was involved in the residential study.

#### 2.3.4.2. Meteorological conditions

Meteorological data, including temperature, wind speed, relative humidity, and precipitation, were obtained from the database collected by the Western Regional Climate Center (WRCC), a division of the National Oceanic and Atmospheric Administration.[46] The measurement station was located in Libby, at latitude 48° 23' 00" and longitude 115° 34' 00".

### 2.3.5. Indoor $PM_{2.5}$ results

Results of the residential $PM_{2.5}$ sampling program are presented in Table 2.3 and Figure 2.14. There were substantial reductions in average and maximum $PM_{2.5}$ observed after the woodstove replacement. Before the changeout, seven homes had 24-hour average $PM_{2.5}$ concentrations above the EPA daily ambient air quality standard of 35 $\mu g/m^3$, with the maximum observed 24-hour average concentration in one home at 118 $\mu g/m^3$. After the stove changeouts, only two of the homes had a 24-hour average concentration above 35 $\mu g/m^3$. The maximum observed 24-hour average concentration in the post-measurements was 86 $\mu g/m^3$. On average, $PM_{2.5}$ levels decreased by 71% after new, EPA-certified woodstoves were installed.

**Table 2.3**

Pre- and post-changeout averages for $PM_{2.5}$, organic and elemental carbon, levoglucosan, and the resin acids measured inside 16 homes in Libby.[17]

| Parameter | Before Changeout Mean ± sd | Before Changeout Median | After Changeout Mean ± sd | After Changeout Median | Percent Change | p-value[a] |
|---|---|---|---|---|---|---|
| Average $PM_{2.5}$ ($\mu g/m^3$) | 51.2 ± 32.0 | 34.5 | 15.0 ± 20.8 | 9.5 | -71% | 0.0001 |
| Maximum $PM_{2.5}$ ($\mu g/m^3$) | 434 ± 419 | 266 | 103 ± 167 | 51.5 | -76% | 0.0002 |
| Organic Carbon (OC) ($\mu g/m^3$) | 17.6 ± 8.2 | 14.4 | 12.5 ± 10.6 | 9.4 | -26% | 0.007 |
| Elemental Carbon (EC) ($\mu g/m^3$) | 0.94 ± 0.90 | 0.68 | 0.88 ± 1.87 | 0.29 | -6% | 0.054 |
| Levoglucosan ($ng/m^3$) | 1050 ± 1027 | 652 | 577 ± 988 | 321 | -45% | 0.001 |
| Dehydroabietic Acid ($ng/m^3$) | 80.2 ± 61.1 | 74.1 | 187 ± 128 | 154 | 133% | 0.0001 |
| Abietic Acid ($ng/m^3$) | 3.7 ± 5.7 | 2.8 | 14.5 ± 22.7 | 5.2 | 292% | 0.153 |

[a]Paired t-test on log-transformed data

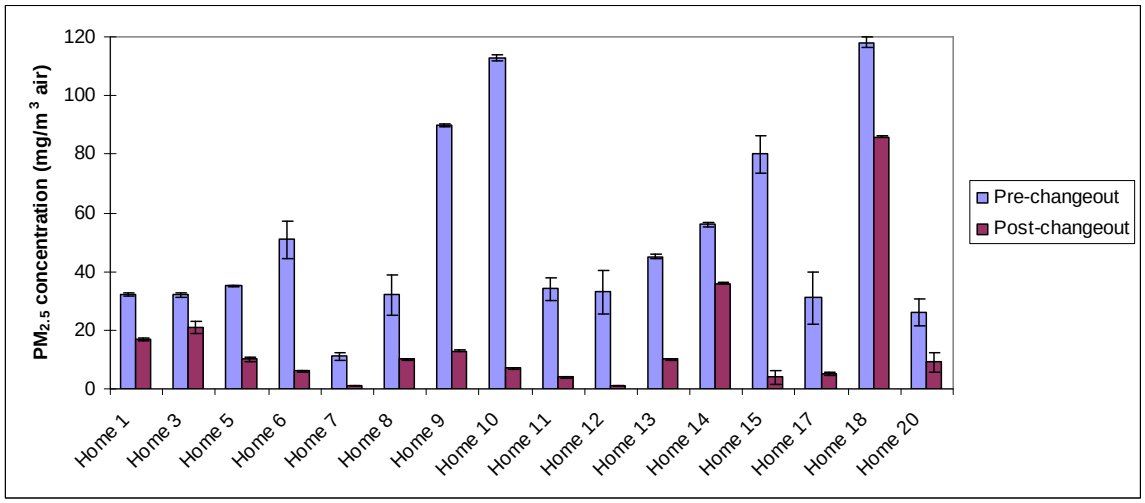


**Figure 2.14**

Pre- and post-woodstove changeout $PM_{2.5}$ mass results from 16 homes in Libby.[17]

### 2.3.6. Tracer results

#### 2.3.6.1. Levoglucosan

Levoglucosan decreased significantly in homes after the installation of an EPA-certified woodstove (Table 2.3 and Figure 2.15). Before the stove changeout,

approximately 2% of the indoor $PM_{2.5}$ mass was composed of levoglucosan, while the measured organic carbon (OC) was composed of approximately 6.0% levoglucosan. For post-changeout measurements, levoglucosan was found to compose 3.8% of the $PM_{2.5}$ mass and 4.6% of the OC. The correlation between levoglucosan and $PM_{2.5}$ in the pre- versus post-changeout samples was consistent, r = 0.69 (p = 0.003) and r = 0.56 (p = 0.025), respectively.

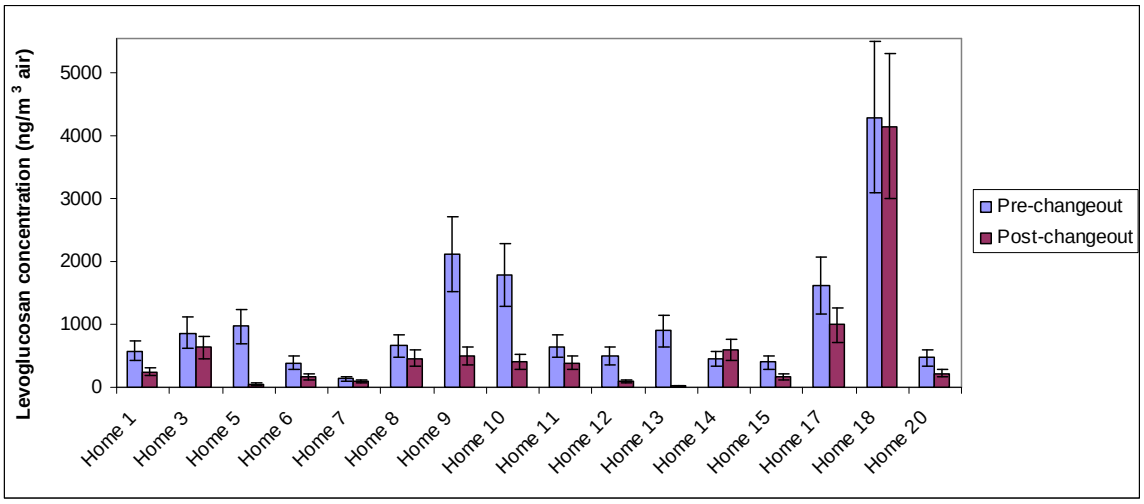


**Figure 2.15**
Pre- and post-woodstove changeout levels of levoglucosan from 16 homes in Libby.[17]

#### 2.3.6.2. Resin acids

The 24-hour concentrations of dehydroabietic acid showed significant increases in all 16 homes following the installation of a new EPA-certified woodstove (Table 2.3 and Figure 2.16a**).** Dehydroabietic acid and $PM_{2.5}$ were strongly correlated in the pre-changeout samples (r = 0.58, p = 0.019), but were not strongly correlated in the post-changeout samples (r = 0.37, p = 0.15).

Abietic acid also increased in most homes, but the overall increase was not statistically significant (p = 0.153) (Figure 2.16b). Abietic acid was present at much lower levels than dehydroabietic acid, resulting in greater uncertainty in the results. There were seven non-detects in the measured samples for abietic acid, including three in the pre-changeout sampling and four in the post-changeout sampling. Similar to dehydroabietic acid, there was a strong correlation between abietic acid and $PM_{2.5\ in}$ the pre-changeout samples (r = 0.64, p = 0.008), but the corresponding correlation in the post-changeout samples was weaker (r = 0.38, p = 0.14).

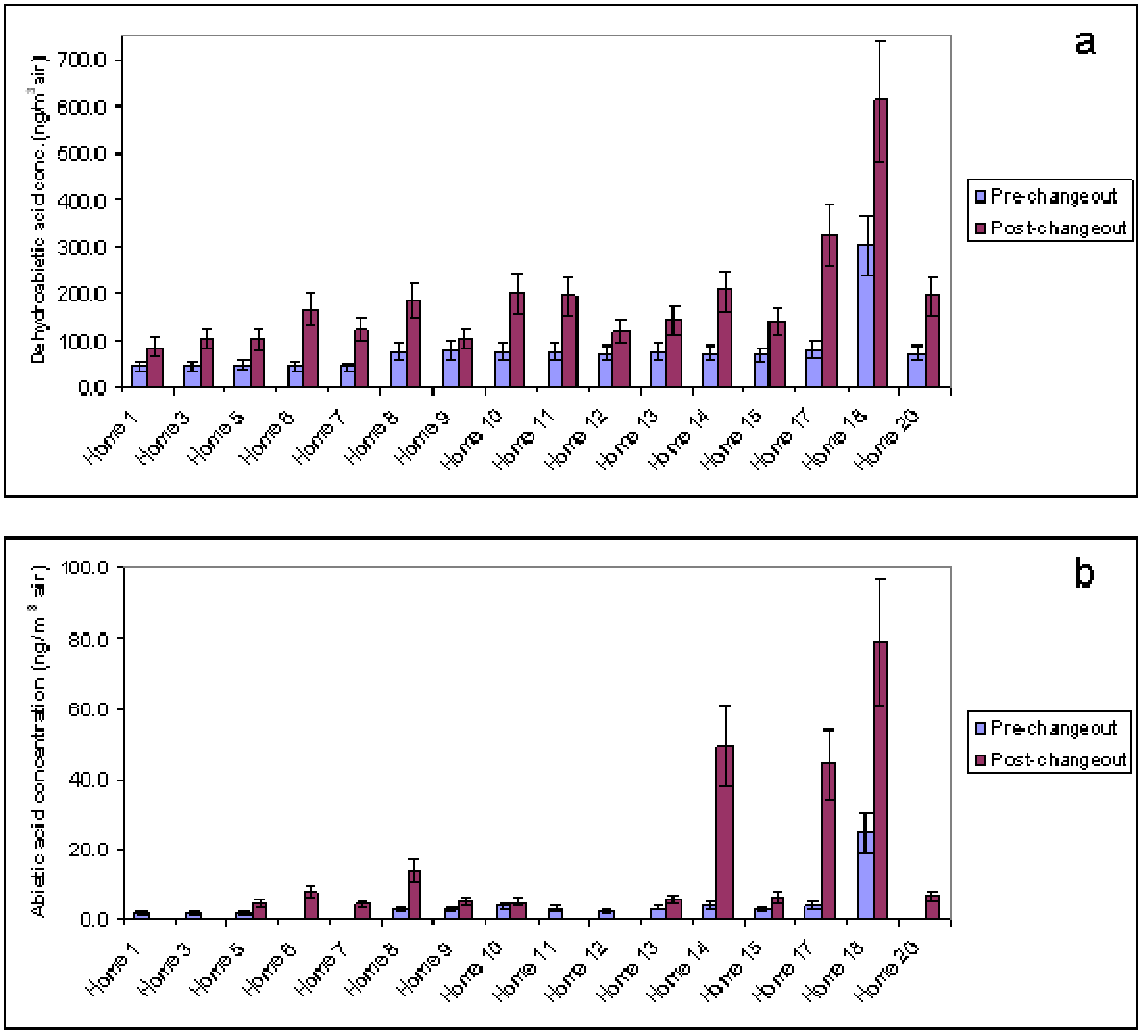


**Figure 2.16**
Pre- and post-woodstove changeout levels of (a) dehydroabietic acid and (b) abietic acid from 16 homes in Libby.[17]

#### 2.3.6.3. Methoxyphenols

The four methoxyphenols that were monitored in this study were vanillin, acetovanillone, guaiacol, and 4-ethylguaiacol. For two of the four methoxyphenols (vanillin and acetovanillone), no trends were observed. The majority of the pre- and post-changeout samples had non-detectable levels of vanillin and acetovanillone, likely due to their higher vapour pressure at indoor temperatures. For guaiacol there were five non-detects, one in the pre-changeout sampling and four in the post-changeout sampling. Pre-changeout measurements of guaiacol were 0.30 ± 0.15 ng/m$^3$ (median 0.28 ng/m$^3$).

Following the installation of the new woodstove, guaiacol decreased in one-half of the homes, but there was not a significant overall decrease ($p = 0.46$). For 4-ethylguaiacol there were three non-detects, all in the post-changeout samples. Pre-changeout concentrations of 4-ethylguaiacol were $0.89 \pm 0.56$ ng/m$^3$ (median 0.72 ng/m$^3$). Concentrations of 4-ethylguaiacol were lower in 11 of the 16 homes following the woodstove changeout, resulting in an average reduction of 6% ($p = 0.24$).

### 2.3.7. Impact of ambient measures on indoor measures

Ambient temperature during the pre-changeout indoor measurements ($34.0 \pm 10.4$ °F) was, on average, higher than ambient temperature during the post-changeout indoor measurements ($25.7 \pm 6.9$ °F) ($p = 0.012$). There was no difference in solar radiation measures or average wind speed on pre-changeout versus post-changeout measurement days ($p = 0.14$ and 0.33, respectively). Ambient $PM_{2.5}$ concentrations were available on both the pre-and post-changeout sample collection days for 14 of the 16 homes. The average 24-hour ambient $PM_{2.5}$ on pre-changeout sample days was $27.0 \pm 14.0$ μg/m$^3$, while the average 24-hour ambient $PM_{2.5}$ concentration on the days of the post-changeout was $18.1 \pm 9.2$ μg/m$^3$ ($p = 0.04$, range of difference for each home = -34 to +11).

If variations in ambient $PM_{2.5}$ levels had a strong influence on reductions in indoor $PM_{2.5}$ following woodstove changeout, we would expect this to be evident in homes which experienced a reduction in ambient $PM_{2.5}$ on post- versus pre-sampling days. When stratifying paired analysis by homes which experienced reductions (n=10) versus increases (n=4) in ambient $PM_{2.5}$ on their corresponding sample days, indoor PM was reduced by 75% ($p < 0.001$) and 62% ($p = 0.07$), respectively. Neither ambient $PM_{2.5}$ nor ambient temperature was significantly associated with indoor $PM_{2.5}$ or indoor levoglucosan in unmatched analysis of variance. These ambient measures also did not have significant interactions with woodstove changeout for changes in indoor PM or levoglucosan.

### 2.3.8. Discussion

The biggest change in air quality observed in this study is the 71% reduction in average $PM_{2.5}$ concentration and 75% reduction in maximum $PM_{2.5}$ concentration after the installation of a new stove. Although there is no non-occupational indoor air quality standard for $PM_{2.5}$, when comparing to the NAAQS this woodstove intervention resulted

in reducing $PM_{2.5}$ concentrations from well above the daily standard of 35 $\mu g/m^3$ to well below the daily standard in most of the homes studied.

The increase in resin acids after the stoves were replaced were contrary to what was expected, however, they were consistent with ambient results in Libby. Previous studies have shown that changes in the relative concentrations of the resin acids can be explained either by changes in the type of fuel burned, or by photolysis of the compounds when particulate matter is exposed to sunlight over a period of time.[36, 39, 49, 57] This is unlikely to be the case in the residential samples since the same wood species were used both pre- and post-changeout. There is also little reason to suspect differences in the age or exposure of the indoor particulate matter to sunlight pre- and post-changeout, especially since the increase was observed in all 16 homes sampled. It is possible that the results were affected by infiltration of ambient particulate matter. However, this cannot be determined without a comprehensive analysis of the ambient meteorological conditions, ambient particulate matter chemistry, and/or measuring the exchange rates within each of the homes. Ambient $PM_{2.5}$ was not significantly associated with indoor $PM_{2.5}$ or indoor levoglucosan in unmatched analysis of variance in this study, nor did ambient $PM_{2.5}$ measures have significant interactions with woodstove changeout for changes in indoor particulate matter or levoglucosan so it is unlikely that this is a major factor in the increase in resin acid levels.

Our findings for the four methoxyphenol markers of woodsmoke were inconsistent, likely because the samples were collected in the indoor environment, where temperatures were more elevated compared to the ambient environment (between 60-80 °F). The ambient studies in Libby show an inverse correlation between the concentrations of these methoxyphenols in ambient $PM_{2.5}$ and temperature.[18] A more useful technique for measuring these four specific methoxyphenols in the indoor environment would be to collect gas phase samples.

There were several limitations to this study that should be considered. First, meteorological conditions cannot be controlled, which can affect wood burning behaviour. The ambient temperature was lower during the post-changeout measures which would likely result in a greater burning activity. Second, the proper usage of newer woodstoves requires some degree of training, and proper usage was difficult to

assess. To partially alleviate this concern, post-changeout measures were conducted after the residents had two to three weeks to use the new woodstove. Third, we could not completely control for resident behaviours (such as tobacco smoke) that affect measures of particulate matter and woodsmoke markers. Our selection of sampling locations was restricted to homes with no reported smoking residents; however one resident (home 18) reported cigarette smoking outside on the patio. It is difficult to assess the impact that this behaviour may have had on sampling, but this home did show much higher levels of $PM_{2.5}$, levoglucosan and resin acids than most other homes in the study.

### 2.4. Laboratory studies

Laboratory studies with both an EPA-certified and an older model, non EPA-certified woodstove were designed to gain more information on the chemical tracer results observed in the ambient and residential studies in Libby. The goal of the laboratory studies was first to replicate the decrease in levoglucosan and increase in resin acids observed and then to attempt to explain these results. Being able to replicate real-world results in a laboratory setting can also be beneficial for future woodsmoke studies, particularly in developing a biomarker for woodsmoke exposure.

#### 2.4.1. Woodstoves

Two different wood burning stoves were used to generate smoke samples. An older model, non-EPA-certified woodstove was used to replicate pre-changeout conditions and a new, EPA-certified woodstove was used to replicate post-changeout conditions. The EPA-certified stove was a non-catalytic model that uses firebox insulation, a large baffle and pre-heated combustion air to create a better environment for complete combustion than the older model stoves.[11] Stoves were each burned for 2 hours at a time on two separate days. A mixture of locally obtained softwoods was used for each burn and the amount and frequency of wood added was recorded.

#### 2.4.2. Sample collection

Aluminium flex tubing was used to direct smoke from the chimney of the stove into a fume hood. A Leland Legacy pump/Personal Environmental Monitor (PEM) sampler fitted with a 37-mm $PM_{2.5}$ quartz filter was used to collect $PM_{2.5}$ for chemical analysis. The sampler was placed at the opening to the flex tubing in the hood and co-

located with a DustTrak $PM_{2.5}$ measurement device. Quartz filter samples were collected every 15 minutes for 2 hours during each stove burn.

### 2.4.3. Sample analysis

Samples were analyzed for the seven selected chemical tracers using the GC-MS method discussed previously in Section 2.2.3.

### 2.4.4. $PM_{2.5}$

$PM_{2.5}$ was monitored using a portable TSI, Inc. DustTrak (Model 8520) that continuously measures $PM_{2.5}$ mass. The DustTrak was co-located with the PEM at the outlet for the stove exhaust to provide a PM concentration for each quartz filter sample. The samplers were moved to keep a $PM_{2.5}$ concentration of between 1 and 10 mg/m$^3$ to avoid overloading the PEM sampler. Average $PM_{2.5}$ was consistent between the two burns from both the older model and EPA-certified model wood stove (Figure 2.17).

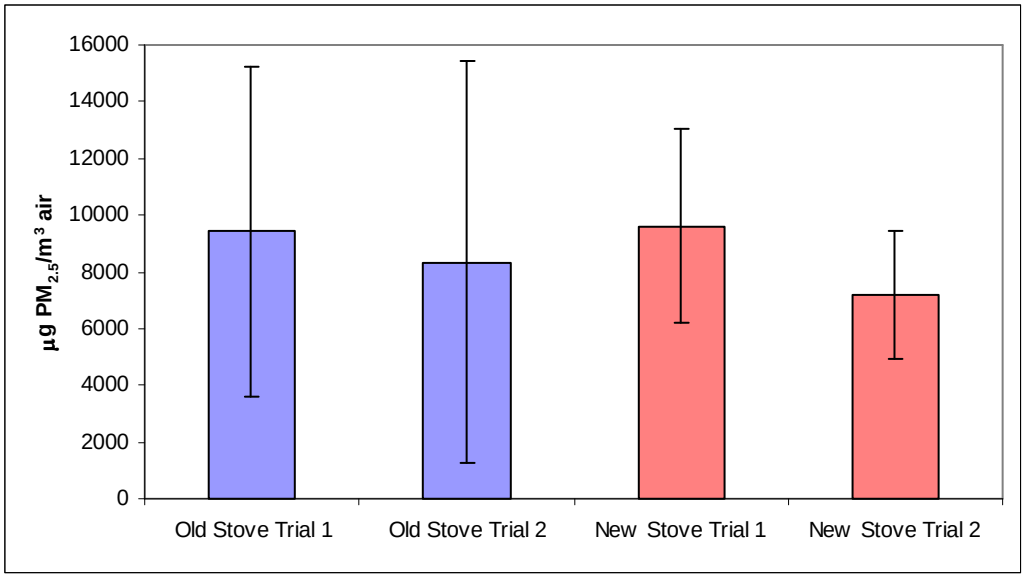


**Figure 2.17**
$PM_{2.5}$ from each of the two laboratory burns using an older model, non EPA-certified model woodstove and a newer model, EPA-certified stove.

### 2.4.5. Results

#### 2.4.5.1. Burn conditions

The amount of wood burned differed by no more than 10% between burns with the same stove or between burns with the two different stoves (Table 2.4). The old stove burned at a higher average temperature and reached a higher maximum temperature than

the new stove did. Both stoves were operating at the low end of the optimum operating temperature (135-300°C) for a wood burning stove.

**Table 2.4**
Average wood burned and average and maximum temperatures from the old and new woodstove laboratory burns.

| | Average amount of wood burned (g) | Burn temperature (oC) Average | Maximum |
|---|---|---|---|
| **Old Stove** | 1087 | 150 | 208 |
| **New Stove** | 994 | 130 | 155 |

### 2.4.5.2. Levoglucosan

Levoglucosan ranged from approximately 1.5-3% of the $PM_{2.5}$ weight during each of the four laboratory burns (Figure 2.18). No significant difference in levoglucosan levels was observed between burns with the same stove or between the two different stoves. The portion of $PM_{2.5}$ made up by levoglucosan in the laboratory studies was significantly less than that observed in Libby (11.8%)[42], however it did fall within previously reported ranges for levoglucosan in particulate matter of 0.8-26%.[20, 50]

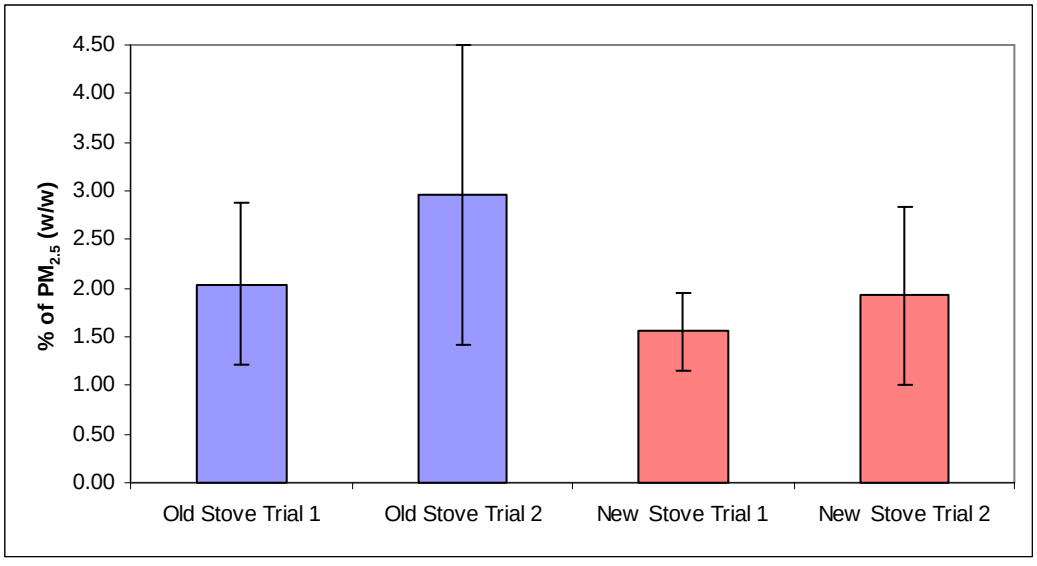


**Figure 2.18**
Levoglucosan as a percentage of $PM_{2.5}$ weight for two laboratory burns using an older model, non EPA-certified model woodstove and a newer model, EPA-certified stove.

### 2.4.5.3. Resin Acids

Dehydroabietic acid ranged from about 2.25% of the $PM_{2.5}$ weight in the first old stove burn to 0.1% in the new stove burn (Figure 2.19). The resin acid data from the second trial with the new stove were discarded because of problems during analysis. The dehydroabietic acid concentration decreased from the average of the two old stove trials to the new stove, however, the decrease was not statistically significant. Abietic acid ranged from 0.1-2.5% between different stove burns (Figure 2.20). The average abietic acid concentration was significantly lower with the newer EPA-certified stove than with the older model stove.

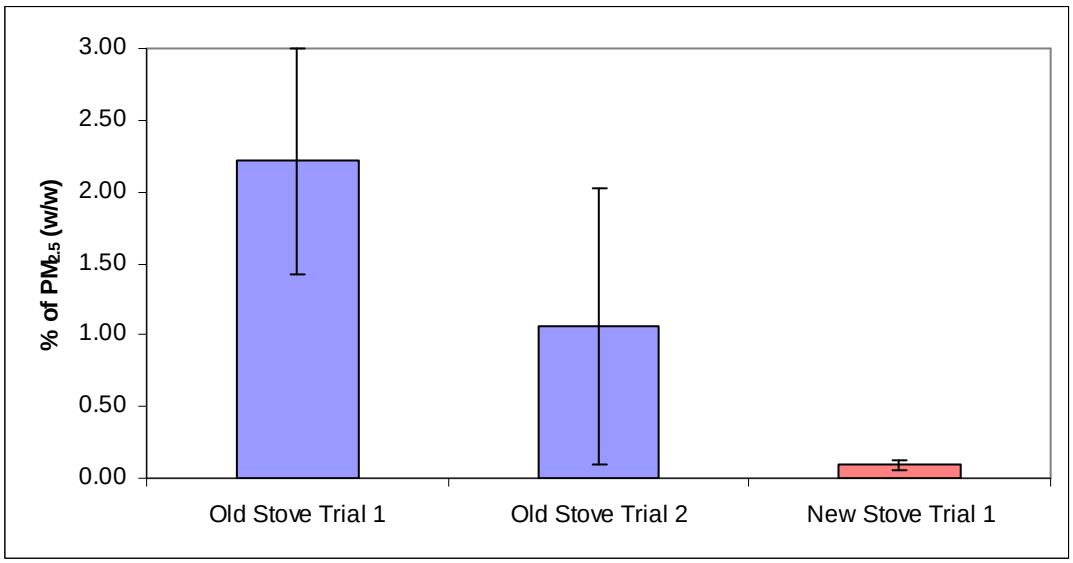


**Figure 2.19**
Dehydroabietic acid as a percentage of $PM_{2.5}$ weight for two laboratory burns using an older model, non EPA-certified model woodstove and a newer model, EPA-certified stove.

These two resin acids represented a combined 1.5% of the total $PM_{2.5}$ mass in Libby before the changeout[42], which is lower than what was observed in the laboratory studies. Reported values for dehydroabietic acid range from 0.3-8.7% and value for abietic acid range from 0.1-1.6%.[23, 24, 51, 52] Most of the reported values are from the burning of specific species of wood, such as Ponderosa or loblolly pine, which can produce different ratios of the resin acids than the mixture of species burned in the laboratory studies or in Libby.

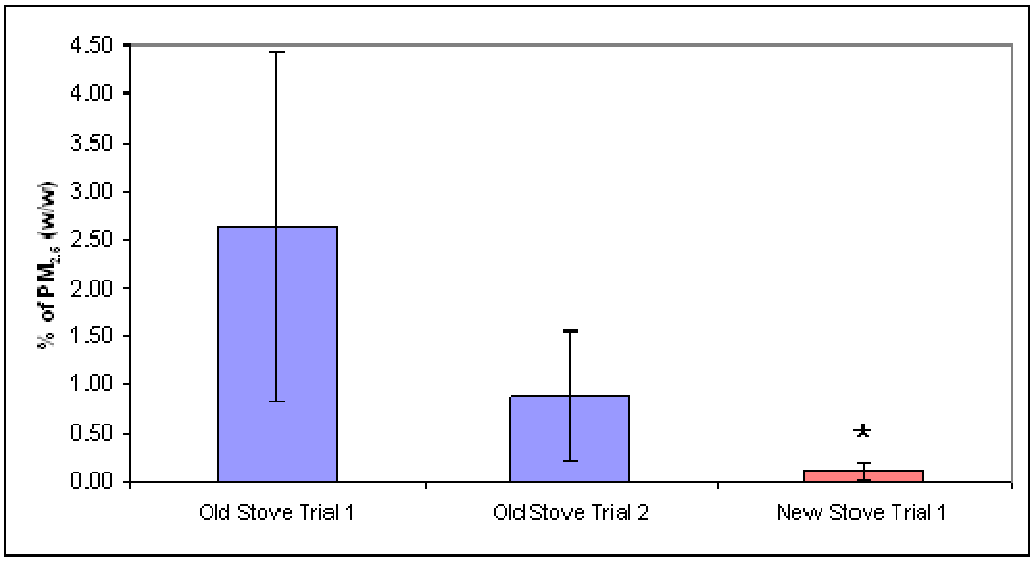


**Figure 2.20**
Abietic acid as a percentage of $PM_{2.5}$ weight for two laboratory burns using an older model, non EPA-certified model woodstove and a newer model, EPA-certified stove. (*difference from average of burns with the old stove is significant at $p<0.05$)

### 2.4.6. Discussion

Contrary to what was previously observed in residential and ambient studies in Libby, levoglucosan showed no significant difference between the EPA certified stove and the older model stove. The resin acids also responded very differently than in previous studies in Libby, showing significantly lower levels in the newer model stove. The burn temperatures were also lower for both stoves in the laboratory studies than what is expected in a real-world setting. We speculate that the decrease in the resin acids is due to operating the stoves below the optimum burn zone. Since the resin acids are simply released from wood during combustion, burning at a lower temperature could result in a lower concentration of resin acids being released in the airborne particulate matter.

## 2.5. Air studies conclusions

### 2.5.1. Libby studies

#### 2.5.1.1. Particulate matter

Ambient $PM_{2.5}$ levels in Libby decreased by 20% over the course of the woodstove changeout. Samples collected inside homes in Libby that had a woodstove replaced, showed an average decrease in $PM_{2.5}$ levels of 71%. Before the changeout,

seven homes had 24-hour average $PM_{2.5}$ concentrations above the EPA daily ambient air quality standard of 35 $\mu g/m^3$, with the maximum observed 24-hour average concentration in one home at 118 $\mu g/m^3$. After the stove changeouts, only two of the homes had a 24-hour average concentration above 35 $\mu g/m^3$ and the highest concentration observed was 86 $\mu g/m^3$.

#### 2.5.1.2. Levoglucosan

Levoglucosan levels decreased by 20% in the Libby ambient study and an average of 45% inside the homes after the woodstove changeout as expected. Levoglucosan was also found to represent a smaller fraction of the particulate matter collected outdoors in Libby after the woodstove changeout. Levoglucosan concentrations were found to strongly correlate to the levels of $PM_{2.5}$ in the Libby ambient studies. Measured levoglucosan concentrations in the Libby PM were comparable to previously reported values for levoglucosan in woodsmoke particulate. Based on these findings, levoglucosan was determined to be a suitable tracer for woodsmoke based particulate matter.

#### 2.5.1.3. Resin Acids

In the initial 2004-2005 study, the concentrations of the resin acids were found to correlate strongly with the levels of $PM_{2.5}$ and the fraction of the $PM_{2.5}$ mass corresponding to each resin acid was with within the range of previously reported values for woodsmoke dominated particulate matter. In both the Libby ambient and residential studies, the levels of both dehydroabietic acid and abietic acid increased or remained the same after the woodstove changeout. Since $PM_{2.5}$ levels decreased outdoors, each resin acid represented a higher percentage of the particulate weight after the changeout than before. The increased levels of resin acids post-changeout indicates that these chemically stable compounds may continue to survive the combustion conditions in the modern stoves, and may actually be released at higher levels due to increased combustion temperatures. This suggests that even though there is a significant decrease in indoor $PM_{2.5}$ following the replacement of an old stove with an EPA-certified stove, there potentially could be an increase in some volatile and semi-volatile compounds due to the difference in burning conditions, or some other factors.[54, 58]

#### 2.5.1.4. Methoxyphenols

The levels of the semivolatile methoxyphenols in the Libby PM were not correlated with levels of $PM_{2.5}$, but were affected by the ambient temperature. Both vanillin and acetovanillone were found to correlate to temperature on the day of sample collection in the Libby ambient study. Guaiacol and 4-ethylguaiacol did not show a correlation to temperature, likely because they were present at very low levels. Many of the residential samples had non-detectable levels of vanillin and acetovanillone. None of the methoxyphenols showed a trend in the Libby residential studies, likely because of the higher temperatures found in the indoor environment. Because of their dependence on temperature and lack of correlation to $PM_{2.5}$ levels, these four methoxyphenols were determined to be poor tracers for woodsmoke particulate matter.

### 2.5.2. Laboratory studies

An older model woodstove and a new, EPA-certified stove were burned in a laboratory setting to attempt to replicate and explain the chemical tracer results observed in Libby. Levoglucosan showed no change between the two types of stoves, while both resin acids showed lower levels in the new stove, contrary to what was expected. The burn temperature for both stoves was lower than optimal burn temperature, which is likely a factor in the contradictory results observed. Further studies are needed to determine if the current results are an artifact of the experimental approach.

Others have also observed differences in the chemical composition of particulate matter depending on air flow during combustion and/or stove design.[54, 58] Purvis et al. observed significant decreases in $PM_{10}$ and $PM_{2.5}$ from a modern wood stove relative to an older design, but also reported unchanged or increased emissions of various semi-volatile organics, including PAHs.[58]

## Chapter 3: Biomarker Studies

Chronic or episodic exposures to biomass smoke present potential health concerns.[17, 59, 60] Intermittent exposures to high levels of biomass smoke can have effects on multiple aspects of human health, including exacerbation of asthma and cardiovascular disease, and alterations in either pulmonary or systemic immunity. Due to the potential health concerns for woodsmoke inhalation, the development of a specific biomarker would be of great importance in assessing the health effects of exposed individuals or communities. A biomarker of wood smoke exposure would be a useful tool to assess individual exposures. A key aspect of such a biomarker would be the ability to account for variables in exposure and individual metabolism. In addition to its ease of use as non-invasive, a urinary biomarker gives a more accurate measurement of actual smoke exposure, as it takes into account individual variations such as breathing rate.[61] A non-invasive biomarker would also be more practical than personal environmental monitoring for measuring occupational exposures (i.e., fire-fighters) or for chronic exposures.[62]

Only a few compounds have thus far been investigated as potential urinary biomarkers for wood smoke exposure. Dills et al. evaluated several methoxyphenols as biomarkers for wood smoke exposure.[44, 63] Subjects were exposed to campfire smoke for 2 hours and personal $PM_{2.5}$ exposure was measured. Propylguaiacol, syringol, methylsyringol, ethylsyringol, and propylsyringol all had peak concentrations in the urine approximately 6 hours after wood smoke exposure. A 12-hour average of these five compounds was found to be the most practical metric for the biomarker of wood smoke exposure to reduce the influence of diet.[63] The sum of urinary concentrations of these five methoxyphenols was shown to have a good correlation to levoglucosan in airborne $PM_{2.5}$; however, urinary levoglucosan was not measured. Another study found that four low molecular weight methoxyphenols (syringol, methylsyringol, ethylsyringol, and propylsyringol) were each moderately correlated with personal exposures of smoke from an indoor cook stove in Guatemala.[7] One drawback to using methoxyphenols as tracers for wood smoke exposure is that they are widely found in foods and can be released into the air by industrial processes. An increase in urinary methoxyphenols after smoke exposure has also not been observed in all settings.[4]

Levoglucosan has been suggested as another potential urinary biomarker for wood smoke exposure. Levoglucosan (1,6-anhydro-B-d-glucopyranose) is a pyrolysis product of cellulose and is one of the major organic components in biomass combustion PM. Levoglucosan is frequently used as a environmental tracer for biomass burning because it is produced at relatively high levels and is stable in the atmosphere.[28, 30] Using levoglucosan as a urinary tracer for woodsmoke exposure would be easy to relate to measurements in particulate matter samples. Levoglucosan represented 2.8–3.8% of $PM_{2.5}$ mass from open burning of foliar fuels[52] and 5.7% of $PM_{2.5}$ mass emissions from prescribed burns of forests in Georgia.[64] During the Montana forest fire season of 2003, levoglucosan concentrations ranged from 900–6000 ng/$m^3$ in the Missoula valley, and were highly correlated with $PM_{2.5}$ mass (r=0.935).[42]

Levels of urinary levoglucosan and methoxyphenols have been measured in subjects after wood smoke exposure from a fire training exercise.[4] The authors reported no significant increase in levoglucosan or methoxyphenols after smoke exposure. This study did not report personal $PM_{2.5}$ or levoglucosan exposure and samples were collected at only one time point after smoke exposure.

There are additional potential limitations with the use of levoglucosan as a quantitative biomarker of exposure to wood smoke. Previously reported values of the ratio of levoglucosan to PM in fireplace emissions span a wide range between 0.8% and 26%.[20, 23, 57] This ratio is dependent upon the type of wood burned, fuel moisture, combustion conditions and the type of combustion device. However, measurements of the ratio of levoglucosan to $PM_{2.5}$ based upon ambient samples collected from wood smoke-dominated airsheds frequently exhibit less variability than the data from laboratory based studies. Ward *et al.* reported a ratio of 4.2±0.5% from samples collected in Missoula during the 2003 fire season[42], whilst Neitzel *et al.* reported a ratio of 8±4% in smoke from controlled burning of forests in Savannah, Georgia.[62] In a community in Montana where wood smoke represented 81% of the wintertime $PM_{2.5}$ mass, the ratio of levoglucosan to $PM_{2.5}$ was 11.2±1.5%.[45] After a wood stove changeout program in the same community, the ratio of levoglucosan to $PM_{2.5}$ was 6.9±0.6%.[45] Nevertheless, the use of urinary levoglucosan as a quantitative marker of exposure to wood smoke would be affected by variability in the levoglucosan emission factor, and would benefit from the

simultaneous characterization of the levoglucosan content of the specific wood smoke. Additionally, levoglucosan is a component of tobacco smoke[65, 66], so exposure to tobacco smoke would either need to be eliminated or corrected for through the determination of secondary biomarkers (e.g., cotinine).

Levoglucosan was investigated as a potential urinary biomarker for woodsmoke exposure through initial laboratory studies with a mouse model and then exposure studies using human subjects. Mouse model studies were done to determine if levoglucosan could be detected in urine after inhalation and if there were any potential confounding factors such as metabolism, interference from other sugars, or interference from exposure to other sources of particulate matter. Human studies were then conducted to verify that the results seen in the mouse model translate to human subjects and to determine if a similar increase in urinary levoglucosan could be observed after smoke exposure. Based upon the varying background levels of levoglucosan observed in the human samples collected, dietary studies were conducted to determine the prevalence of levoglucosan in the average human diet.

### 3.1. Mouse model studies

#### 3.1.1. Necessity

The first steps in developing levoglucosan as a biomarker for woodsmoke exposure were to demonstrate that levoglucosan could be detected in urine after exposure, that our method was specific for the sugar, and that it was specific for woodsmoke exposures compared to other types of particulate matter. The initial studies can be performed using a mouse model to simplify the experimental conditions and avoid additional human exposure to potentially harmful substances such as woodsmoke. Using the mouse model can reduce the number of unknowns as the subjects are all genetically identical. The mouse model also allows for more complete control of the diet and environment than human subject studies do, making it a good model for preliminary studies before human subject studies are done.

#### 3.1.2. Recovery study

Mice were instilled with three different levels of levoglucosan (5 μg, 25 μg, and 250 μg) and urine samples were collected at 2, 4, 6, and 8 hours post instillation and pooled for analysis to determine recovery. Pre-instillation samples were also collected

and levoglucosan was not detected in any of them. The higher doses (250 µg and 25 µg) averaged about 40% recovery of the total levoglucosan instilled. The lower dose (5 µg) appeared to be below the level of detection and did not result in consistent values. These results indicate that levoglucosan is detectable in urine following introduction into the lungs and that a significant amount is recoverable within 8 hours of exposure.

### 3.1.3. Time course

To determine the average time for levoglucosan to pass through the body, mice were instilled with 250 µg of levoglucosan and samples were collected at 0, 1, 2, 4, 6, 8, 10, and 24 hours post instillation. No levoglucosan was detected in the "pre" and "24 hrs post" samples (Figure 3.1), and levoglucosan was detected at all other time points (1-10 hours). In this study, an average of more than 50% of the instilled levoglucosan was recovered. Over 85% of the levoglucosan that was recovered appeared in samples collected within 4 hours of instillation. These results suggest that the majority of levoglucosan is recovered within 4 hours of instillation and none remains in the body 24 hours after exposure.

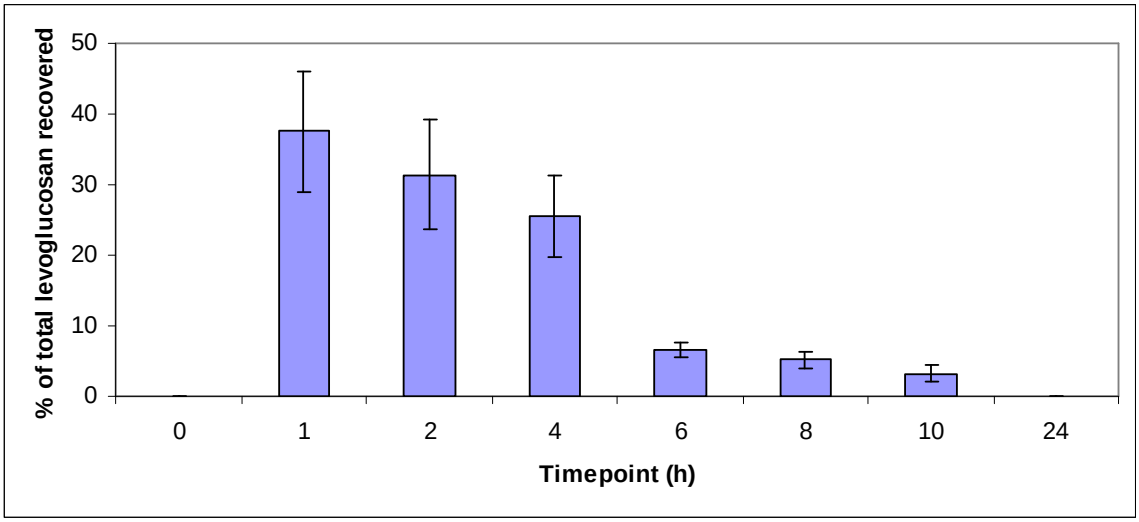


**Figure 3.1**
Levoglucosan recovery in urine over time in mice instilled intranasally with levoglucosan. The total amount of levoglucosan recovered was determined for each animal and the percent of total recovered levoglucosan was calculated for each mouse and averaged.[67]

### 3.1.4. Sugar selectivity

As there is a high degree of structural similarity between levoglucosan and other sugars, and a potential for other sugars to be metabolized to levoglucosan, a direct comparison between levoglucosan and three related sugars was performed. The sugars used in this study were glucose, mannosan, and galactosan (Figure 3.2). Mice were instilled with 250 μg of the desired sugar and urine samples were collected at 2, 4, and 6 hours post exposure and pooled for analysis. Samples were also collected before instillation and no levoglucosan was detected in "pre" instillation samples from any group (n=18). One mouse from the glucose-instilled group had detectable levels of levoglucosan, but it was significantly lower than that detected in the levoglucosan-instilled mice (Figure 3.3). None of the mice instilled with mannosan or galactosan had detectable levels of levoglucosan. As expected, all of the levoglucosan-treated mice had high levels of urinary levoglucosan. These data confirm that glucose is not metabolized into levoglucosan and other sugars of similar structure do not interfere with the analysis.

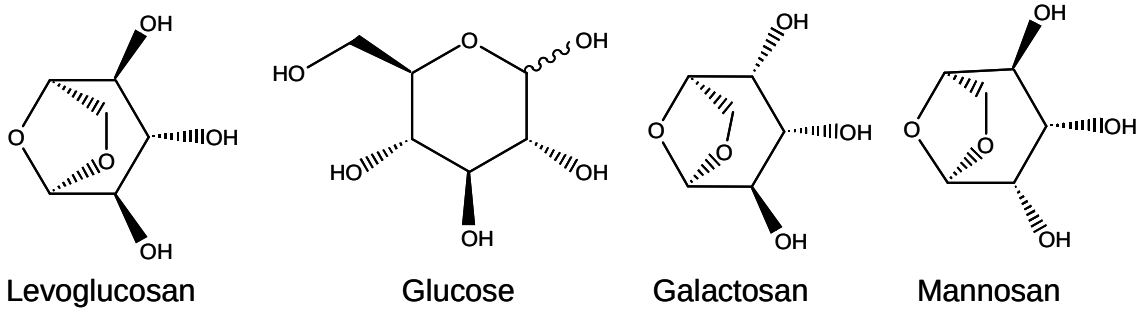


**Figure 3.2**

Comparative structures of levoglucosan, glucose, galactosan, and mannosan.

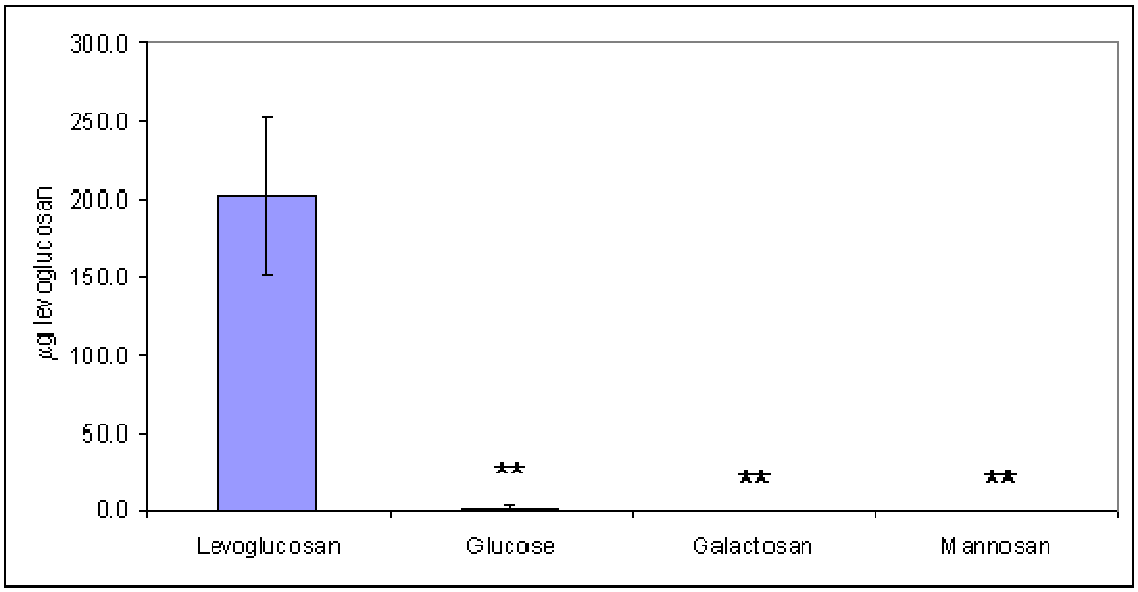


**Figure 3.3**
Levoglucosan detection specificity when instilled intranasally in mice compared to glucose, galactosan, and mannosan.[67] (** difference from levoglucosan instillation is significant at $p<0.01$)

### 3.1.5. Diesel/Missoula/PM comparison

Mice were instilled with three different types of particulate matter and collected from diesel exhaust, Missoula ambient air, and wood smoke along with a phosphate buffer blank (PBS) to test the specificity of levoglucosan recovery. Mice were instilled with 125 µg of the specified PM and samples were collected at 2, 4, and 6 hours post instillation and pooled for analysis. The levels of levoglucosan detection in the Missoula particulate matter-treated and diesel exhaust particulate matter-treated mice were slightly above that in the PBS controls, whereas the wood smoke-treated mice had significantly higher levels of levoglucosan detected in the urine (Figure 3.4). The wood smoke PM was analyzed and determined to be about 27% levoglucosan (Table 3.1). Calculating for the amount levoglucosan recovered, it appears that the average value (8.01 µg) is at about 24% recovery when assuming that each mouse was instilled with 33.25 µg of levoglucosan (26.6% of 125 µg). These data indicate that levoglucosan at sufficient levels in woodsmoke particulate matter can be detected in urine following intranasal instillation, and is a specific marker to woodsmoke when compared to other sources of particulate matter.

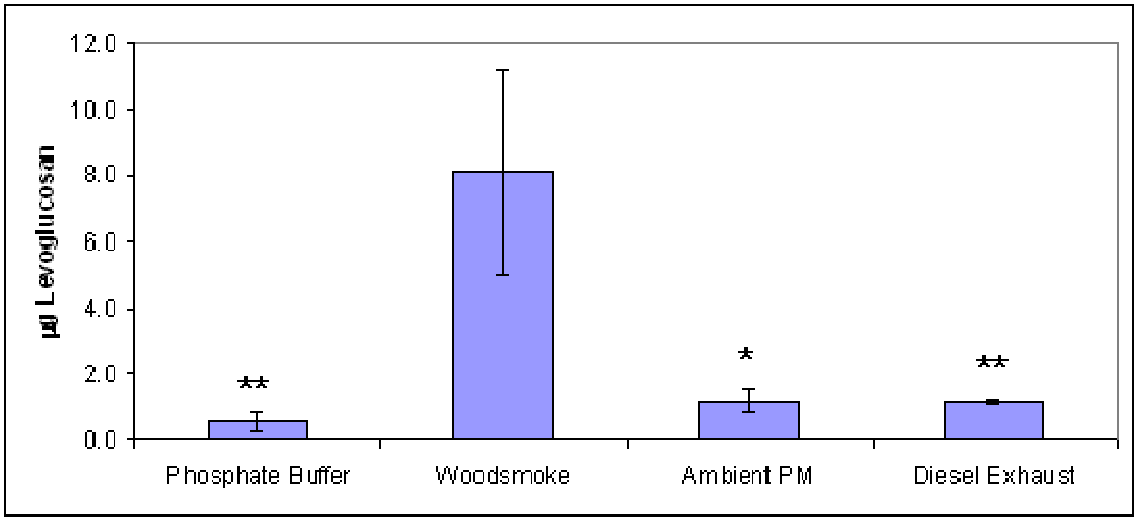


**Figure 3.4**
Levoglucosan specificity from woodsmoke particulate compared to phosphate buffer, Missoula ambient particulates, and diesel exhaust particulates.[67] (* difference from wood smoke particulate instillation is significant at $p<0.05$, ** difference from wood smoke particulate instillation is significant at $p<0.01$)

**Table 3.1**
Average levoglucosan composition of particulate matter collected from three different sources.[67]

| Particle source | Concentration in PBS | Percent levoglucosan |
|---|---|---|
| Ambient air | 5 | 0.42 ± 0.006 |
| Diesel exhaust | 5 | 0.00 |
| Woodsmoke | 5 | 26.6 ± 1.4 |

### 3.1.6. Mouse smoke exposure

Two groups of mice were exposed to high levels of wood smoke for 2 hours and urine samples were collected at 2, 4, and 6 hours post exposure and pooled for analysis. For the woodsmoke inhalation experiments one exposure averaged 3.14 mg/m$^3$ and the second exposure averaged 3.75 mg/m$^3$ where Figure 3.5 presents a representative graph of the time course of $PM_{2.5}$ mass concentration in 1-min increments. The combination of the two separate exposures is summarized in Table 3.2. Where only 1 of 14 air-exposed controls was positive for levoglucosan, 10 of 13 (76.9%) samples collected from smoke-exposed mice contained detectable levels of levoglucosan (Figure 3.6). In calculating

particle deposition, each mouse was potentially exposed to 0.85 µg of levoglucosan (see section 5.11.4.1 for calculation). The average levoglucosan recovered from the 13 smoke-exposed mice was 0.574 µg (Figure 3.6), or ~67% of the calculated exposure. These results support the use of levoglucosan as a specific biomarker of exposure to woodsmoke.

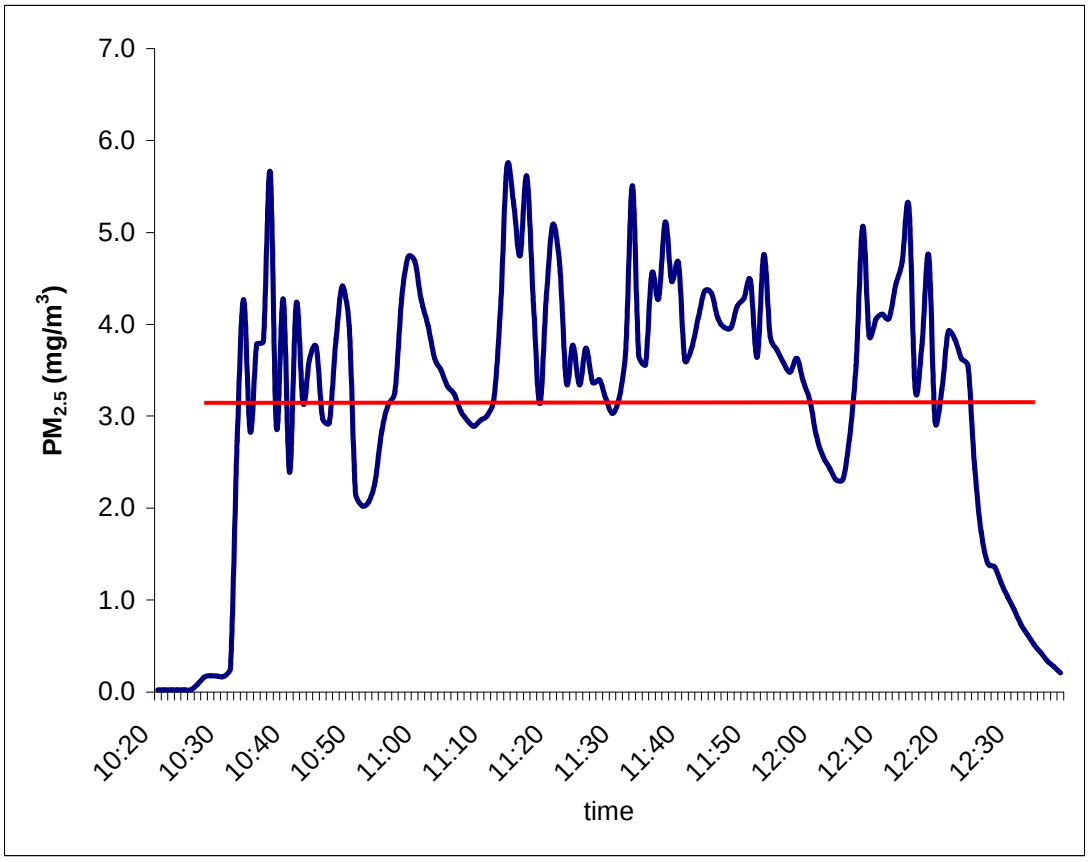


**Figure 3.5**

Representative time course $PM_{2.5}$ for one of the mouse smoke exposures. The average exposure was 3.142 mg/m$^3$ over the 2-hour exposure time, represented by the red line.[67]

**Table 3.2**

Summary of separate woodsmoke inhalation exposures, samples collected, and levoglucosan analysis.[67]

| Exposure | # of mice | Urine samples collected | Positive for levoglucosan |
|---|---|---|---|
| Air only 1 | 6 | 6/6 | 0/6 |
| Air only 2 | 10 | 8/10 | 1/8 |
| Total | 16 | 14/16 | 1/14 |
| Smoke 1 | 6 | 6/6 | 4/6 |
| Smoke 2 | 11 | 7/11 | 6/7 |
| Total | 17 | 13/17 | 10/13 |

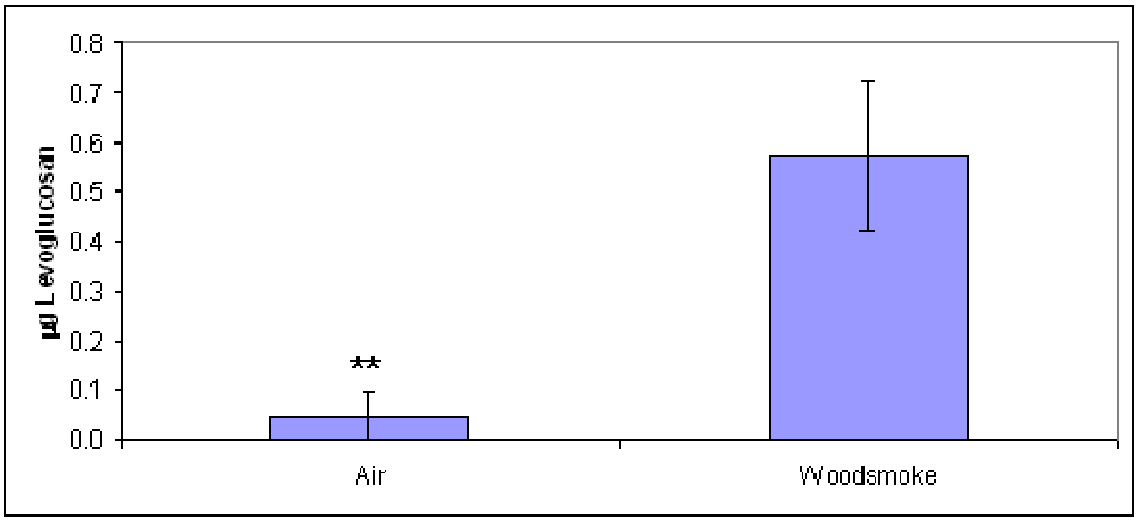


**Figure 3.6**
Average urinary levoglucosan for mice exposed to woodsmoke or clean air.[67]
(** difference from woodsmoke exposure is significant at $p<0.01$)

### 3.1.7. Discussion

One major concern in developing a biomarker is metabolism and loss in the body. The initial recovery study was designed to check for metabolism and determine levoglucosan recovery after intranasal instillation. Woodsmoke particulate matter was also instilled to determine average recovery. While only 24% was recovered in the woodsmoke particulate matter instillations, a minimum level of 40% recovery was obtained in both pure levoglucosan instillation as well as the woodsmoke inhalation studies. A greater yield was seen in the glucose comparison study (~80% average), possibly due to timing of sample collection. While urine samples are collected at specific time points, the actual amounts vary between animals and time points and it is likely that not all urine released during the study period is collected.

Another issue of concern in developing levoglucosan as a biomarker is the potential for other sugars of similar structure to be metabolized to levoglucosan. While the metabolism of glucose is well understood, these experiments showed that it is not altered to levoglucosan and, therefore, the levoglucosan detected in the urine is from instilled levoglucosan only (Figure 3.3, p. 43). Two other sugar anhydrides with similar structure to levoglucosan (mannosan and galactosan) were also instilled and neither was metabolized into levoglucosan, suggesting that any levoglucosan measured in the urine is due to exposure to levoglucosan and not conversion from a different sugar.

In the recovery studies from instilled particles, our concentrated woodsmoke particulates in the experimental setting contained 26% levoglucosan (Table 3.1, p. 44), while the ambient air samples contained less than 0.5% levoglucosan, and no levoglucosan was detected in the diesel exhaust particles. Levoglucosan was detected in the urine of mice instilled with each of these three types of particulates, as well as the mice given a blank PBS instillation. Levoglucosan was not expected to be present in the PBS or diesel exhaust instilled mice as neither of these treatments contained levoglucosan, however, the measured levels were significantly lower than the woodsmoke instilled mice.

More than three-fourths (76.9%) of the mice exposed to woodsmoke in the inhalation studies had detectable levels of levoglucosan in the urine. While the contribution of particle ingestion (i.e., from grooming) cannot be discounted, this experiment still confirms that levoglucosan can be detected in urine after exposure to woodsmoke. Although levoglucosan was not detected in the urine from three smoke-exposed mice, it is likely that this is an error from manual urine sampling as these samples had lower volumes than most. Another concern is the fact that one air-exposed animal had a detectable levoglucosan in its urine sample. However, it was only 1 of 14 sham-exposed animals, and the level detected (0.649 μg) was still below the average level detected in PBS-instilled controls (0.788 μg) in Figures 3.6 and Table 3.2 (p. 46 and 45), so it is likely it is due to contamination during collection or analysis.

## 3.2. Human exposure studies

The objective of these studies was to evaluate the potential of levoglucosan as a biomarker in human urine after exposure to wood smoke in several different controlled settings.

### 3.2.1. Libby schoolchildren study

#### 3.2.1.1. Ambient and indoor air sampling

Ambient $PM_{2.5}$ mass on the day of the urine collection was 5.9 $\mu g/m^3$. $PM_{2.5}$ mass inside the school on the day of the urine sampling was 41.1 $\mu g /m^3$. Levoglucosan in the particulates inside the school was 98.5 $ng/m^3$ on the day of sampling.

#### 3.2.1.2.Urinary levoglucosan

Spot urine samples were collected from 14 grade-school children in Libby, MT to evaluate the presence of levoglucosan in urine after prolonged exposure to low levels of wood smoke. All of the subjects live within the Libby airshed, which has moderately elevated levels of ambient wood smoke from wintertime domestic woodstove use. Levoglucosan was detected in all 14 urine samples. The mean (±sd) creatinine-adjusted levoglucosan concentration was 55 (±94) ng/mg creatinine. Information was collected on the type of home heating and present of tobacco smokers in the home of each student. Urinary levoglucosan concentrations by selected factors are presented in Table 3.3. Woodstoves were reported as the primary heating source for 9 of the 14 homes. Average urinary levoglucosan among children living in woodstove homes was slightly higher than among children living in homes without woodstoves, but this difference was not significant. Smoking was reported in 6 of the 14 homes, and children's urinary levoglucosan was associated with parent-reported household smoking ($p = 0.003$).

Urinary cotinine was evaluated to further assess the association with exposure to environmental tobacco smoke, but urinary cotinine concentrations did not correspond with reported household smoking. Urinary cotinine (ng/mg creatinine) was 28 (±30) among the six children living in homes with reported smoking and 22 (±44) among the eight children living in homes with no reported smoking ($p = 0.27$). There was not a strong correlation between urinary cotinine and urinary levoglucosan concentrations ($r = 0.27$, $p = 0.36$).

**Table 3.3**
Average urinary levoglucosan in children by various factors.[67]

| Factor | Number | Urinary levoglucosan (ng/mg creatinine) | ρ-value |
|---|---|---|---|
| **Sex** | | | |
| Female | 2 | 18.9 ± 23.6 | 0.27 |
| Male | 12 | 61.2 ± 100.0 | |
| **Woodstove in home** | | | |
| No | 5 | 42.9 ± 48.8 | 0.89 |
| Yes | 9 | 62.0 ± 113.4 | |
| **Time of sample collection** | | | |
| Morning | 8 | 30.6 ± 42.9 | 0.09 |
| Afternoon | 6 | 87.9 ± 133.8 | |
| **Smoking in home** | | | |
| No | 8 | 14.3 ± 11.1 | 0.003 |
| Yes | 6 | 109.6 ± 127.7 | |
| **Urinary cotinine (ng/mg creatinine)** | | | |
| < 10 | 7 | 66.6 ± 130.2 | 0.54 |
| ≥ 10 | 7 | 43.8 ± 40.8 | |
| **Total** | 14 | 55.2 ± 93.5 | |

### 3.2.2. Controlled smoke exposures

#### 3.2.2.1. Laboratory woodstove exposures

Subjects were exposed to wood smoke generated with an older model, non-EPA-certified wood stove in a controlled setting. Individual exposures ranged from 1.15-1.97 mg/m$^3$ $PM_{2.5}$. Urinary levoglucosan measurements from the subjects in the controlled wood stove smoke exposure showed no consistent response to the exposure (Figure 3.7a and b). In exposure trial #1, one subject showed an increase in urinary levoglucosan 10 hours post exposure, while the other three subjects showed no change (Figure 3.7a). Because of this inconclusive result, a second exposure was carried out using the same subjects plus one additional subject. In exposure trial #2, subjects also showed a variable response of either no change in urinary levoglucosan or multiple peaks within 24 hours post exposure (Figure 3.7b). One subject showed an elevated level of urinary levoglucosan beginning 12.75 hours post exposure and for the remainder of the monitoring time (24 hours post exposure). Another subject showed a small increase in urinary levoglucosan 8.5 hours post exposure, but all other points were the same as pre-

exposure. All subjects showed a low level of urinary levoglucosan pre-exposure, suggesting that levoglucosan is present in the diet or from other airborne sources.

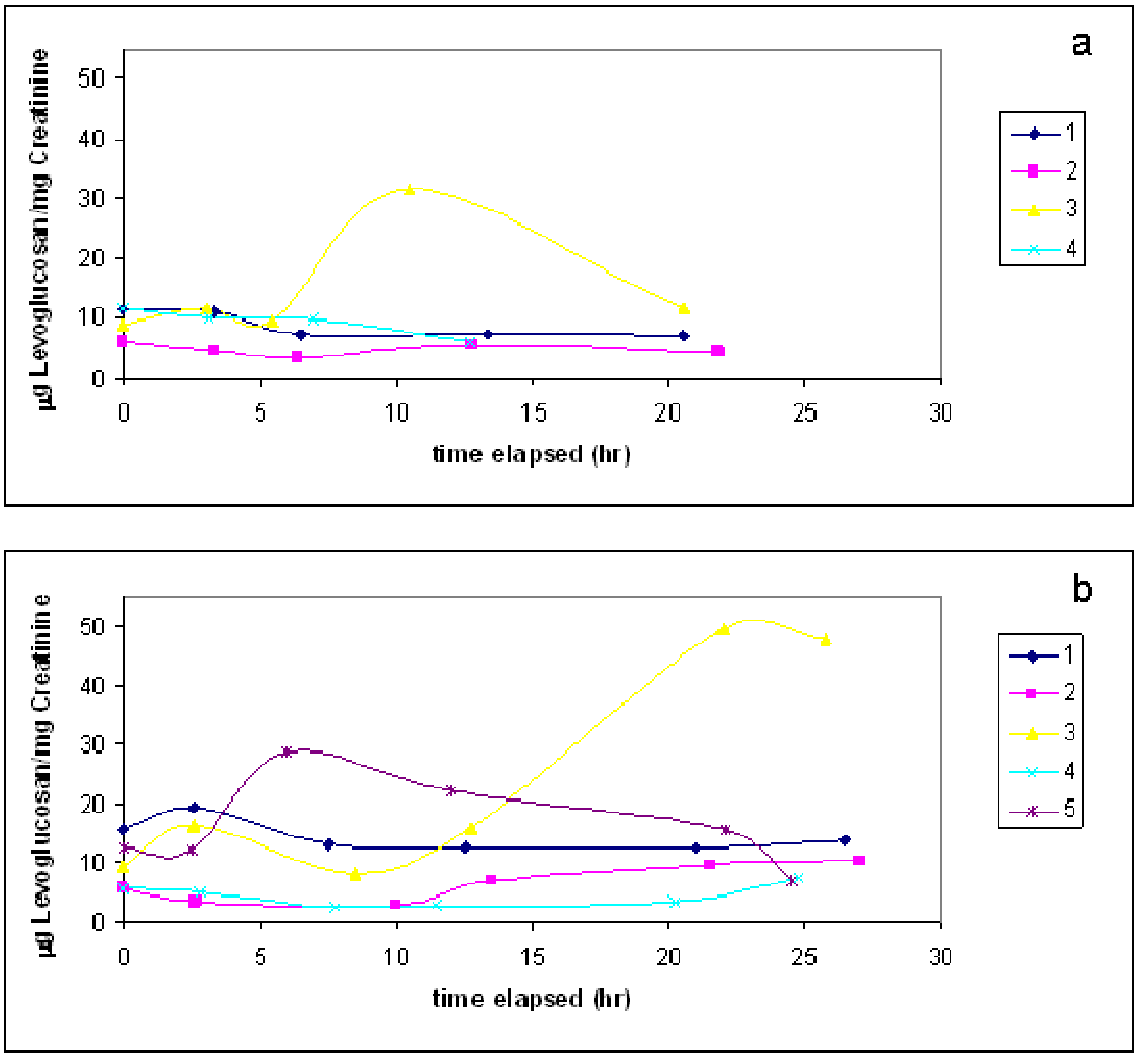


**Figure 3.7**
Urinary levoglucosan for each subject after 2 controlled smoke exposures from an older model wood stove. Smoke exposure occurred between time 0 and 2 hours.[68] (a) first exposure trial (b) second exposure trial.

### 3.2.2.2. Campfire exposure study

Nine subjects were exposed to $PM_{2.5}$ generated from a campfire for 2 hours in a controlled setting. Individual exposures ranged from 0.84-2.99 mg/m$^3$ $PM_{2.5}$, and from 76-256 µg/m$^3$ levoglucosan.[63] Urinary levoglucosan levels from the subjects in the campfire wood smoke exposure showed no consistent response to the exposure (Figure

3.8). Seven of the nine subjects had measurable levels of urinary levoglucosan at the zero time point. Several of the subjects showed only low levels throughout the entire study. Others showed peaks of urinary levoglucosan before or more than 24 hours after the exposure. Only one of the subjects (#1 at 9.75 hours post exposure) showed a maximum urinary levoglucosan level within 24 hours of the exposure, while three had a maximum before the exposure, and five had a maximum more than 24 hours post-exposure. Several subjects also showed multiple levoglucosan peaks. The initial intent of this campfire exposure was to evaluate urinary methoxyphenols, so subjects were asked to avoid smoked or grilled foods and other sources of smoke. Foods containing caramel as a potential source of levoglucosan were not monitored or restricted as the initial intention of this study was to measure methoxyphenols.

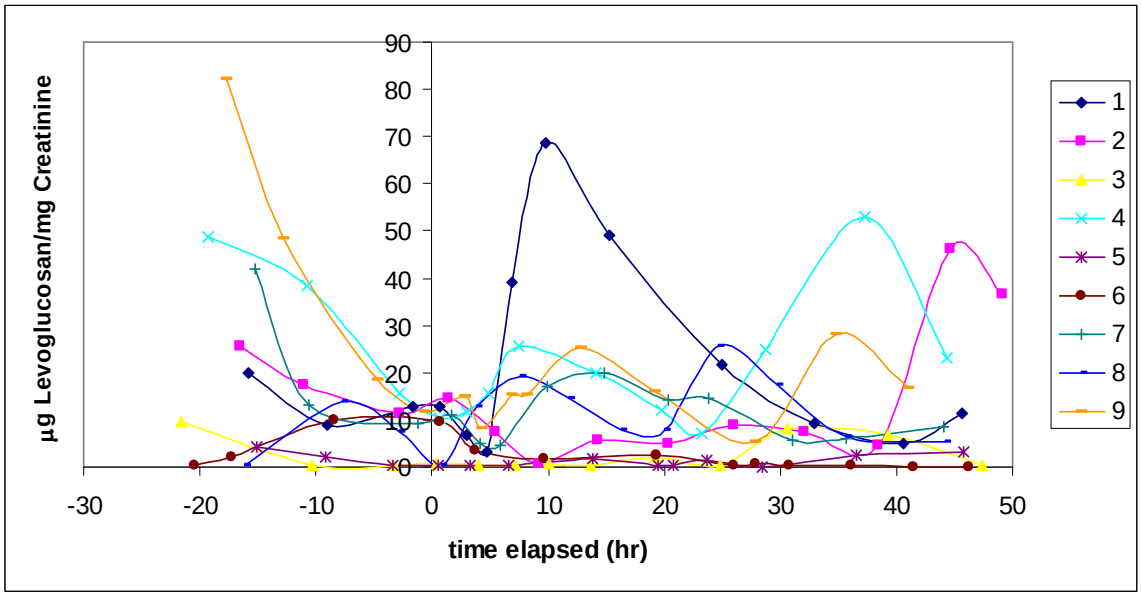


**Figure 3.8**
Urinary levoglucosan for each subject after controlled smoke exposure from a campfire. Smoke exposure occurred between time 0 and 2 hours.[68]

### 3.2.2.3. Discussion

Levoglucosan was detected in the urine samples from all 14 children exposed to low levels of woodsmoke typical of a community with extensive wood stove use, suggesting the potential for further investigation of this biomarker in humans under controlled experimental settings or in observational studies with rigorous exposure assessment. Urinary levoglucosan in these children could not be clearly associated with

the presence of a woodstove in their homes. While previous studies in this community have demonstrated the potential for high woodsmoke-derived ambient $PM_{2.5}$ concentrations[69] as well as high indoor $PM_{2.5}$ concentrations in the grade school attended by the child subjects[70], we did not have specific information about the level of $PM_{2.5}$ inside the homes. Such sources of PM exposures outside the home may have diluted any effect with woodstoves that we may have otherwise been able to detect. The finding that urinary levoglucosan concentrations were slightly higher among those children that provided a sample in the afternoon rather than the morning suggests the potential influence of ambient or in-school exposures on urinary levoglucosan, but it is difficult to draw conclusions on this point with the limited number of observations.

Tobacco smoke is also a potential source of levoglucosan exposure and should be considered when evaluating urinary levoglucosan.[71] Our findings for environmental tobacco smoke exposure and urinary levoglucosan levels were also inconsistent. Parent-reported smoking in the home was strongly associated with urinary levoglucosan, but cotinine, a known biomarker of exposure to tobacco smoke, was not associated with urinary levoglucosan. It is possible that there was exposure misclassification when exposure to tobacco smoke is based on parent-report. The pharmacokinetics of cotinine are also likely quite different from that of levoglucosan. Indeed our findings suggested that the majority of levoglucosan is excreted within a few hours whereas the half-life for cotinine is close to 1 day.[72] The varied urine sample collection times could account for the discrepancy in our findings with respect to parent-reported smoking versus biochemical evaluation of children's exposure to environmental tobacco smoke. The inconsistent results from this convenience sample study suggest a need to perform more controlled smoke exposure studies.

Some subjects from our controlled laboratory smoke exposure studies showed elevated urinary levoglucosan, but this was not consistently observed for all subjects. For the campfire smoke exposure study, both $PM_{2.5}$ and levoglucosan personal breathing zone exposures were measured for each subject. No overall associations were observed when comparing average personal breathing zone exposures with average urinary levoglucosan concentrations from 0 to 12 hours post-exposure ($r=0.26$ ($p=0.49$) for $PM_{2.5}$ and $r=0.22$ ($p=0.56$) for levoglucosan). For the two wood stove exposure trials, personal breathing

zone concentrations of $PM_{2.5}$ did not show a correlation to 12-hour urinary levoglucosan measurements, with r=0.27 (p=0.48). Twelve hour averages were chosen because previous studies with mice (levoglucosan instillation, $PM_{2.5}$ instillation, and wood smoke exposure) suggest that this is sufficient time to observe any changes in urinary levoglucosan levels.[67] Given the small sample sizes for each of these studies and the high degree of inter-individual variability we had limited power to detect patterns of response to smoke exposure.

During the laboratory exposure studies, subjects were exposed to elevated levels of wood smoke particulate matter representative of high exposure (acute) scenarios. In the two controlled wood stove exposure studies, individual exposures ranged from 1.15-1.97 $mg/m^3$, while in the campfire study individual $PM_{2.5}$ exposures ranged from 0.84-2.99 $mg/m^3$. For reference, these levels are 24 to 85 times higher than the EPA's 24 hour standard for $PM_{2.5}$ of 0.035 $mg/m^3$. The $PM_{2.5}$ levels used in the controlled exposure studies reported in this manuscript are more than 30 times higher than the ambient levels measured in Libby[45], and more than 200 times higher than the $PM_{2.5}$ levels inside the school in Libby.[17] The levoglucosan measured in the campfire exposure studies reported here is 1000 times higher than the level measured inside the school. Levels of $PM_{2.5}$ for wildland firefighters have been reported at $1.054 \pm 0.415$ $mg/m^3$, which is comparable to the levels in our two exposure studies.[62] Particulate exposure from wood burning cook stoves in developing countries have been measured from 0.097-3.50 $mg/m^3$.[73]

The results observed in the controlled human smoke exposure studies were not consistent with those of the mouse exposure studies. In the human studies, all of the subjects had elevated levels of urinary levoglucosan before exposures, whereas in the mice most were non-detects. This is likely due to differences in diet between mice and humans. The diet was much easier to control in the mouse studies. The food provided to the mice was analyzed for levoglucosan and none was detected, whereas the extent of levoglucosan from the human diet is unknown. It is also possible that there is a difference in metabolism or uptake of levoglucosan between mice and humans, as little is known about these mechanisms. Based on the low exposures in the Libby school study previously reported and the strong influence of diet, we speculate that the results

observed in that study were caused by dietary influences and are not likely correlated to wood smoke exposure.

### 3.3. Diet studies

Levoglucosan in human urine was first reported in 1986 by Dorland et al. using one-dimensional thin-layer chromatography.[74] In this study, levoglucosan was observed in approximately 20% of all urine samples screened at levels ranging from 0 up to 0.85 mg/mL. There was no apparent correlation with age or disease and levoglucosan levels, however, it is suggested that the source was likely dietary polysaccharides that have been heated.[74] Such dietary exposures are a concern for the use of levoglucosan as an exposure marker since levoglucosan has also been reported in several types of caramel.[75] It is also possible that dietary exposures are responsible for the inconclusive results seen in the human smoke exposure studies. The objective of these studies was to investigate the effect of diet on urinary levoglucosan levels and determine if they could be reduced or corrected for in using urinary levoglucosan as a biomarker for wood smoke exposure.

#### 3.3.1. Caramel study

Subjects each consumed five cubes of caramel, for an average of 42.2 g consumed per person (sd=0.49). The caramel was found to have approximately 5.3 mg of levoglucosan per cube, giving an average individual exposure of 26.5 mg of levoglucosan, compared to individual levoglucosan exposures for the campfire study of 0.076-0.256 mg. Pre-consumption urine samples showed an average of 18.3 (±10.2) μg of levoglucosan per mg of creatinine. All nine subjects showed an increase in levoglucosan levels of at least 2 times the pre-consumption value beginning 2 hours post exposure. Eight of the subjects had the highest levoglucosan readings 2 hours post-exposure, while one was highest in the 6 hour post-exposure sample, suggesting that levoglucosan has a short residence time in the human body when consumed in the diet. Average levoglucosan levels decreased 12 hours post consumption and returned to pre-consumption values for seven of the nine subjects (Figure 3.9). The other two subjects showed initial decreases in levoglucosan 6 and 12 hours post consumption, but then showed an increased levoglucosan level again 24 hours post-consumption. In both the pre-consumption and 24 hour post-consumption samples, all of the subjects showed a low

level of levoglucosan, suggesting again that there are likely other sources for levoglucosan in the diet or elsewhere. The average level of urinary levoglucosan measured after caramel consumption was more than 5 times higher than the average urinary levoglucosan measured after either of the two wood stove smoke exposure trials (Figure 3.10).

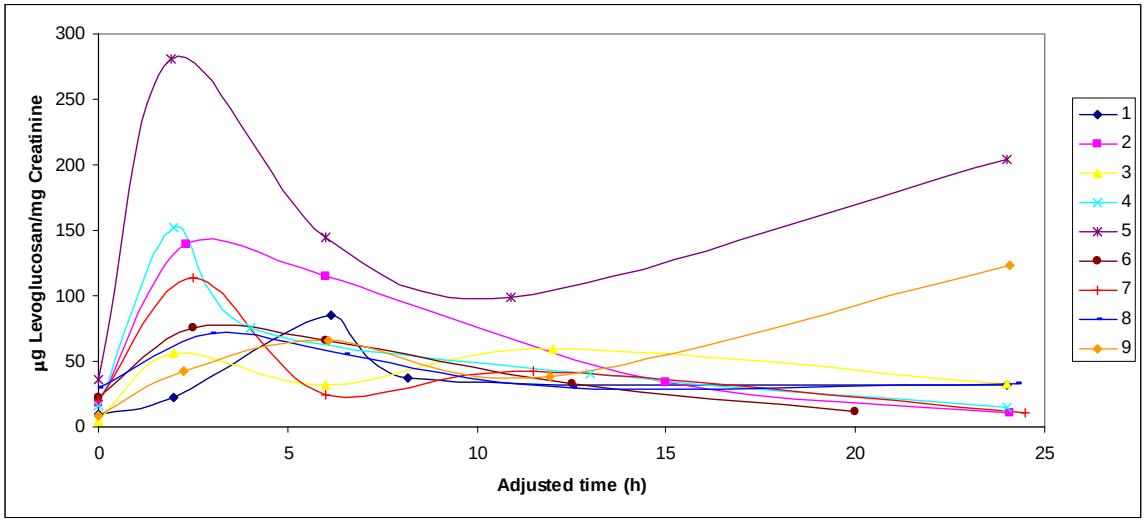


**Figure 3.9**
Individual levoglucosan levels during the caramel study. Sample time points have been adjusted so that time zero is beginning of caramel consumption.

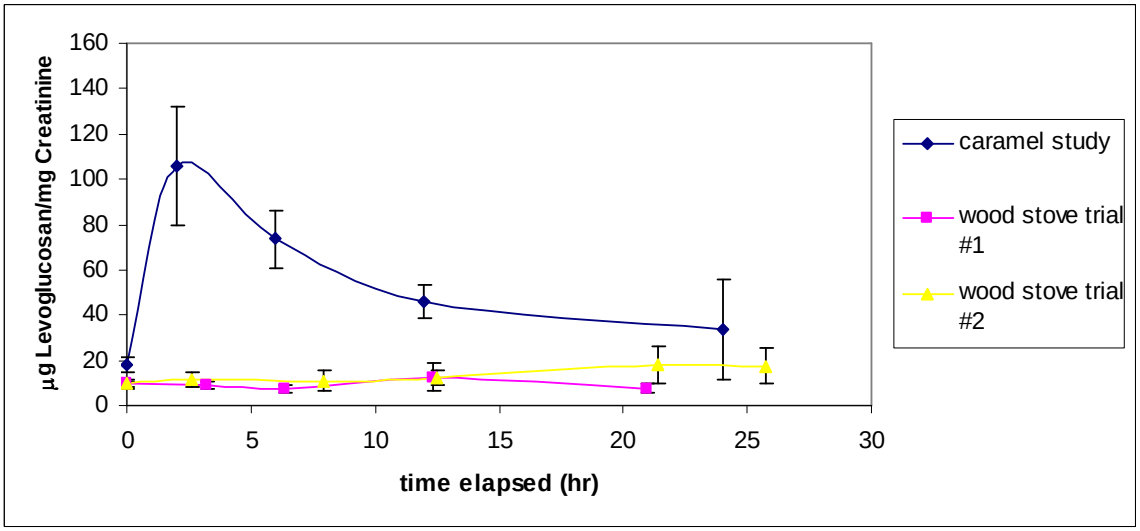


**Figure 3.10**
Average levoglucosan values at each time point during the caramel consumption study and two wood smoke exposure trials.[68] (** difference from pre-exposure is significant at $p<0.01$)

### 3.3.2. Fasting study

Twenty one subjects were asked to fast for 14 hours to determine the effect of diet on urinary levoglucosan levels and the approximate residence time for levoglucosan in the body. Subjects were asked to provide one urine sample first thing in the morning after fasting for approximately 12 hours and a second urine sample 2 hours later after 14 hours of fasting. The subjects had a wide range of urinary levoglucosan levels after both 12 and 14 hours of fasting (Figure 3.11). All subjects showed lower levels of levoglucosan at the 14 hour time point than the 12 hour time point. After 14 hours of fasting, 5 of the 21 subjects still had detectable levels of urinary levoglucosan

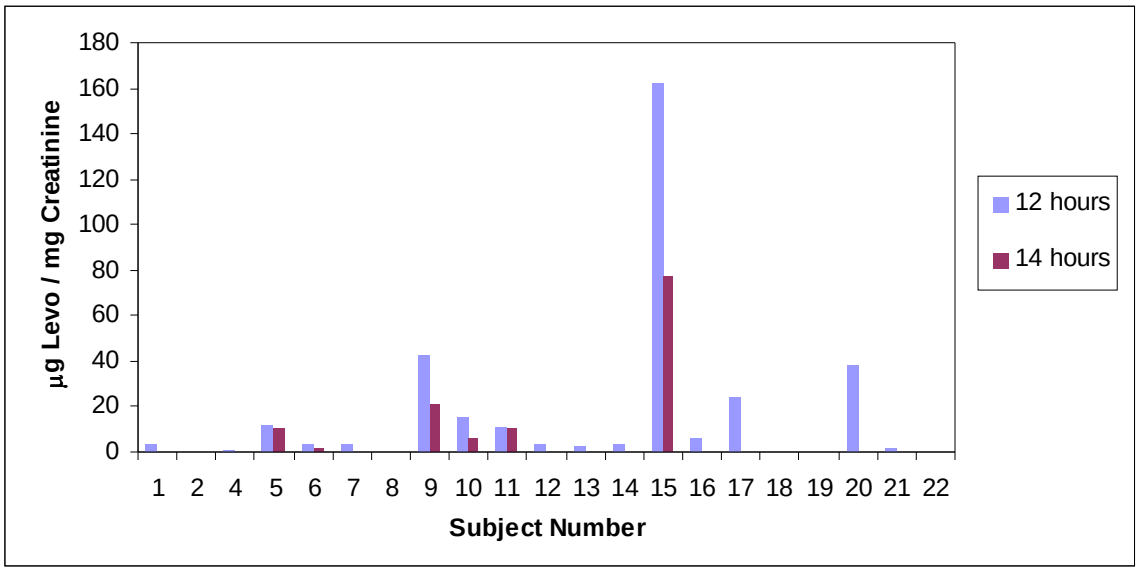


**Figure 3.11**
Urinary levoglucosan levels for 21 subjects after 12 and 14 hours of fasting.

### 3.3.3. Food investigations

Twenty three different foods were tested for levoglucosan to try to develop a levoglucosan-free diet that could be followed to obtain a blank, levoglucosan free urine sample. If a levoglucosan-free diet could be developed, subjects could follow it before and during a controlled smoke exposure to determine the amount of levoglucosan in urine from wood some particulates. Foods were determined to contain no levoglucosan if the chromatogram contained only the deuterated standard and a clean, level baseline at the time of levoglucosan elution. Of the foods tested, only caramel and wheat bread toast contained detectable levels of levoglucosan. Eight of the foods tested (Table 3.4) were

found to contain no levoglucosan. The majority of the foods tested showed inconclusive results, either due to matrix effects or high baseline noise. Many of the foods with inconclusive results contain high levels of other sugars, which were simultaneously extracted and analyzed with levoglucosan, and could interfere with the analysis.

**Table 3.4**
List of foods tested for levoglucosan content sorted by result.

| Positive for levoglucosan | Inconclusive | Negative for levoglucosan |
|---|---|---|
| caramel | lettuce | black beans |
| toast | salsa | wheat bread |
| | corn chips | hummus |
| | tortillas | french bread |
| | red pepper | pasta |
| | V8 | Ragu pasta sauce |
| | Special K cereal | apples |
| | carrots | strawberries |
| | rice | |
| | coffee | |
| | tomato | |
| | cherries | |
| | popcorn | |

### 3.3.4. Discussion

The caramel study confirmed that ingested levoglucosan appears in the urine within an hour of exposure. Most of the subjects had levels that returned to background levels within 10 hours of exposure. We speculate that the two subjects that had increased levels again at 24 hours post-exposure inadvertently consumed food containing levoglucosan and this increase is not associated with the original caramel consumption. The fasting study further confirmed that most people have low levels of urinary levoglucosan. All of the subjects had lower levels after 14 hours of fasting, however, a significant portion of the subjects did still have elevated levels. There were no noticeable trends regarding sex or diet for the subjects that still had elevated levoglucosan after 14 hours of fasting. The variability of levoglucosan levels after 14 hours of fasting makes it difficult to eliminate background levoglucosan before an exposure study. The food tests were mainly inconclusive due to high levels of other sugars and matrix effects. In developing a levoglucosan-free diet, foods were only determined to be negative if there was no uncertainty. Because levoglucosan is formed from glucose and heat, it is likely

that any food that has been cooked could contain levoglucosan. Pre-exposure samples for all of the studies averaged between 10 and 20 μg levoglucosan/mg creatinine, suggesting that most people are exposed to levoglucosan through diet even when avoiding caramel-containing or smoked foods. The food studies suggest that developing a reasonable levoglucosan-free diet is not possible as most of the foods were not determined to be free of levoglucosan.

### 3.4. Methoxyphenols

Vanillin, acetovanillone, guaiacol, and 4-ethylguaiacol were also investigated as potential urinary biomarkers of woodsmoke exposure. The methoxyphenols in urine commonly form adducts with proteins and must be deconjugated before analysis. After adjusting the pH to a suitable range for the deconjugation enzyme, the methoxyphenols were deconjugated for 18 hours and then extracted from the urine using a solid phase extraction cartridge. The samples were then derivatized and analyzed using GC-MS.

With this current procedure, recoveries were 82 ± 46 % for vanillin, 173 ± 74% for acetovanillone, 124 ± 15% for guaiacol, and 135 ± 10 % for 4-ethylguaiacol. The high recoveries and errors were likely due to methoxyphenols present in the "blank" urine samples from foods or other sources. The background levels were subtracted out of each spiked sample, but reproducibility of the background recovery was poor.

Different deconjugation conditions and methods were investigated, but a reliable method could not be developed. Deconjugation time, sample pH, addition of urease, concentration of the deconjugation enzyme, and brand of the deconjugation enzyme were all investigated in attempt to improve the spike recoveries, but no satisfactory results were obtained due to a combination of low recovery or low precision. An unspiked urine sample was prepared and analyzed eight times, and RSD values for the recoveries of the chosen methoxyphenols ranged from 15-32, with the exception of 4-ethyl guaiacol which was not present in any of the samples. A previously published acid deconjugation[63] was also investigated with little success. Recoveries from urine using this method were 72.6 ± 27.6% for vanillin, 90.3 ± 36.4% for acetovanillone, 100.7 ± 82.3% for guaiacol, and 69.8 ± 17.2% for 4-ethylguaiacol, corrected for background levels of each compound.

## 3.5. Conclusions

### 3.5.1. Mouse model

The mouse studies present strong evidence for the use of levoglucosan as a biomarker of woodsmoke exposure. The objectives of the mouse studies were met as it was demonstrated that levoglucosan could be detected in urine after exposure, that our method was specific for the sugar, and that it was specific for woodsmoke exposures compared to other types of particulate matter. Both direct instillation and inhalation exposures appeared to have similar kinetics for detection in mouse urine as both were measured with similar recoveries.

### 3.5.2. Human studies

The initial study of urine collected from schoolchildren in Libby, MT showed that levoglucosan can be detected in urine samples. No correlation was found between urinary levoglucosan levels and several different factors, including the presence of a woodstove in the homes. This study was able to validate the sample preparation and analysis method for human urine, however more controlled smoke exposure studies were necessary to determine the influence of smoke exposure on urinary levoglucosan levels.

The results from the controlled laboratory smoke exposure studies suggest that there is not a consistent increase in urinary levoglucosan in humans following an exposure to wood smoke. In both of our controlled wood smoke studies, some subjects had an increase in urinary levoglucosan after smoke exposure, while other subjects exhibited higher levoglucosan levels before exposure. None of the urinary levoglucosan levels measured showed a correlation to $PM_{2.5}$ or levoglucosan exposure. Both studies also further confirm that there is a relatively high and variable background level of levoglucosan present in all urine samples. Since most occupational or chronic $PM_{2.5}$ exposures are at levels similar to or lower than those used in this study, detectable increases in urinary levoglucosan above the background after biomass smoke exposures are unlikely.

### 3.5.3. Diet studies

The caramel study suggests that levoglucosan levels are subject to a strong short-term dietary influence. The average level of urinary levoglucosan measured after caramel consumption was more than 5 times higher than the average urinary

levoglucosan measured after either of the two wood stove smoke exposure trials, suggesting that even a small amount of dietary levoglucosan will likely have a greater influence on urinary levoglucosan levels than exposure to wood smoke, even at high levels. Fasting for 14 hours was not sufficient to eliminate levoglucosan from the urine of all subjects in the study, suggesting that fasting is not a viable option to eliminate dietary influences. A levoglucosan-free diet was not able to be developed due to matrix difficulties during analysis.

While these complicating factors diminish the potential use of levoglucosan as a biomarker of biomass smoke exposure in community-wide studies, carefully controlled studies may prove to be useful in developing levoglucosan as a tool in controlled laboratory studies. Urinary levoglucosan has been shown to increase in mice after wood smoke exposure[67], so it also could still be useful in studies with mice and potentially other animals where the diet is easily controlled or does not contain levoglucosan.

### 3.5.4. Methoxyphenols

The four methoxyphenols investigated were not found to be suitable tracers for woodsmoke in urine. A consistent extraction method with accurate and precise recoveries was not able to be developed. Three of the methoxyphenols were detected in the "blank" urine samples collected, suggesting that other sources of methoxyphenol exposure would likely interfere with woodsmoke exposures. Methoxyphenols were also not found to be a good indicator of woodsmoke levels in particulate matter due to their volatility and correlation to ambient temperature, so relating urinary levels to levels in the air would be difficult.

# Chapter 4: Filter Analysis Methods

Methods have been published for the analysis of multiple organic compounds in fine particulate matter[19-24, 31], as well as for the specific determination of methoxyphenols[25] and levoglucosan.[26-28, 32] Resin acids such as dehydroabietic acid have been specifically investigated in forest fire smoke[49], but not in residential wood smoke. Analysis of a large number of organic compounds can be costly and is not always necessary when investigating $PM_{2.5}$ from a single source. However, no method optimized for simultaneous determination of selected biomass burning tracers from multiple classes of compounds has been published.

The method described here combines analysis of residential wood smoke particulate for methoxyphenols and levoglucosan with a third group of wood smoke indicators, resin acids, for which no specific method has been optimized and reported. Conditions from the two published methods for methoxyphenols and levoglucosan have also been further optimized for faster analysis of particulates composed primarily of wood smoke.

## 4.1. Prior Work and Method development

Various solvent systems have been reported in the literature for the extraction of organic compounds from particulate matter, including mixtures of ethyl acetate/triethyl amine[25, 26, 48], dichloromethane/methanol[28, 31], methanol/water/dichloromethane[19, 20], and hexane/benzene/proponal.[20, 39, 50] Soxhlet extraction using Hydromatrix, dichloromethane, and acetone has also been used to extract levoglucosan from particulate matter.[33] A comparison of the ethyl acetate and dichloromethane based solvent systems showed no difference in their performance.[33, 76] Ethyl acetate with triethylamine was chosen for this study because it does not utilize benzene, a known carcinogen, or dichloromethane, a suspected carcinogen.[77] Aqueous extraction has been reported for levoglucosan determination[78], but this method will not extract the methoxyphenols and resin acids as well. Protic solvents such as water and methanol are also not compatible with the derivatization conditions for levoglucosan and the resin acids.

### 4.1.1. Levoglucosan and resin acids

A variety of different derivatization conditions were investigated for levoglucosan, dehydroabietic acid, and abietic acid. Levoglucosan can be derivatized

with TMSI[26], BSTFA/TMCS[19, 28, 31, 39, 78], or BSTFA/TMCS plus pyridine[21, 32, 33, 49, 76], while the resin acids are derivatized with a mixture of BSTFA and TMCS.[19, 20, 31, 49] These compounds can also be analysed as their methyl ester derivatives.[20, 23, 24, 43, 50, 51]

The mixture of BSTFA/TMCS is a weaker set of conditions and typically requires heating for 3+ hours to completely derivatize levoglucosan from particulates. Under these conditions, several derivatization products of levoglucosan were frequently observed in our samples, suggesting the reaction was not going to completion. TMSI is a stronger reagent, and can derivatize both types of compounds, but causes much noisier peaks and appears to degrade the resin acids. A mixture of the three reagents (BSTFA/TMCS/TMSI) as well as a BSTFA/TMCS/pyridine mixture was investigated. Both were found to be effective at derivatizing the compounds in less than 60 minutes. The TMSI has the same function as the pyridine and also has additional trimethylsilyl groups that can react with the analytes. The first mixture was chosen because it contains a greater amount of silylating reagents and therefore should experience fewer problems with the varying amounts of unknown compounds present in wood smoke. We also found it necessary to evaporate the extracts to dryness before derivatization. When the solvent is not removed, levoglucosan is not completely derivatized under the chosen conditions.

#### 4.1.2. Methoxyphenols

The methoxyphenols can be analysed as trimethylsilyl derivatives, however this was not found to be optimal under our conditions. The current conditions for TMS derivatization require the solvent to be evaporated first. This is not favourable for the methoxyphenols because they are volatile and were found to evaporate with the solvent. To avoid these problems, a different set of derivatization conditions was used for the methoxyphenols, generating the acetate derivatives. Levoglucosan and the resin acids do not form acetate derivatives under the conditions used.

Derivatization conditions for the methoxyphenols were adopted from Simpson *et al.*[25] The four chosen methoxyphenols all derivatize quickly, so the reaction time was reduced from 3 hours to 1 hour. Simpson's work suggests that the syringol type methoxyphenols require longer to derivatize than the others, so the time can be reduced when these compounds are not being analysed for. The original conditions of 50 μL of a

4:3 mixture of acetic anhydride: triethylamine produced double peaks of identical mass spectra on the chromatograms for both vanillin and guaiacol, for unknown reasons. The amount of derivatizing reagents was reduced to 30 μL of a 2:3 mixture in 250 μL of sample because this was found to be sufficient for the compounds to be analysed and did not produce the double peaks.

#### 4.1.3. Sample extraction

Some sample filters were extracted a second time with a fresh volume of solvent and an additional 30 minutes of sonication to check the efficiency of the extraction procedure Levoglucosan was the only tracer detected in the second extract, and all levels measured were less than 6% of the first extraction, so the extraction procedure was determined to be sufficient. Increasing the sonication time or volume of solvent also had little or no effect on the recovery.

### 4.2. Materials

Vanillin 99%, acetovanillone 98%, guaiacol 99+%, 4-ethyl guaiacol 98%, and levoglucosan 99+% were purchased from Acros Organics (Geel, Belgium). Abietic acid 90-95% was purchased from Alfa Aesar (Ward Hill, MA). Dehydroabietic acid (technical grade) was purchased from Pfaltz and Bauer Inc (Waterbury, CT). Ethyl acetate (reagent grade), triethylamine (reagent grade), and acetonitrile (HPLC grade) were purchased from Fisher Scientific (Hampton, NH). N-*O*-bis(trimethylsilyl)trifluoroacetamide (derivatization grade, 99+ %), trimethylchlorosilane (97%), and trimethylsilylimidazole (derivatization grade) were purchased from Sigma (St. Louis, MO). D-Vanillin (ring-5-D1) 98%, D-guaiacol (methoxy-D3) 98%, D-levoglucosan (D7) 98%, and D-stearic acid (D35) 98% were purchased from Cambridge Isotope Laboratories (Andover, MA). All compounds were used as received except dehydroabietic acid, which was purified chromatographically in the lab using silica gel and mobile phase of dichloromethane (dehydroabietic acid purity estimated to be 85+% after purification).

### 4.3. Standards

Deuterated compounds of the same or similar structure to the chosen tracers were employed as internal standards in the procedure to eliminate the possible effects of incomplete extraction from the filters and other variations throughout the analysis period.

The chosen internal standards were D-vanillin as a standard for vanillin and acetovanillone, D-guaiacol as a standard for guaiacol and 4-ethylguaiacol, D-levoglucosan as a standard for levoglucosan, and D-stearic acid as a standard for abietic and dehydroabietic acid. The solutions containing D-stearic acid, D-vanillin, and D-guaiacol were prepared in ethyl acetate, while D-levoglucosan was prepared in acetonitrile.

### 4.4. GC-MS conditions

Analysis was performed on an Agilent 6890N Gas Chromatograph with an Agilent 5973 Mass Spectrometer. An HP-5MS column ((5%-Phenyl)-methylpolysiloxane) was used with dimensions of 0.25 mm ID x 30 m length x 0.25 $\mu$m film thickness. A volume of 2 μL was injected for each analysis into a Split/Splitless FocusLiner$^{TM}$ for HP, single taper p/w quartz wool liner. Split injection was used to analyse for levoglucosan with a split ratio of 50:1 and splitless injection was used to analyse for the remaining compounds. The inlet temperature was set to 250°C and the auxiliary transfer line temperature was set at 280°C. The temperature program was started at 40C for 1.5 minutes, ramped at 30°C/min to 190°C, 20°C/min to 210°C, and then 50°C/min to a final temperature of 300°C, which was held for 1.5 minutes. The mass spectrometer was operated with a solvent delay of 4.00 minutes and the mass range from 40-450 was scanned. Single ion monitoring was also used during detection for guaiacol, D-guaiacol, and 4-ethyl guaiacol with ions 124, 127, and 137. For all compounds, highly selective quantitation was performed using the signal for representative ions extracted from the total ion chromatogram. (see Table 4.1 and Figure 4.1)

**Table 4.1**

Calibration range, quantification ions, and linearity of the calibration for the tracers.[18]

| Compound | # of Calibration Points | Concentration Range | $R^2$ | Quantification Ion |
|---|---|---|---|---|
| Levoglucosan | 4 | 25-125 µg/mL | 0.9925 | 217 |
| D-Levoglucosan | | 80 µg/mL | | 220 |
| Dehydroabietic acid | 7 | 5-20 µg/mL | 0.982 | 239 |
| Abietic acid | 7 | 200-1000 ng/mL | 0.9383 | 256 |
| D-Stearic acid | | 1 µg/mL | | 376 |
| Vanillin | 7 | 0.2-3 µg/mL | 0.9908 | 151 |
| Acetovanillone | 7 | 0.2-3 µg/mL | 0.9722 | 166 |
| D-Vanillin | | 2 µg/mL | | 153 |
| Guaiacol | 4 | 2-20 ng/mL | 0.9948 | 124 |
| 4-Ethylguaiacol | 4 | 5-40 ng/mL | 0.9969 | 137 |
| D-Guaiacol | | 30 ng/mL | | 127 |

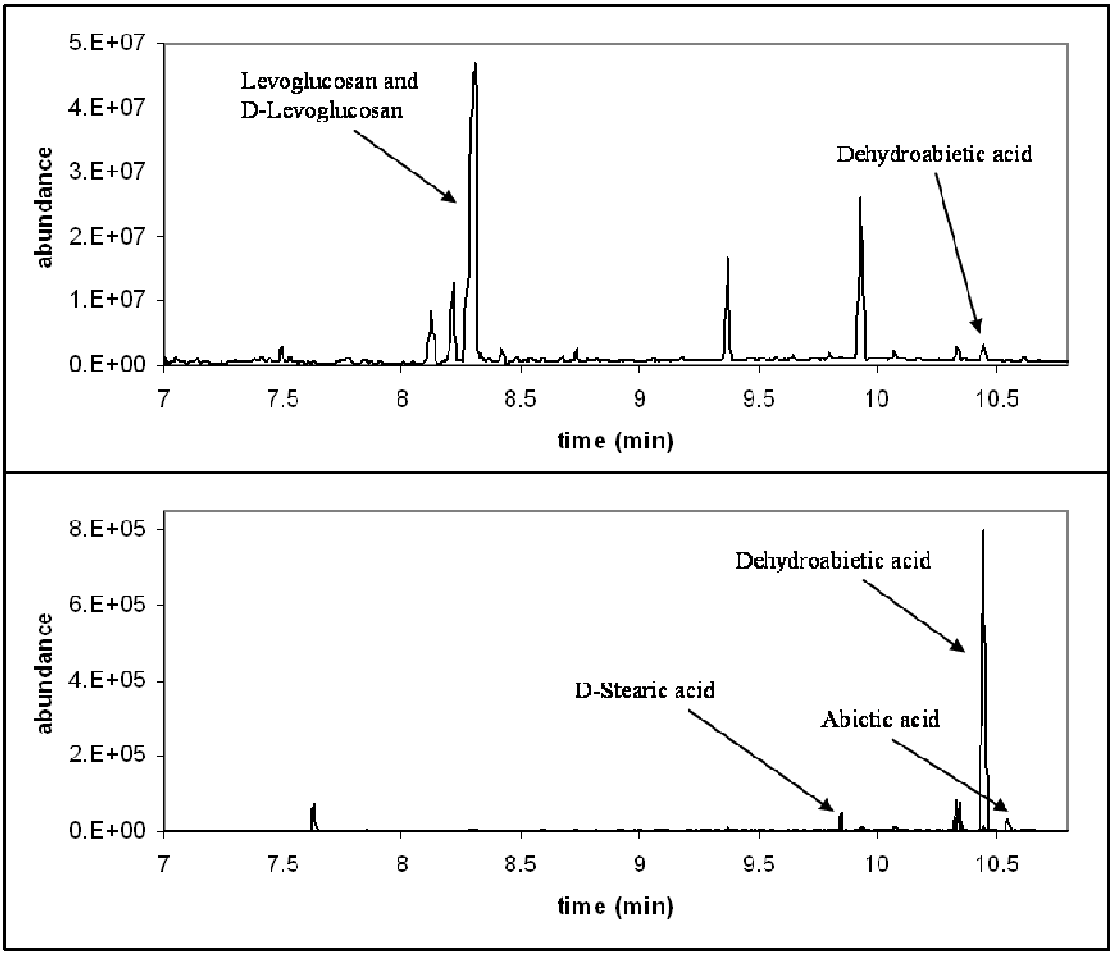


**Figure 4.1**

Total ion chromatogram (top) showing an example of a sample from Libby analysed for levoglucosan and resin acids and an extracted ion chromatogram (bottom), showing the specific ion peaks used to quantify the resin acids. The chromatograms were started at 7.00 minutes to eliminate the peaks from the derivatizing reagents.[18]

### 4.5. Sample preparation method

One half of the quartz filter was analyzed for chemical markers of woodsmoke at The University of Montana following the analytical method described here.[18] The method employed for the chemical markers of woodsmoke was adapted from methods reported previously.[25, 51] Half of each 47-mm quartz filter sample was placed in a 30 mL vial and spiked with the 4 deuterated standards (D-vanillin, D-guaiacol, D-levoglucosan, and D-stearic acid). The vials were capped and left at room temperature to allow the standards to be absorbed onto the filter. After half an hour, 20 mL of ethyl acetate with 3.6 mM triethylamine (TEA) was added and the samples were sonicated for half an hour to extract the desired compounds. After sonication, the filter was removed and the extract was filtered through a Whatman 0.45 μm nylon filter to remove particulates. The volume of the solvent was adjusted to 0.5 mL through evaporation under a stream of air in an oil bath at 45 °C. The sample was then divided into two 250 μL portions. One portion was derivatized with 30 μL of a freshly prepared 2:3 mixture of acetic anhydride to triethylamine to be analysed for methoxyphenols. The other portion was evaporated to dryness under a stream of air at room temperature and then derivatized with 75 μL N-*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA), 10 μL trimethylchlorosilane (TMCS), and 10 μL trimethylsilylimidazole (TMSI) to be analysed for levoglucosan and the resin acids. Methoxyphenols were analysed as acetate derivatives, while levoglucosan and the resin acids were analysed as trimethylsilyl derivatives. Both portions were heated in an oil bath at 70 °C for 1 hour to allow the derivatization to go to completion. Upon removal from the oil bath, the portion for methoxyphenols was transferred to a GC vial for analysis. The portion for levoglucosan and the resin acids was diluted to 250 μL with ethyl acetate containing 3.6 mM TEA and then was transferred to a GC vial for analysis.

#### 4.5.1. Organic Carbon/Elemental Carbon analysis

For the Organic Carbon/Elemental Carbon analyses, one half of the exposed $PM_{2.5}$ filters were shipped in coolers to Chester LabNet (Tigard, Oregon) for analysis by Thermal Optical Reflectance. Chester LabNet employs a comprehensive analytical laboratory QA/QC program.

### 4.6. Calibration

Calibration standards were prepared containing variable concentrations of each selected tracer (see Table 4.1, p. 65) and a fixed concentration of the corresponding deuterated internal standard. The fixed concentration of deuterated internal standard was selected to match the concentration expected from extraction of internal standard spiked on the filters, assuming 100% recovery. The standards were derivatized and analysed on the GC-MS according to the determined conditions for each type of compound. The ratio of the peak area of the tracer to the peak area of the deuterated standard was found for each calibration standard. A calibration curve was prepared by plotting the ratio of the two peak areas versus the concentration of the tracer. Linearity was determined for each calibration curve, and all had $R^2$ values of at least 0.93 (see Table 4.1). The concentration of extracted analytes was determined by measuring the ratio of the peak area for the analyte to that of the corresponding deuterated standard, and reading the concentration from the appropriate calibration curve. Recoveries were calculated for filters spiked with the analytes at known amounts corresponding to typical levels seen in actual sample filters (see Table 4.1).

### 4.7. QA/QC program

A comprehensive QA/QC program was employed throughout the sampling program, including the analysis of blank filters, spikes, instrument calibration and routine instrument maintenance. Using a Bios DryCal flow meter, the flow rate on the Leland pump/Personal Environment Monitor (PEM) was measured both before and after each sampling event. Quartz filter field blanks were collected for approximately every 10 samples. Field personnel followed the recommended maintenance and cleaning schedules for the DustTrak and Leland/PEM as described in their respective manuals throughout the program. The DustTrak was zeroed prior to each sampling event, with results documented on datasheets.

#### 4.7.1. Blanks

Field and lab blanks were analysed to monitor for contamination. The field blanks were stored with the samples to check for contamination in transport and storage, while the lab blanks were prepared daily at the same time as the samples to check for contamination in the analysis procedure. For every 10 filters that were analysed, one

sample and one spiked blank filter were analysed in duplicate to check reproducibility (typically one of each per day of analysis).

#### 4.7.1.1.Ambient sampling program

Levoglucosan, abietic acid, and the methoxyphenols were detected on less than 1/3 of the blanks analyzed (n=39) with the ambient filters collected in Libby. Average concentrations of each analyte and the number of blanks on which each analyte was detected are provided in Table 4.2. Dehydroabietic acid was detected at a low level on every blank except one. An average value of dehydroabietic acid on the blanks was calculated and subtracted from all reported values to correct for this background. The other analytes were not corrected for average blank concentrations. None of the blank filters were positive for more than four of the compounds, suggesting that contamination of $PM_{2.5}$ samples/blanks during transport or storage did not occur. The presence of different compounds on the blanks is likely due to small contamination during the sample preparation or analysis.

**Table 4.2**

Average percent recovery from spiked clean filters (95% confidence interval, n=69), average concentration of each analyte on the blank filters, and number of blanks detected for each analyte for the ambient and residential studies.[17, 45]

| | | Ambient | | Residential | |
|---|---|---|---|---|---|
| Compound | % recovery | Average on blanks ($ng/m^3$)[a] | # of blanks detected[b] | Average on blanks ($ng/m^3$)[a] | # of blanks detected[c] |
| Levoglucosan | 102 (6.1) | 249.2 | 7 | 12.5 | 2 |
| Dehydroabietic acid | 65 (3.1) | 105.5 | 38 | 108.8 | 7 |
| Abietic acid | 64 (4.5) | 3.23 | 1 | 6.22 | 1 |
| Vanillin | 99 (2.8) | 5.94 | 7 | 28.68 | 2 |
| Acetovanillone | 104 (4.2) | 2.24 | 1 | 27.23 | 2 |
| Guaiacol | 110 (5.5) | 0.10 | 12 | 1.57 | 6 |
| 4-Ethylguaiacol | 111 (5.0) | 0.15 | 11 | 1.87 | 6 |

[a]Average of only the blanks that contained the given analyte at a concentration above the detection limit.
[b]n=39
[c]n=7

#### 4.7.1.2.Residential sampling program

For blanks analyzed with the residential sampling program in Libby, levoglucosan was detected on two of seven blank filters, but at a very low concentration (Table 4.2). Dehydroabietic acid was detected at low levels on all seven of the blanks, but abietic acid

was only detected on one. An average concentration of dehydroabietic acid on the blanks was determined and subtracted from every sample to correct for this. The levels of dehydroabietic acid detected on the blanks were less than 30% of the average levels measured on the samples. For the methoxyphenols, acetovanillone and vanillin were only detected on two blanks, but at relatively high levels. Since these two methoxyphenols are semi-volatile and were not detected in most of the residential samples, the data were not used and their presence on the blanks is inconsequential. Guaiacol and 4-ethylguaiacol were detected on most of the blanks at higher levels than expected based on the blanks from the ambient studies, but again the guaiacol and 4-ethylguaiacol data from residential studies had little utility and their presence on blanks is inconsequential. None of the blank filters were positive for more than four of the compounds, suggesting that contamination during transport or storage did not occur.

### 4.7.2. Recovery

Clean quartz filters were spiked with known levels of all seven compounds and four deuterated internal standards and passed through the extraction procedure daily in parallel with the samples analysed (at least one spiked filter for every 10 samples analyzed). Recovery was calculated from these spiked filters to monitor method efficiency and instrument performance. The spike solutions were all prepared in ethyl acetate, except for levoglucosan, which was prepared in acetonitrile. Calibration standards were also made at least once a week to monitor solutions and instrument calibration. A full set of calibration standards was analyzed whenever new solutions were prepared or instrument maintenance was performed, or as necessary when indicated by the single calibration point analyzed weekly (no less than one full set analyzed per month).

Five of the compounds had recoveries near 100%, while the two resin acids exhibited low, but consistent, recoveries.[18] The recoveries were close to 100% for levoglucosan, vanillin, acetovanillone, guaiacol, and 4-ethylguaiacol (Table 4.2). The resin acids both exhibited recoveries that were significantly lower than 100%, but they were still reproducible. The reported values for the two resin acids were adjusted to correct for this low recovery, allowing the values to be compared to other published values.

There is no isotope-labelled resin acid commercially available and attempts to synthesize one were unsuccessful. D-35 stearic acid was chosen as a standard because it has the same functional group and similar molecular weight as the resin acids, but a different carbon backbone. We speculate that this difference in structure gave the standard different interactions with particulates and filters than the resin acids and caused a difference in extraction efficiency and recovery. However, a second extraction of the filters does not show any resin acids, so it is unlikely that the resin acids are remaining on the filter. The resin acids could also be remaining in the particles after extraction from the sample filter and are then being removed and discarded during filtration with the nylon membrane filter. Another possible explanation is incomplete derivatization of the resin acids under the current conditions. A longer derivatization time and stronger conditions were investigated, but no change in recovery was observed. While the exact cause of the low recoveries is unknown, the recovery for both resin acids was reproducible and the low recovery can thus be corrected for if desired.

### 4.8. Detection limits

Minimum detection limits (MDLs) for each of the parameters measured are presented in Table 4.3. For $PM_{2.5}$ mass, MDLs are reported in the DustTrak manual. MDLs for OC and EC were reported by the contracted laboratory in μg. To calculate the final MDLs, the values for OC and EC, respectively, were divided by the average volume collected with the Leland pump / PEM during each sample run (results reported in $\mu g/m^3$).

MDLs for levoglucosan, the resin acids, and the methoxyphenols were also calculated. The peak height of each concentration of standard was ratioed to the peak height of the deuterated internal standard, and a calibration curve was created. Peak to peak noise was estimated before and after the standard peak in each file to give an average value. Three times the standard deviation, taken as 1/5 of the peak-to-peak noise, was used as the MDL. The value of 3/5 of the peak-to-peak noise was ratioed to the deuterated internal standard and the corresponding ratio was used to calculate the MDL from the calibration curves. Samples below the detection limit were assigned a value of ½ the detection limit for all subsequent data analysis.

**Table 4.3**
Minimum detection limits for $PM_{2.5}$, organic and elemental carbon, and the seven selected tracers for wood smoke in the air.

| Parameter | MDL |
|---|---|
| $PM_{2.5}$ | 0.001 mg/m$^3$ |
| Organic Carbon | 0.098 µg/m$^3$ |
| Elemental Carbon | 0.007 µg/m$^3$ |
| Levoglucosan | 7.7 ng/m$^3$ |
| Dehydroabietic Acid | 0.6 ng/m$^3$ |
| Abietic Acid | 0.5 ng/m$^3$ |
| Vanillin | 0.9 ng/m$^3$ |
| Acetovanillone | 0.5 ng/m$^3$ |
| Guaiacol | 0.03 ng/m$^3$ |
| 4-Ethylguaiacol | 0.1 ng/m$^3$ |

## 4.9. Sample collection

### 4.9.1. Ambient

Ambient particulate samples were collected on the roof of the Lincoln County Annex in Libby, MT throughout the last several winters as previously reported[18]. Samples were collected every 6 days during the winter months (November through February) following the EPA compliance schedule, starting in November 2004 and ending in February 2008. A BGI PQ200 $PM_{2.5}$ Federal Reference Method (FRM) $PM_{2.5}$ sampler (BGI, Inc., Waltham, MA) was fitted with a quartz filter for each sample day to collect the ambient $PM_{2.5}$. Pre-fired 47-mm quartz filters (fired at 500°C for 2.5 hours) were purchased from Chester LabNet (Tigard, OR), and delivered to Lincoln County personnel in a cooler. Clean quartz filters were stored in a refrigerator at approximately 2°C prior to sample collection. Following sample collection, the quartz filter samples were stored in a freezer at -20°C until analysis. Approximately 24 m$^3$ of air was sampled during each 24-hour episode. Quartz filter field blanks were also collected periodically throughout the program to address artifact contamination.

### 4.9.2. Residential

From October 2006 through March 2007, a residential sampling program initially evaluated the indoor air quality within 21 homes in Libby before a woodstove changeout. Homes were eligible for this sampling program if there was a planned woodstove changeout for the winter of 2006/2007 and there were no residents who smoked inside

the home. Seventeen of the homes were single family residences and four of the homes were mobile homes. Only two of the homes had attached garages, but one of these garages was used for storage rather than vehicles. Eighteen of the homes reported woodstoves as their primary heating source. The average square footage of sampled homes was approximately 1500, and the average annual wood usage for heating was six cords. The type of wood typically burned for home heating in Libby includes softwoods such as Douglas fir and larch. Five of the original 21 homes were discarded from the study for various reasons, leaving a sample size of 16 homes (Table 4.4).

**Table 4.4**
Reasons for excluding homes from the Libby residential study.[17]

| Home Number | Reason |
|---|---|
| 2 | As noted on sampling datasheet: "possibly trouble getting fire lit, using new stove, chimney blocked?" |
| 4 | As noted on sampling datasheet: "didn't use their new woodstove much during the 24 hours, and utilized mostly electric heat." |
| 16 | Resident switched to pellet stove rather than woodstove. |
| 19 | Resident moved prior to post-changeout measurement. |
| 21 | Home needed a new chimney; no post-measurements conducted. |

One 24-hour sampling event was conducted within each of the homes prior to the woodstove changeout, and a follow-up 24-hour sampling event occurred two to three weeks after the installation of the new EPA-certified woodstove. During each sampling event within the home, two types of air samplers were deployed, including a portable TSI, Inc. DustTrak (Model 8520) that continuously measured $PM_{2.5}$ mass, and one Leland Legacy pump/Personal Environmental Monitor (PEM) sampler fitted with a 37-mm $PM_{2.5}$ quartz filter.

For each of the homes, indoor samples were collected within the same room of the residence that the woodstove was located, usually in the common area (living room). Both the DustTrak and Leland/PEM were co-located during the sampling event, and placed approximately 3-5 feet off of the ground. The DustTrak measured $PM_{2.5}$ mass (1 minute interval averages) throughout the 24-hour sampling period. During sampling,

indoor air was drawn through the quartz filter at an average flow rate of 9.8 L/min for 24 hours, collecting a total volume of ~14,080 L of indoor air.

### 4.9.3. Laboratory studies

Woodsmoke emitted from an older model, non-EPA-certified woodstove or an EPA-certified model was directed by aluminum flex tubing into a fume hood for sample collection. $PM_{2.5}$ concentrations were regulated using several in-line valves, with continuous $PM_{2.5}$ measurement conducted using a TSI DustTrak (TSI, Inc., Minneapolis, MN). The type of wood used was a mixture of locally obtained softwoods (Douglas fir, larch, and Ponderosa pine). Fires were started with 4 g of paper and 20 g of kindling, and maintained by the addition of pre-weighed wood batches (50.00-54.99 g) approximately every 5 min. The remaining ash and unburned wood was removed from the stove after each burn and weighed to determine the total amount of wood burned. The combustion conditions during the burns ranged from flaming to smoldering. Each stove was burned for 2 hours at a time on two separate days. A DustTrak was used to monitor background $PM_{2.5}$ in the laboratory beginning 8 hours before each burn until approximately 12 hours after the burn was completed. This information was also used to ensure that $PM_{2.5}$ levels returned to a background concentration before the next burn and no cross contamination was occurring in the air in the laboratory between burns.

$PM_{2.5}$ samples were collected using a Leland Legacy pump/Personal Environmental Monitor (PEM) sampler fitted with a 37-mm $PM_{2.5}$ quartz filter. The PEM was co-located with a DustTrak $PM_{2.5}$ measurement to determine the total amount of $PM_{2.5}$ collected on each sample. Filters were changed every 15 minutes to avoid overloading the sample pump. One background sample was collected for 30 minutes in the laboratory before the beginning of each burn to check for other sources of $PM_{2.5}$ in the air. After collection, quartz filters were cut in half and stored in a freezer until analysis. One half of each filter was analyzed for the seven selected tracers of woodsmoke according to the previously reported GCMS method, and the remaining half was stored in a freezer for future studies. The resin acid data from the second trial with the EPA-certified stove was discarded because of problems during sample analysis.

### 4.10. Statistical analysis

Data from ambient samples were analyzed by Student's t-test as appropriate using the Data Analysis tool of Microsoft Excel. All significance was determined with $p<0.05$ unless otherwise noted. For comparison of pre- and post-changeout residential samples, data were log transformed to approximate normality and paired t-tests were conducted (SAS 9.1, Cary, NC). Pearson correlations were used to evaluate associations between $PM_{2.5}$ and chemical markers of woodsmoke.

### 4.11. Conclusions

A method was developed for the determination of chemical tracers for biomass burning in particulate matter. The method provides excellent recoveries for levoglucosan, vanillin, acetovanillone, guaiacol, and 4-ethylguaiacol and adequate and reproducible recoveries for dehydroabietic acid and abietic acid. The sensitivity and precision of the method are good for all of the selected compounds. The method has been applied to the analysis of ambient and residential samples collected in Libby, MT, as well as to the analysis of laboratory samples of wood smoke particulate generated in Missoula.

# Chapter 5: Biomarker Analysis Methods

## 5.1. Materials

Levoglucosan (99+%) was purchased from Acros Organics (Geel, Belgium). Ethyl acetate (reagent grade), ethanol (95%) and triethylamine (reagent grade) were purchased from Fisher Scientific (Hampton, NH). N-*O*-bis(trimethylsilyl)trifluoroacetamide (derivatization grade, 99+ %), trimethylchlorosilane (97%), trimethylsilylimidazole (derivatization grade), and urease (type C-3 from *Canavalia ensiformis*) were purchased from Sigma (St. Louis, MO). All chemicals were used as received.

## 5.2. Standards

Deuterated levoglucosan was employed as an internal standard in the procedure to eliminate possible matrix effects and other variations throughout the analysis period. D-levoglucosan (D7) 98% was purchased from Cambridge Isotope Laboratories (Andover, MA). The solution containing D-levoglucosan was prepared in distilled water and stored in the refrigerator.

## 5.3. Development

The method used was adapted from Tetsuo *et al.* based upon observations and equipment available in our laboratory.[79] A combination of evaporation in a vacuum manifold and a freeze-dry system was used to remove all of the water from the samples. All water must be removed because water can also react with the derivatizing reagents and interferes with the analysis. The vacuum manifold alone required 24+ hours to remove all of the visible water, and approximately 1/4 samples still contained traces of water that were evident upon analysis. A higher amount of urease was investigated but was not found to be necessary. Significantly smaller amounts of urease were found to be insufficient in eliminating all urea present. Derivatization with a combination of BSTFA, TMCS, and TMSI was determined to be the most effective.[18] Using this combination of compounds allows for the potential to investigate other woodsmoke tracers in urine samples as well.

## 5.4. Urine analysis method

### 5.4.1. Human urine samples

A GC-MS method developed in our laboratory based upon a previously published method was used for analysis of the urine samples.[67, 68, 79] First, 100 µL of human urine

was placed in an eppendorf tube. Approximately 30 Units of urease was added and the samples were placed in an oil bath at 37°C for 30 minutes. To precipitate out the protein, 900 µL of ethanol was added and the samples were centrifuged for 8 minutes. The supernatant was then transferred to a clean eppendorf tube and the remaining solids were discarded. The sample was then dried in a vacuum manifold for 6+ hours to evaporate the ethanol. To ensure the samples were completely dry, 100 µL of distilled water as added and then the samples were lyophilized until dry (minimum of 4 hours). The remaining solids were derivatized with 75 µL of BSTFA, 10 µL of TMCS, and 10 µL of TMSI in an oil bath at 70°C for 1 hour. After derivatization, the samples were diluted to 0.5 mL with ethyl acetate containing 3.6 mM TEA and were transferred to GC-MS vials for analysis.

#### 5.4.2. Mouse urine samples

Mouse urine samples were analyzed using the same method as human urine samples, except for the following changes.[67] Only 50 µL of sample was analyzed, unless the total sample volume was less than 50 µL in which case the entire sample was analyzed. Protein was precipitated with 500 µL of ethanol. The final dilution on the samples for analysis was 250 µL and the concentration of the deuterated standard was adjusted accordingly.

### 5.5. GC-MS conditions

All urine samples were analyzed on an Agilent 6890N Gas Chromatograph with an Agilent 5973 Mass Spectrometer. An HP-5MS column ((5%-Phenyl)-methylpolysiloxane) was used with dimensions of 0.25 mm ID x 30 m length x 0.25 µm film thickness. A volume of 2 µL was injected for each analysis into a Split/Splitless FocusLiner™ for HP, single taper liner packed with quartz wool. Split injection was used to analyze for levoglucosan with a split ratio of 50:1. Helium was used as the carrier gas at an initial flow rate of 1mL/min through the column. The inlet temperature was set to 250°C and the auxiliary transfer line temperature was set at 280°C. The temperature program was started at 40°C for 1.5 minutes, ramped at 30°C/min to 175°C, 20°C/min to 220°C, held for 2 minutes at 220°C, and then ramped at 50°C/min to a final temperature of 300°C, which was held for 1.5 minutes for a total run time of 13.95 minutes. The mass spectrometer was operated with a solvent delay of 4.00 minutes and the mass range

from 40-450 was scanned. For all compounds, highly selective quantitation was performed using the signal for representative ions extracted from the total ion chromatogram. Levoglucosan was analyzed using an m/z of 217, while m/z 220 was used for D7-levoglucosan. These two ions were selected for analysis because they are predominant ions in the mass spectra that are semi-unique to the compounds of interest and represent the same fragment in the normal and deuterated levoglucosan.

**5.6. Calibration**

Blank urine was collected from mice that had not been exposed to woodsmoke or other potential sources of levoglucosan. Potential blank samples were analyzed using the GC-MS method to verify the absence of levoglucosan. Blank samples from several mice were combined and this pool was used as the matrix for the calibration curves. Calibration standards were prepared containing 10, 25, 50, 80, or 125 ppm levoglucosan with a fixed concentration of 80 ppm D-levoglucosan (Cambridge Isotope Laboratories, Andover, MA) as an internal standard. The D7-levoglucosan had a mass of 169 before derivatization, compared to 162 for levoglucosan. The standards were derivatized and analyzed on the GC-MS according to the procedure described above. A calibration curve was prepared by plotting the ratio of the two peak areas versus the concentration of the tracer and the $R^2$ value was 0.9634. The concentration of analytes in the samples was determined by measuring the ratio of the peak area for the analyte to that of the corresponding deuterated standard, and reading the concentration from the appropriate calibration curve. For human exposure studies, distilled water was used as the calibration matrix due difficulty finding a human urine sample containing no levoglucosan and the limited amount of blank mouse urine available. $R^2$ values for the calibration curves used in human exposure studies were 0.96 or above.

**5.7. Recovery**

Spikes in distilled water were prepared daily with the samples to monitor instrument performance and solution composition (no less than 1 spike per 10 samples analyzed). Average recovery of levoglucosan was 107±9.5% (n=15) for the mouse exposure studies. For human exposure studies, average levoglucosan recovery was 104±4.1% (n=21).

### 5.8. Blanks

Blanks of distilled water were analyzed daily with the samples (no less than 1 blank per 10 samples) to monitor for contamination during analysis. Distilled water was chosen as the matrix for blank because of the limited volume of blank mouse urine available and presence of levoglucosan in pre-exposure human urine samples. Levoglucosan was not detected in any of the blanks for the mouse (n=15) or human (n=21) exposure studies, confirming that no contamination occurs during sample preparation or analysis.

### 5.9. Detection limits

Detection limits for the method were defined as the concentration of analyte that gives an instrument response that is 3 times the standard deviation of the instrumental baseline signal. The detection limit for levoglucosan in the final ethyl acetate extract was determined to be 0.92 μg/ml (1.8 ng injected, 37 pg on-column), which equates to a detection limit of 0.23 μg in 100 μl of urine sample with the dilutions used for analysis. Samples below the detection limit were assigned a value of ½ the detection limit for calculations.[80-82]

### 5.10. Statistical analysis

All data were analyzed using SAS 9.1 (Cary, NC) or Microsoft Excel 2003. Data were analyzed by analysis of variance and t-test as appropriate. Urinary levoglucosan and urinary cotinine were compared by Pearson correlation. The descriptive urinary levoglucosan data presented in the text, tables and figures were untransformed unless noted.

### 5.11. Mouse model

#### 5.11.1. Mice

Balb/c mice (Jackson Laboratory, Bar Harbor, ME) were used for all *in vivo* studies. The Balb/c strain is utilized for a wide variety of studies and has been well characterized in multiple models. Animals were housed in microisolators on a 12:12-h light-dark cycle. The mice were given food and deionized water *ad libitum*. To ensure that the mice were treated properly as to minimize discomfort and suffering, all animal procedures were approved by the University of Montana Institutional Animal Care and Use Committee.

### 5.11.2. Instillations

For intranasal instillations, animals were anesthetized using 0.1 cc Ketamine (1:4 in sterile phosphate buffered solution) by intraperitoneal injection. Mice were then instilled with 25 µl of specified treatment dissolved in a phosphate buffer system. Treatments included levoglucosan, glucose, or concentrated particulates from wood smoke, ambient Missoula air, and diesel exhaust. Treatment regimen consisted of a single instillation followed by urine collection within 24 hours.

### 5.11.3. Directly instilled PM

Mice were directly instilled with multiple kinds of PM including: (1) $PM_{2.5}$ harvested from the ambient air of Missoula, Montana, (2) woodsmoke $PM_{2.5}$ harvested from a non EPA-certified woodstove, and (3) diesel exhaust. Particles were weighed, re-suspended in sterile phosphate buffer, and sonicated in a water bath for 1 minute immediately prior to instillations. Mice were instilled with 125 µg of particle, and urine was collected at 2, 4, and 6 hrs post instillation and pooled into a single sample per mouse for analysis.

#### 5.11.3.1. Ambient air

A versatile aerosol concentration enrichment system (VACES)[83] particle concentrator was used to harvest $PM_{2.5}$ from the ambient air in Missoula, Montana. Air samples were collected by the concentrator on the roof of a three-story building at The University of Montana. The concentrator has three parallel sampling lines (concentrators) that simultaneously collected fine PM, each at a set flow rate of 110 LPM. The fine fraction (all particles less than 2.5 µm) was concentrated by drawing air samples through two parallel lines, and using 2.5 µm cut-point pre-impactors to remove larger sized particles. These particles were then drawn through a saturation-condensation system that grows the particles to 2-3 µm droplets before being concentrated by virtual impaction. Particles and water-soluble fraction are then collected in glass impinger Biosamplers, and then lyophilized to harvest the ambient $PM_{2.5}$.

#### 5.11.3.2. Wood smoke

The VACES particle concentrator was used to collect smoke-$PM_{2.5}$ emitted from a non-EPA-certified woodstove. In a controlled simulation, a non-EPA-certified woodstove (Englander, England Stove Works, Inc.) was loaded with a mixture of locally

obtained softwoods (Douglas fir, larch, and Ponderosa pine), with the smoke pumped into a modified inhalation chamber to allow the smoke to cool and age (residence time was no more than 2 minutes). The combustion conditions during the burns ranged from flaming to smoldering. The VACES was then utilized to harvest smoke $PM_{2.5}$ into a deionized water dropout (Biosampler, SKC, Inc.). At the conclusion of the smoke-PM harvesting trial, there were clearly two fractions that were collected, including a water-soluble fraction and a water-insoluble black tar-like material that coated the inside of the impinger. Because we were unable to get the water-insoluble fraction in a suitable solution with which to instill mice, only the water-soluble fraction of the harvested $PM_{2.5}$ was used in this study. However, it was determined by GC-MS that only the water-soluble fraction contained detectable levels of levoglucosan.

#### 5.11.3.3. Diesel exhaust

National Institute of Standards and Technology (NIST) particulate matter standard reference material (SRM) for diesel PM (SRM 1650) was purchased and utilized in this study.

### 5.11.4. Smoke exposure

Woodsmoke emitted from an older model, non-EPA-certified woodstove was directed by aluminum flex tubing into a modified inhalation chamber. $PM_{2.5}$ concentrations inside the chamber were regulated using several in-line valves, with continuous $PM_{2.5}$ measurement conducted using a TSI DustTrak (TSI, Inc., Minneapolis, MN). The type of wood used was a mixture of locally obtained softwoods (Douglas fir, larch, and Ponderosa pine). Fires were started with 4 g of paper and 20 g of kindling, and maintained by the addition of pre-weighed wood batches (50.00-54.99 g) approximately every 5 min. The combustion conditions during the burns ranged from flaming to smoldering.

Mice were placed in individual slots in an animal housing unit, within the exposure chamber, which is composed of perforated metal to allow diffusion of woodsmoke through the compartments. Mice were exposed in two separate groups for 2 hours each at a target concentration between 3-4 $mg/m^3$. During the combustion process temperature and CO readings were constantly observed. Urine samples were collected at

2, 4, and 6 hours post exposure and pooled for analysis. Procedures in this section are approved in the AUP 050-06 at the University of Montana.

#### 5.11.4.1. Exposure calculation

In calculating particle deposition, a minute ventilation (mv) of 42.6 cc/min and a minimum 20% deposition (d) (derived empirically from $PM_{2.5}$ deposition models) were assumed based on previously published studies (Hsieh and Oberdorster 1999; Kleeman *et al.* 1999). Taking the average exposure (ae) of 3.14 mg/m$^3$ for 2 hrs (t), it was calculated that approximately 3.2 μg of $PM_{2.5}$ was deposited in the lungs of exposed mice [(ae) x (mv) x (t) x (d) = amount deposited]. Based on a previously determined levoglucosan concentration of 26.6% in the particulate (Table 3.1, p. 44), each mouse was potentially exposed to 0.85 μg of levoglucosan during the 2 hour wood smoke exposure period.

### 5.11.5. Sample collection

Mouse urine samples were collected in sterile 1.5 ml eppendorf tubes at designated time points. For "pre" samples, urine was collected just prior to intraperitoneal injection. Briefly, one researcher handled the animals by grasping from behind and positioning such that the fluid was collected in pre-labeled tubes by a second researcher. Samples were stored at -20°C until analyzed.

## 5.12. Subject selection

### 5.12.1. Libby schoolchildren study

A convenience sample of 14 grade school children in Libby, MT was used initially to evaluate the presence of levoglucosan in urine. All children were non-Hispanic white with a mean age of 8.5 years, ranging from 7 to 10 years old. The residences of these subjects were located within the Libby, MT airshed, an area with moderately elevated levels of ambient woodsmoke from wintertime domestic woodstove usage. Information was collected from parents on the type of home heating and whether or not there were tobacco smokers in the household. Spot urine samples were collected at the school between 8:30 am and 2:00 pm, aliquoted, and stored at -80°C. Eight samples were collected in the morning (i.e., before noon) and six samples were collected in the afternoon. All human sample collection procedures, including documentation of parental permission and child assent, were approved by the University of Montana Institutional Review Board.

#### 5.12.1.1. Indoor and ambient air monitoring

Indoor air monitoring at the children's school and ambient air monitoring at a central site located less than one-quarter mile from the school was conducted to determine these subjects' potential exposure to wood smoke particulate matter. A Sioutas impactor PM sampler with Leland Legacy pump (SKC, Inc., Eighty Four, PA) was fitted with Teflon filters to measure the gravimetric mass of five size fractions of the indoor PM. The size fractions included >2.5 μm, 1.0-2.5 μm, 0.5-1.0- μm, 0.25-0.5 μm, and <0.25 μm. A collocated $PM_{2.5}$ cyclone (BGI, Inc., Waltham, MA) was fitted with a 47-mm pre-fired quartz filter for subsequent analysis of specific chemical markers of woodsmoke, including levoglucosan. The previously discussed GC-MS method (section 4.4) was used to analyzed all filters for levoglucosan.[18] Ambient $PM_{2.5}$ data were collected from the Montana Department of Environmental Quality's $PM_{2.5}$ compliance site for the town of Libby is located approximately one half mile from the school.

### 5.12.2. Lab smoke exposures

Subjects were healthy, non-smoking adults between the ages of 18 and 65. Beginning 24 hours before the exposure and continuing until the completion of the study, subjects were asked to avoid exposure to smoke of any type. Previous studies with mice have demonstrated that 86% of levoglucosan instilled intranasally in mice is recovered within 4 hours of exposure, so 24 hours was determined to be sufficient to avoid any effects from prior wood smoke or levoglucosan exposure.[67] Subjects were also asked to avoid consuming a variety of foods, including smoked or grilled foods, bacon, foods containing artificial wood smoke flavoring, and foods containing caramel that could potentially interfere with study results. People with asthma or other lung diseases were excluded from the study. Two separate exposure trials were conducted using smoke generated from an older-model wood stove. Four male subjects participated in the first trial, and the same four male subjects plus one female subject participated in the second trial. All procedures were approved by the University of Montana Institutional Review Board.

#### 5.12.3. Campfire study

Samples were obtained from a previous campfire smoke exposure study published by Dills *et al.*[63], designed to measure urinary methoxyphenols before and after exposure to wood smoke.

#### 5.12.4. Caramel study

Subjects were healthy, non-smoking adults between the ages of 18 and 65. Beginning 24 hours before the exposure and continuing until the completion of the study, subjects were asked to avoid exposure to smoke of any type. Subjects were also asked to avoid consuming a variety of foods, including smoked or grilled foods, bacon, foods containing artificial wood smoke flavoring, and foods containing caramel that could potentially interfere with study results. People with diabetes were excluded from the study. All procedures were approved by the University of Montana Institutional Review Board.

#### 5.12.5. Fasting study

Subjects were healthy, non-smoking adults between the ages of 18 and 65. Beginning 24 hours before the exposure and continuing until the completion of the study, subjects were asked to avoid exposure to smoke of any type. All procedures were approved by the University of Montana Institutional Review Board.

### 5.13. Human smoke exposures

#### 5.13.1. Campfire exposure

Samples were obtained from a previous campfire smoke exposure study published by Dills *et al.*[63], designed to measure urinary methoxyphenols before and after exposure to wood smoke. Subjects were exposed to wood smoke from a continuous open fire for 2 hours, and all urine voided by the subjects was collected (as separate voids) beginning 24 hours prior to the study up until 48 hours after the exposure (Table 5.1). Samples were stored at -80°C and remained frozen during shipment. One personal $PM_{2.5}$ sample was collected for each subject using the Harvard Personal Environmental Monitor for $PM_{2.5}$ and analyzed as previously reported for various chemicals in wood smoke, including levoglucosan.[63]

**Table 5.1**

Overview of exposures and sample collection for each human exposure study.

| Type of exposure | Subjects | Time Point Number | Sample collection time (hours post exposure)[a] | length of exposure | nondetects/total number of samples |
|---|---|---|---|---|---|
| campfire smoke | 9 | 1-13[b] | samples collected ad libitum over 72 hours, beginning 24 hours before exposure | 2 hours | 26/117 |
| wood stove | 4 | 1 | 0 | 2 hours | 0/20 |
| | | 2 | 3.2 (0.2) | | |
| | | 3 | 6.3 (0.6) | | |
| | | 4 | 12.3 (1.3) | | |
| | | 5 | 20.9 (0.8) | | |
| wood stove | 5 | 1 | 0 | 2 hours | 0/30 |
| | | 2 | 2.6 (0.1) | | |
| | | 3 | 7.9 (1.5) | | |
| | | 4 | 12.5 (0.8) | | |
| | | 5 | 21.4 (0.8) | | |
| | | 6 | 25.7 (1.1) | | |
| caramel | 9 | 1 | 0 | N/A | 0/45 |
| | | 2 | 2.3 (0.4) | | |
| | | 3 | 6.1 (0.2) | | |
| | | 4 | 13.3 (2.8) | | |
| | | 5 | 23.6 (1.3) | | |
| fasting | 21 | 1 | 12 | N/A | 5/21 |
| | | 2 | 14 | | 13/21 |

[a]Average sample collection time for each time point (standard deviation)
[b]Subjects each had 3-4 pre exposure samples and 9-10 post exposure samples

### 5.13.2. Lab smoke exposure

Subjects were asked to collect spot urine samples immediately pre-exposure, and at various time points post-exposure. In the first trial, four post-exposure samples were collected from each subject, and a fifth time point was added for the second trial so that two samples were collected the morning after exposure (Table 5.1). Smoke was generated within an enclosed laboratory using an older model, non-EPA-certified wood stove. Locally available softwood species (Douglas fir, larch, and Ponderosa pine) were used for the exposure. Fires were started with 4 g of paper and 20 g of kindling, and maintained by the addition of pre-weighed wood batches (50.00-54.99 g) approximately every 5 min. Within-room $PM_{2.5}$ concentrations were monitored continuously using a TSI DustTrak (TSI, Inc., Minneapolis, MN). It is important to note that the DustTrak is not a Federal Reference Method (FRM) sampler. DustTrak measurements have been shown to be reasonably precise (R2 = 0.859) when compared with an FRM sampler.[84] However, the results presented here were not validated using a co-located FRM sampler from which a correction factor (i.e. wood smoke PM correction factor) could be developed.

Each subject wore a DustTrak to determine personal $PM_{2.5}$ exposures during the two trials. Personal breathing zone monitoring for the study subjects began approximately 1 hour before the exposure, throughout the approximately 2 hour exposure trials, and for 6 hours after smoke exposure to monitor any other potential sources of $PM_{2.5}$. For both the in-room and personal breathing zone sampling, 60-second intervals were recorded. Levoglucosan in the air was not measured during the wood stove smoke exposure trials.

### 5.14. Human diet studies

#### 5.14.1. Caramel study

Nine non-smoking subjects between the ages of 18 and 65 (6 female, 3 male) participated in the caramel study. Subjects were asked to consume cubes of caramel in a short period of time (no more than 30 minutes). Subjects each consumed five cubes of caramel, for an average of 42.2 g consumed per person (sd=0.49). Urine samples were collected immediately before exposure, and 2, 6, 12, and 24 hours after exposure (Table 5.1).

#### 5.14.2. Fasting study

Twenty two non-smoking subjects between the ages of 18 and 65 (12 male, 10 female) participated in the fasting study. Subjects were asked to avoid eating or drinking anything other than water for 14 hours during the study. Subjects were asked to begin fasting after dinner one evening and fast overnight for at least 14 hours. Subjects were asked to record everything eaten for the last meal prior to beginning the fast. Urine samples were collected after fasting for 12 hours (or after waking in the morning) and 14 hours (completion of the study). The first 12-hour sample represents an accumulation of urine overnight, so it could still contain compounds from meals eaten the previous night. The 14-hour sample was designed to represent urine composition after fasting. Upon receipt at the laboratory, samples were divided into aliquots for analysis. One aliquot was stored in a refrigerator until analysis and the remaining aliquots were placed in a freezer for long-term storage. One subject was discarded from the study due to errors during sample creatinine analysis.

### 5.15. Food analysis

A list of foods to test for levoglucosan was determined by selecting foods that could make-up three complete meals for one day, were primarily non-baked or heavily sweetened, and did not have high fat contents for easier analysis. The foods selected were lettuce, salsa, corn chips, tortillas, red pepper, Special K cereal, carrots, rice, tomatoes, cherries, popcorn, black beans, wheat bread (both unaltered and as burned toast), hummus, French bread, pasta, Ragu™ pasta sauce, apples, and strawberries. Coffee and V8™ were the two beverages tested. Caramel was also analyzed to verify the extraction method was working since it is known to be positive for levoglucosan. Foods such as pasta and rice were prepared according to directions, but without the addition of any seasonings (such as salt or butter). Other foods with a higher fat content such as milk, turkey lunchmeat, cheddar cheese, peanut butter, and sour cream were initially investigated. They were mixed with methanol before analysis to remove some protein and fat and simplify the matrix; however, the composition of these foods made lyophilization difficult. Because of difficulties in analysis of the fatty foods and also the inconclusive results obtained for many of the other foods with a simpler matrix, GC-MS analysis of these foods was not performed.

For analysis, the foods were first lyophilized at least overnight or for up to several days until completely dry. They were then ground into a powder to homogenize the sample and stored in the freezer. Approximately 1 gram was weighed out and spiked with D-levoglucosan, to be used for method validation. The samples were then extracted by mixing with 8 mL of solvent and placing them in a sonicator for 1 hour. Ethyl acetate, ethanol, and water were all investigated as potential extraction solvents and water was determined to be the most effective through spikes and tests with caramel extraction. The samples were then filtered through a Whatman 0.45 μm nylon filter to remove any solids. The sample was lyophilized again and then derivatized with the previously used mixture of BSTFA, TMCS, and TMSI for 1 hour at 70°C. After derivatization, the samples were centrifuged to remove any remaining solids and the supernatant was transferred to a vial and analyzed using GC-MS. Levoglucosan levels in the foods were not calculated, they were simply tested for the presence or absence of levoglucosan.

### 5.16. Methoxyphenols

#### 5.16.1. Analysis method

Two milliliters of urine was placed in a vial. The pH was adjusted to 5.0 with 1 M acetic acid, and 0.25 mL of 1 M acetate buffer (pH 5.5) is added. The methoxyphenols were deconjugated with 20 μL of β-glucuronidase from *Helix Pomatia* (purchased from Sigma) in an oil bath at 37 °C for 18 hours. The deuterated standards were then spiked into the urine and the pH was lowered further with the addition of approximately 100 μL of 1 M sulfuric acid. The entire sample was passed through a preconditioned Oasis HBL solid-phase extraction column to extract the methoxyphenols. The column was then rinsed with 2 mL of 10 mM HCl. Air was drawn through the column for 2-3 minutes and then the column was allowed to air dry for at least 30 minutes. To remove the methoxyphenols from the column, 2 mL of ethyl acetate containing 3.6 mM TEA was passed through the cartridge and collected in a test tube. The volume was then reduced to 0.5 mL under a stream of air in an oil bath at 40 °C. The samples were transferred to an eppendorf tube and derivatized with 30 μL of a freshly prepared 2:3 mixture of acetic anhydride: triethyl amine in an oil bath at 70 °C for 1 hour. After derivatization, 1,3,5-trimethoxybenzene was added as an internal standard. The samples were then analyzed on the GC-MS using splitless injection.

#### 5.16.2. Calibration

Standard solutions for calibration and sample spikes were prepared in ethanol. Calibration curves were prepared by spiking the standards into 0.5 mL of ethyl acetate with 3.6 mM triethylamine, followed by derivatization and analysis in the same fashion as the samples. The correlation coefficients for the calibration curves are all 0.97 or higher. Each compound was calibrated individually against the internal standard (1,3,5 trimethoxybenzene).

#### 5.16.3. Recovery

The recoveries of the deuterated compounds that were spiked into the sample were calculated and used to adjust the recovery of the other compounds to account for any lost during the extraction procedure. Spike recoveries from water were 83±12 % for vanillin, 69±12 % for acetovanillone, 109±9 % for guaiacol, and 104±8% for 4-ethylguaiacol.

### 5.17. Creatinine analysis

Human urine samples were analyzed for creatinine using a creatinine ELISA kit purchased from Cayman Chemical Company (Ann Arbor, MI). Creatinine analysis was performed in the same week as analysis for levoglucosan. Standards and samples were analyzed in duplicate. Values were used to normalize levoglucosan measurements to account for dilution. Creatinine analysis for the campfire smoke exposure was performed as previously reported as part of the original study and was not repeated at the time of levoglucosan analysis.[63]

### 5.18. Cotinine analysis

Human urine samples from the Libby kid study were analyzed by ELISA for cotinine (Calbiotech, Spring Valley, CA) to evaluate exposure to tobacco smoke. Cotinine results for this study were normalized with urinary creatinine values.

## Chapter 6: Conclusions

A method was developed for the analysis of seven selected chemical tracers for woodsmoke in particulate matter. The method used solvent extraction to remove the compounds from the particulate matter and GC-MS analysis. The developed method was applied to samples collected as part of several studies in Libby, MT during the course of a woodstove changeout program.

A study of the ambient particulate matter in Libby found that $PM_{2.5}$ decreased by 20% and levoglucosan decreased by 50% after the woodstove changeout. The two resin acids measured did not decrease during the changeout, suggesting that the chemistry of the particulate matter is changing as the stoves are replaced. The methoxyphenols measured did not show any trends throughout the changeout, but two were found to correlate to ambient temperature on the day of sample collection.

In a residential study inside homes in Libby, samples were collected before and after installation of a new, EPA-certified woodstove. $PM_{2.5}$ and levoglucosan both decreased inside homes after installation of the new stove, while both resin acids increased.

Laboratory studies with an older-model and EPA-certified model stove were completed in an attempt to replicate the change in particulate matter chemistry observed in Libby. Levoglucosan showed no change between the two types of stoves, while both resin acids showed lower levels in the new stove, contrary to what was expected. The results observed in the laboratory setting are likely due to low burn temperatures and small amounts of wood being burned.

A method was also developed for the determination of levoglucosan in urine and it was used to investigate levoglucosan as a urinary biomarker for woodsmoke exposure. Initial studies with the mouse model showed that levoglucosan instilled intranasally could be recovered at 40+% within 4 hours of exposure. Specificity tests showed that exposure to other sugars of similar structure did not affect levoglucosan recovery. Exposure to other sources of PM also did not affect levoglucosan recovery. Inhalation of woodsmoke particulates did cause an increase in urinary levoglucosan in mice. All together, the mouse studies suggested levoglucosan could be a suitable urinary biomarker for woodsmoke exposure.

Initial human studies showed a background level of urinary levoglucosan. Looking at a sample of schoolchildren in Libby, no correlation was found between urinary levoglucosan and woodstoves or smoking in the home. Controlled laboratory smoke studies also did not show an increase in urinary levoglucosan after exposure from either a woodstove or a campfire. Diet studies showed a large increase in urinary levoglucosan after consumption of caramel, suggesting that diet is a big factor in human levels. Fasting for up to 14 hours was not sufficient to eliminate the dietary influences. Foods were tested for levoglucosan content, but most tests were inconclusive due to matrix effects. The effects of dietary levoglucosan are too great to be overcome by exposure to higher levels of woodsmoke.

Because of the prevalence of levoglucosan in the human diet, it is not a suitable urinary tracer for woodsmoke exposure in most settings. It could still be used in studies with animal models where the diet can be closely controlled and does not contain levoglucosan.

## 6.1. Future work

### 6.1.1. Air studies

#### 6.1.1.1. Libby studies

Organic and elemental carbon analysis was performed on the particulate matter in Libby, MT before the woodstove changeout began (winter of 2003/2004), but follow up data from after the completion of the woodstove changeout have not yet been investigated. Samples were collected during the winter of 2008/2009 for this analysis, but data have not been analyzed and compared to the pre-changeout data. Organic carbon is analyzed in four separate fractions based upon temperature and these fractions were found to respond differently to the woodstove changeout. We hope to determine which OC fraction each of the monitored chemical tracers belongs to and determine if the changes in organic carbon composition are consistent with the changes in PM chemistry observed.

A complete analysis of all organic compounds found in the Libby particulate matter was also performed, but data have not yet been analyzed. The data from the two winters will provide a more complete picture of the change in PM chemistry occurring in

Libby. From this list, we hope to choose a few other compounds (such as other resin acids) to retroactively look for in the samples that have already been analyzed.

A follow-up study should be carried out in Libby to determine if the reductions in ambient $PM_{2.5}$ and levoglucosan are sustained in the long term. A similar study could also be conducted in the previously-studied homes to determine if reductions in indoor $PM_{2.5}$ and levoglucosan are sustainable. As the stoves age and the residents' vigilance about using them decreases, it is possible that the improved PM emissions will again rise and the chemistry will change. Samples have been collected inside many of the homes in the Libby residential study 1 and 2 years post-changeout to see if the observed changes in particulate matter continue. Analysis of the samples is not complete as the data are more complex to compare when other possible sources of variation are considered, such as meteorological conditions. Additional indoor studies could also be performed to determine if similar reductions in the levels of various air toxics, including PAHs, are observable.

#### 6.1.1.2. Laboratory studies

With a more suitable location for the controlled laboratory studies, the older and EPA-certified woodstoves could be burned at higher temperatures to better replicate real-world conditions. The previous laboratory stove studies can be repeated with larger amounts of wood, higher burn temperatures, and longer burns. Burning the stoves in this manner will replicate the operating conditions seen in the real-world, providing more information about the chemistry of the PM. We hope to replicate the PM changes between the old and new stoves seen in Libby. Being able to re-create the PM chemistry also can be useful in future exposure studies, making laboratory smoke exposures closer to real-life exposures.

#### 6.1.1.3. Other compounds

As more information is obtained about the composition of woodsmoke particulate matter, additional compounds are found to be changing during stove replacements. New compounds that are indicative of woodsmoke in particulate matter such as galactosan, mannosan, pimaric acid, isopimaric acid, and several PAHs (particularly retene, fluoranthene, pyrene, and benzo[a]pyrene) can be added to the tracer analysis to obtain a more complete picture of the effects of changing out a woodstove.

### 6.1.2. Biomarker studies

Other potential compounds to use as a urinary biomarker of woodsmoke exposure are being investigated, including hydroxyl-PAHs, S-phenylmercapturic acid and S-benzylmercapturic acid. These are metabolites of compounds that have been detected in the airborne particulate matter. It would also be useful to investigate other biological fluids for potential biomarkers such as exhaled breath condensate or blood rather than urine.

## References


1.) Slaughter, J. C.; Lumley, T.; Sheppard, L.; Koenig, J. Q.; Shapiro, G. G., Effects of ambient air pollution on symptom severity and medication use in children with asthma. *Annals of Allergy, Asthma, & Immunology* **2003,** 91, (4), 346-353.

2.) Jaffe, D. H.; Singer, M. E.; Rimm, A. A., Air pollution and emergency department visits for asthma among Ohio Medicaid recipients, 1991-1996. *Environmental Research* **2003,** 91, (1), 21-28.

3.) Laden, F.; Schwartz, J.; Speizer, F. E.; Dockery, D. W., Reduction in fine particulate air pollution and mortality: extended follow-up of the Harvard Six Cities Study. *American Journal of Respiratory and Critical Care Medicine* **2006,** 173, (6), 667-672.

4.) Hinwood, A. L.; Trout, M.; Murby, J.; Barton, C.; Symons, B., Assessing urinary levoglucosan and methoxyphenols as biomarkers for use in woodsmoke exposure studies. *Science of the Total Environment* **2008,** 402, (1), 139-146.

5.) Boman, B. C.; Forsberg, A. B.; Jaervholm, B. G., Adverse health effects from ambient air pollution in relation to residential wood combustion in modern society. *Scandinavian Journal of Work, Environment & Health* **2003,** 29, (4), 251-260.

6.) Park, E.; Lee, K., Particulate exposure and size distribution from wood burning stoves in Costa Rica. *Indoor Air* **2003,** 13, (3), 253-259.

7.) Clark, M.; Paulsen, M.; Smith, K. R.; Canuz, E.; Simpson, C. D., Urinary Methoxyphenol Biomarkers and Woodsmoke Exposure: Comparisons in Rural Guatemala with Personal CO and Kitchen CO, Levoglucosan, and PM2.5. *Environmental Science & Technology* **2007,** 41, (10), 3481-3487.

8.) United States Environmental Protection Agency, National ambient air quality standards for particulate matter: Final Rule. Federal Register: **2006**; Vol. 71, pp 61143-61233.

9.) United States Environmental Protection Agency. Air quality criteria for particulate matter; EPA/600/P-99/002bF (Research Triangle Park, NC, USA, **2004**).

10.) United States Environmental Protection Agency, Standards of Performance for New Stationary Sources. In Federal Register: **1988**; Vol. 53, p Subpart AAA.

11.) United States Environmental Protection Agency, Burn Wise. www.epa.gov/burnwise. Accessed **2006-2010**.

12.) Hidinger, J. O. National policy impacts derived from the Crested Butte wood stove study, *84th Annual Meeting of The Air and Waste Management Association,* **1991**.

13.) Arnold, S.; Pierce, G.; Kenniston, G. E.; Osterburg, R. D., The Crested Butte experiment: an air quality analysis scheme to identify particulate impacts associated with a woodstove replacement program. *Proceedings, Annual Meeting - Air & Waste Management Association* **1991,** 84th, (Vol. 16), Paper 91/130 4.

14.) Eagle, B.; Houck, J. E. *Final Report. Phase I of the Libby, Montana Woodstove Changeout Program*; OMNI Environmental Services: **2007**a.

15.) Eagle, B.; Houck, J. E. *Final Report. Phase II of the Libby, Montana Woodstove Changeout Program*; OMNI Environmental Services: **2007**b.

16.) Ward, T.; Rinehart, L.; Lange, T., The 2003/2004 Libby, Montana PM2.5 Source Apportionment Research Study. *Aerosol Science and Technology* **2006,** 40, (3), 166-177.

17.) Ward, T. J., Palmer, C., Bergauff, M., Hooper, K., and Noonan, C., Results of a Residential Indoor PM2.5 Sampling Program Before and After a Woodstove Changeout. *Indoor Air* **2008,** 18, 408-415.

18.) Bergauff, M.; Ward, T.; Noonan, C.; Palmer, C. P., Determination and evaluation of selected organic chemical tracers for wood smoke in airborne particulate matter. *International Journal of Environmental Analytical Chemistry* **2008,** 88, (7), 473-486.

19.) Nolte, C. G.; Schauer, J. J.; Cass, G. R.; Simoneit, B. R. T., Highly Polar Organic Compounds Present in Wood Smoke and in the Ambient Atmosphere. *Environmental Science and Technology* **2001,** 35, (10), 1912-1919.

20.) Fine, P. M.; Cass, G. R.; Simoneit, B. R. T., Chemical Characterization of Fine Particle Emissions from the Fireplace Combustion of Woods Grown in the Southern United States. *Environmental Science and Technology* **2002,** 36, (7), 1442-1451.

21.) Mazzoleni, L. R.; Zielinska, B.; Moosmueller, H., Emissions of Levoglucosan, Methoxy Phenols, and Organic Acids from Prescribed Burns, Laboratory Combustion of Wildland Fuels, and Residential Wood Combustion. *Environmental Science & Technology* **2007,** 41, (7), 2115-2122.

22.) McDonald, J. D.; Zielinska, B.; Fujita, E. M.; Sagebiel, J. C.; Chow, J. C.; Watson, J. G., Fine Particle and Gaseous Emission Rates from Residential Wood Combustion. *Environmental Science and Technology* **2000,** 34, (11), 2080-2091.

23.) Fine, P. M.; Cass, G. R.; Simoneit, B. R. T., Chemical Characterization of Fine Particle Emissions from the Wood Stove Combustion of Prevalent United States Tree Species. *Environmental Engineering Science* **2004,** 21, (6), 705-721.

24.) Rogge, W. F.; Hildemann, L. M.; Mazurek, M. A.; Cass, G. R.; Simoneit, B. R. T., Sources of Fine Organic Aerosol. 9. Pine, Oak, and Synthetic Log Combustion in Residential Fireplaces. *Environmental Science and Technology* **1998,** 32, (1), 13-22.

25.) Simpson, C. D.; Paulsen, M.; Dills, R. L.; Liu, L. J. S.; Kalman, D. A., Determination of Methoxyphenols in Ambient Atmospheric Particulate Matter: Tracers for Wood Combustion. *Environmental Science and Technology* **2005,** 39, (2), 631-637.

26.) Simpson, C. D.; Dills, R. L.; Katz, B. S.; Kalman, D. A., Determination of levoglucosan in atmospheric fine particulate matter. *Journal of the Air & Waste Management Association* **2004,** 54, (6), 689-694.

27.) Engling, G.; Carrico, C. M.; Kreidenweis, S. M.; Collett, J. L.; Day, D. E.; Malm, W. C.; Lincoln, E.; Wei, M. H.; Iinuma, Y.; Herrmann, H., Determination of levoglucosan in biomass combustion aerosol by high-performance anion-exchange chromatography with pulsed amperometric detection. *Atmospheric Environment* **2006,** 40, (Suppl. 2), S299-S311.

28.) Simoneit, B. R. T.; Schauer, J. J.; Nolte, C. G.; Oros, D. R.; Elias, V. O.; Fraser, M. P.; Rogge, W. F.; Cass, G. R., Levoglucosan, a tracer for cellulose in biomass burning and atmospheric particles. *Atmospheric Environment* **1998,** 33, (2), 173-182.

29.) Schmidl, C.; Marr, I. L.; Caseiro, A.; Kotianova, P.; Berner, A.; Bauer, H.; Kasper-Giebl, A.; Puxbaum, H., Chemical characterization of fine particle emissions from wood stove combustion of common woods growing in mid-European Alpine regions. *Atmospheric Environment* **2008,** 42, (1), 126-141.

30.) Fraser, M. P.; Lakshmanan, K., Using Levoglucosan as a Molecular Marker for the Long-Range Transport of Biomass Combustion Aerosols. *Environmental Science and Technology* **2000,** 34, (21), 4560-4564.

31.) Simoneit, B. R. T., Biomass burning - a review of organic tracers for smoke from incomplete combustion. *Applied Geochemistry* **2002,** 17, (3), 129-162.

32.) Poore, M. W., Levoglucosan in PM2.5 at the Fresno supersite. *Journal of the Air & Waste Management Association* **2002,** 52, (1), 3-4.

33.) Larsen, R. K., III; Schantz, M. M.; Wise, S. A., Determination of levoglucosan in particulate matter reference materials. *Aerosol Science and Technology* **2006,** 40, (10), 781-787.

34.) Fine, P. M.; Cass, G. R.; Simoneit, B. R. T., Organic compounds in biomass smoke from residential wood combustion: Emissions characterization at a continental scale. *Journal of Geophysical Research, [Atmospheres]* **2002,** 107, (D21), ICC11/1-ICC11/9.

35.) Cain, C.; Carlin, J. *Libby Annual PM2.5 Standard Technical Support Document*; September 2007.

36.) Corin, N. S.; Backlund, P. H.; Kulovaara, M. A. M., Photolysis of the Resin Acid Dehydroabietic Acid in Water. *Environmental Science and Technology* **2000,** 34, (11), 2231-2236.

37.) Rigol, A.; Lacorte, S.; Barcelo, D., Sample handling and analytical protocols for analysis of resin acids in process waters and effluents from pulp and paper mills. *Trends in Analytical Chemistry* **2003,** 22, (10), 738-749.

38.) Peng, G.; Roberts, J. C., Solubility and toxicity of resin acids. *Water Research* **2000,** 34, (10), 2779-2785.

39.) Simoneit, B. R. T.; Rogge, W. F.; Mazurek, M. A.; Standley, L. J.; Hildemann, L. M.; Cass, G. R., Lignin pyrolysis products, lignans, and resin acids as specific tracers of plant classes in emissions from biomass combustion. *Environmental Science and Technology* **1993,** 27, (12), 2533-41.

40.) Hawthorne, S. B.; Miller, D. J.; Barkley, R. M.; Krieger, M. S., Identification of methoxylated phenols as candidate tracers for atmospheric wood smoke pollution. *Environmental Science and Technology* **1988,** 22, (10), 1191-6.

41.) Hawthorne, S. B.; Miller, D. J.; Langenfeld, J. J.; Krieger, M. S., PM-10 high-volume collection and quantitation of semi- and nonvolatile phenols, methoxylated phenols, alkanes, and polycyclic aromatic hydrocarbons from winter urban air and their relationship to wood smoke emissions. *Environmental Science and Technology* **1992,** 26, (11), 2251-62.

42.) Ward, T. J.; Hamilton, R. F.; Dixon, R. W.; Paulsen, M.; Simpson, C. D., Characterization and evaluation of smoke tracers in PM: results from the 2003 Montana wildfire season. *Atmospheric Environment* **2006,** 40, (36), 7005-7017.

43.) Schauer, J. J.; Cass, G. R., Source Apportionment of Wintertime Gas-Phase and Particle-Phase Air Pollutants Using Organic Compounds as Tracers. *Environmental Science and Technology* **2000,** 34, (9), 1821-1832.

44.) Dills, R. L.; Zhu, X.; Kalman, D. A., Measurement of Urinary Methoxyphenols and Their Use for Biological Monitoring of Wood Smoke Exposure. *Environmental Research* **2001,** 85, (2), 145-158.

45.) Bergauff, M. A.; Ward, T. J.; Noonan, C. W.; Palmer, C. P., The effect of a woodstove changeout on ambient levels of PM2.5 and chemical tracers for woodsmoke in Libby, Montana. *Atmospheric Environment* **2009,** 43, (18), 2938-2943.

46.) Western Regional Climate Center, Station Daily Time Series: Libby Montana. *http://www.wrcc.dri.edu/cgi-bin/rawMAIN.pl?mtMLIB,* Accessed **Nov 2004-June 2008**.

47.) United States Environmental Protection Agency, PM2.5 mass weighing laboratory standard operating procedures for the performance evaluation program. *Quality assurance guidance document*, Quality Assurance Guidance Document. Method Compendium.: **October 1998**.

48.) Jimenez, J.; Wu, C.-F.; Claiborn, C.; Gould, T.; Simpson, C. D.; Larson, T.; Liu, L. J. S., Agricultural burning smoke in eastern Washington - part I: Atmospheric characterization. *Atmospheric Environment* **2006,** 40, (4), 639-650.

49.) Leithead, A.; Li, S.-M.; Hoff, R.; Cheng, Y.; Brook, J., Levoglucosan and dehydroabietic acid: Evidence of biomass burning impact on aerosols in the Lower Fraser Valley. *Atmospheric Environment* **2006,** 40, (15), 2721-2734.

50.) Fine, P. M.; Cass, G. R.; Simoneit, B. R. T., *Environmental Engineering Science* **2004,** 21.

51.) Schauer, J. J.; Kleeman, M. J.; Cass, G. R.; Simoneit, B. R. T., Measurement of Emissions from Air Pollution Sources. 3. C1-C29 Organic Compounds from Fireplace Combustion of Wood. *Environmental Science and Technology* **2001,** 35, (9), 1716-1728.

52.) Hays, M. D.; Geron, C. D.; Linna, K. J.; Smith, N. D.; Schauer, J. J., Speciation of Gas-Phase and Fine Particle Emissions from Burning of Foliar Fuels. *Environmental Science and Technology* **2002,** 36, (11), 2281-2295.

53.) Ward, T. J.; Palmer, C. P.; Houck, J. E.; Navidi, W. C.; Geinitz, S.; Noonan, C. W., Community Woodstove Changeout and Impact on Ambient Concentrations of Polycyclic Aromatic Hydrocarbons. *Environmental Science & Technology* **2009,** 43, 5345-5350.

54.) Jordan, T. B.; Seen, A. J., Effect of Airflow Setting on the Organic Composition of Woodheater Emissions. *Environmental Science and Technology* **2005,** 39, (10), 3601-3610.

55.) Jenkins, P.; Phillips, T.; Mulberg, J.; Hui, S., Activity patterns of Californians: use and proximity to indoor pollutant sources. *Atmospheric Research* **1992,** 26A, 2141-2148.

56.) Fishbein, L.; Henry, C., Introduction: workshop on the methodology for assessing health risks from complex mixtures in indoor air. *Environmental Health Perspectives* **1991,** 85, 3-5.

57.) Fine, P. M.; Cass, G. R.; Simoneit, B. R. T., Chemical Characterization of Fine Particle Emissions from Fireplace Combustion of Woods Grown in the Northeastern United States. *Environmental Science and Technology* **2001,** 35, (13), 2665-2675.

58.) Purvis, C. R.; McCrillis, R. C.; Kariher, P. H., Fine Particulate Matter (PM) and Organic Speciation of Fireplace Emissions. *Environmental Science and Technology* **2000,** 34, (9), 1653-1658.

59.) Bates, M. N.; Khalakdina, A.; Pai, M.; Chang, L.; Lessa, F.; Smith, K. R., Risk of tuberculosis from exposure to tobacco smoke: a systematic review and meta-analysis. *Archives of Internal Medicine* **2007,** 167, (4), 335-343.

60.) Mishra, V.; Dai, X.; Smith, K. R.; Mika, L., Maternal exposure to biomass smoke and reduced birth weight in Zimbabwe. *Annuals of Epidemiology* **2004,** 14, (10), 740-747.

61.) Needham, L. L.; Calafat, A. M.; Barr, D. B., Uses and issues of biomonitoring. *International Journal of Hygiene and Environmental Health* **2007,** 210, (3-4), 229-238.

62.) Neitzel, R.; Naeher, L.; Paulsen, M.; Dunn, K.; Stock, A.; Simpson, C., Biological monitoring of smoke exposure among wildland firefighters: A pilot study comparing urinary methoxyphenols with personal exposures to carbon monoxide, particulate matter, and levoglucosan. *Journal of Exposure Science and Environmental Epidemiology* **2009,** 19, 349-358.

63.) Dills, R. L.; Paulsen, M.; Ahmad, J.; Kalman, D. A.; Elias, F. N.; Simpson, C. D., Evaluation of Urinary Methoxyphenols as Biomarkers of Woodsmoke Exposure. *Environmental Science & Technology* **2006,** 40, (7), 2163-2170.

64.) Lee, S.; Baumann, K.; Schauer James, J.; Sheesley Rebecca, J.; Naeher Luke, P.; Meinardi, S.; Blake Donald, R.; Edgerton Eric, S.; Russell Armistead, G.; Clements, M., Gaseous and particulate emissions from prescribed burning in Georgia.*Environmental science & technology* **2005,** 39, (23), 9049-56.

65.) Saint-Jalm, Y., Qualitative analysis of the hydroxyl fraction of cigarette smoke. *Annales du Tabac Sect. 1* **1981,** 18, 41-48.

66.) Schumacher, J. N.; Green, C. R.; Best, F. W.; Newell, M. P., Smoke composition. An extensive investigation of the water-soluble portion of cigarette smoke. *Journal of Agricultural and Food Chemistry* **1977,** 25, (2), 310-20.

67.) Migliaccio, C. T.; Bergauff, M. A.; Palmer, C. P.; Jessop, F.; Noonan, C. W.; Ward, T. J., Urinary Levoglucosan as a Biomarker of Wood Smoke Exposure: Observations in a Mouse Model and in Children. *Environmental Health Perspectives* **2009,** 117, (1), 74-79.

68.) Bergauff, M. A.; Ward, T. J.; Noonan, C. W.; Migliaccio, C. T.; Simpson, C. D.; Evanoski, A. R.; Palmer, C. P., Urinary Levoglucosan as a Biomarker for Wood Smoke: Results of Human Exposure Studies. *Journal of Exposure Science and Environmental Epidemiology* **2010,** in press.

69.) Ward, T. J.; Rinehart, L. R.; Lange, T., The 2003/2004 Libby, Montana PM2.5 source apportionment research study. *Aerosol Science and Technology* **2006,** 40, 166-177.

70.) Ward, T. J.; Noonan, C. W.; Hooper, K., Indoor PM2.5 Concentrations in Libby, Montana Schools. *Environmental Monitoring and Assessment* **2007,** 130, 1-3.

71.) Sakuma, H.; Ohsumi, T.; Sugawara, S., Particulate phase of cellulose cigarette smoke. *Agricultural and Biological Chemistry* **1980,** 44, 555-561.

72.) Heinrich-Ramm, R.; Wegner, R.; Garde, A. H.; Baur, X., Cotinine excretion (tobacco smoke biomarker) of smokers and non-smokers: comparison of GC/MS and RIA results. *International Journal of Hygiene and Environmental Health* **2002,** 205, (6), 493-9.

73.) Naeher, L. P.; Brauer, M.; Lipsett, M.; Zelikoff, J. T.; Simpson, C. D.; Koenig, J. Q.; Smith, K. R., Woodsmoke health effects: a review. *Inhalation Toxicology* **2007,** 19, (1), 67-106.

74.) Dorland, L.; Wadman, S. K.; Fabery de Jonge, H.; Ketting, D., 1,6-Anhydro-b-D-glucopyranose (b-glucosan), a constituent of human urine. *Clinica Chimica Acta* **1986,** 159, (1), 11-16.

75.) Ratsimba, V.; Fernandez, J. M. G.; Defaye, J.; Nigay, H.; Voilley, A., Qualitative and quantitative evaluation of mono- and disaccharides in D-fructose, D-glucose and sucrose caramels by gas-liquid chromatography-mass spectrometry Di-D-fructose dianhydrides as tracers of caramel authenticity. *Journal of Chromatography, A* **1999,** 844, (1-2), 283-293.

76.) Schkolnik, G.; Rudich, Y., Detection and quantification of levoglucosan in atmospheric aerosols: a review. *Analytical and Bioanalytical Chemistry* **2006,** 385, (1), 26-33.

77.) World Health Organization, Re-Evaulation of some organic chemicals, hydrazine, and hydrogen peroxide. International Agency for Research on Cancer. Vol. 71.

78.) Gorin, C. A.; Collett, J. L., Jr.; Herckes, P., Wood smoke contribution to winter aerosol in Fresno, CA. *Journal of the Air & Waste Management Association* **2006,** 56, (11), 1584-1590.

79.) Tetsuo, M.; Zhang, C.; Matsumoto, H.; Matsumoto, I., Gas chromatographic-mass spectrometric analysis of urinary sugar and sugar alcohols during pregnancy. *Journal of Chromatography, B: Biomedical Sciences and Applications* **1999,** 731, (1), 111-120.

80.) Nehls, G. J.; Akland, G. G., Procedures for handling aerometric data. *Journal of the Air Pollution Control Association* **1973,** 23, 180.

81.) Helsel, D. R., More than obvious: better methods for interpreting nondetect data. *Environmental Science and Technology* **2005,** 39, (20), 419A-423A.

82.) Hornung, R. W.; Reed, L. D., Estimation of average concentration in the presence of nondetectable values. *Applied Occupational and Environmental Hygiene* **1990,** 5, (1), 46-51.

83.) Kim, S.; Jaques, P. A.; Chang, M.; Froines, J. R.; Sioutas, C., Versatile aerosol concentration enrichment system (VACES) for simultaneous in vivo and in vitro evaluation of toxic effects of ultrafine, fine and course ambient particles Part I: Development and laboratory characterization. *Journal of Aerosol Science* **2001,** 32, (11), 1281-1297.

84.) Yanosky, J. D.; Williams, P. L.; MacIntosh, D. L., A comparison of two direct-reading aerosol monitors with the federal reference method for PM2.5 in indoor air. *Atmospheric Environment* **2002,** 36, 107-113.