Utilizing Biomarkers to Assess Prevalence and Trends of Substance Use During Pregnancy in Canada

by

Kaitlyn Elisabeth Delano

A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
Graduate Department of Pharmacology and Toxicology
University of Toronto

© Copyright by Kaitlyn Delano 2015
Utilizing Biomarkers to Assess Prevalence and Trends of Substance Use During Pregnancy in Canada

Kaitlyn Elisabeth Delano
Doctor of Philosophy
Department of Pharmacology and Toxicology
University of Toronto
2015

Abstract

Substance use during pregnancy is associated with numerous risks to both mother and fetus. Studies of the prevalence and trends of substance use during pregnancy have predominantly relied on maternal self-report, which is known to be unreliable and inaccurate. The emerging use of biomarkers has presented researchers and clinicians with the opportunity to assess this complex matter in a more objective and reliable manner. The overall objective of the research described in this thesis is to utilize biomarkers to assess prevalence rates and trends of substance use during pregnancy in Canada. This objective was addressed through three studies focusing on distinct populations. The first study focuses on a highly specific population of methadone-using pregnant women involved in social services, and found that rates of polydrug use (specifically opioid use) were similar to a negative control group, with approximately half of individuals continuing to use additional opioids during pregnancy. The second study highlights a routine urine drug screen program within the obstetric unit of a regional hospital; we conclude that the prevalence of substance use in this population is close to three times the national average, and that drug use during pregnancy is associated with more maternal and neonatal complications. Lastly, the third study aims to estimate the prevalence of heavy fetal alcohol exposure through the analysis of fatty acid ethyl esters in meconium samples collected nationwide, and reveals that the prevalence ranges between 1.16
and 2.40%, the equivalent of at least 1,800 new cases of Fetal Alcohol Spectrum Disorder in Canada each year. Overall, this research provides new insights into the prevalence and trends of substance use during pregnancy, and aims to guide future public education initiatives and highlight the continuing need for more effective, accessible treatment options for pregnant women who struggle with substance use.
Acknowledgments

It is with my deepest and sincerest gratitude that I would like to thank the following individuals for their guidance and support over the past four years of my graduate studies:

Firstly, to my supervisor, Dr. Gideon Koren, for your mentorship, guidance and support and for the countless opportunities I have been blessed with during my time with the Motherisk Department.

To my committee members, Dr. Bhushan Kapur and Dr. Martin Zack, for the constructive criticism and ensuring that I constantly strive for the best. I would specifically like to thank Dr. Kapur for the continued guidance, analytical expertise and unwavering support you have given me throughout my studies.

To Dr. Shinya Ito for being an outstanding example of distinguished professionalism in any circumstance and for providing guidance and support, especially throughout my final year at Motherisk.

To the entire Motherisk Department who has welcomed me into such an amazingly supportive, close-knit community with open arms. To Dr. Joey Gareri, for seeing my potential and allowing me to fulfill a leadership role within the laboratory. To Dr. Katarina Aleksa, for always being the voice of the graduate students and helping with any issues we encountered along our studies. To Dr. Prateek Lala, for your friendship and support for the entire graduate student unit and consistently going above and beyond your responsibilities.

To the Motherisk Laboratory, for sticking together no matter the circumstances. Specific thanks go out to Paula Walasek, Chitra Rao and Maggie Lee for your amazing expertise, help you have given me in the laboratory, and always being there to change a broken fibre.

To my collaborators at the MIREC Study Group and Miramichi Regional Hospital for allowing me to work with such upstanding and hard working individuals so that we could meet our common goals.

To my fellow graduate students, both past and present, for being there to lend a helping hand, an open ear and a critical eye. You were always the ones to get me through the rough times and help celebrate the good times.
To my friends, both near and far, for your love and support through thick and thin. You all make me strive to be a better person and friend and for that I am indebted. To ADG, your words travel with me wherever I go, have struck a chord in both my mind and soul, and allow me to forget anything and remember everything.

To my Uncle Randy and Grandmama, I will never forget you or the inspiration you have given me to go down this path. I truly hope I make you proud each and every day.

Lastly, to my parents, words really can’t describe how much you both mean to me and how grateful I am to call you Mom and Dad. The unconditional love and support you have shown me never goes unnoticed or unappreciated. I love you both from the bottom of heart.
Table of Contents

ACKNOWLEDGMENTS ....................................................................................... IV

TABLE OF CONTENTS ................................................................................... VI

LIST OF TABLES ........................................................................................... IX

LIST OF FIGURES .......................................................................................... X

LIST OF APPENDICES .................................................................................. XI

LIST OF ABBREVIATIONS .............................................................................. XII

CHAPTER 1 INTRODUCTION .......................................................................... 1

1  SUBSTANCE USE ......................................................................................... 1
  1.1  SUBSTANCE USE BY WOMEN ............................................................. 1
  1.2  SUBSTANCE USE DURING PREGNANCY ......................................... 2
  1.3  RISK FACTORS OF SUBSTANCE USE DURING PREGNANCY .......... 3
  1.4  TREATMENT OF SUBSTANCE USE DURING PREGNANCY ............ 7
  1.5  SUMMARY ............................................................................................ 9

2  ADVERSE OUTCOMES OF SUBSTANCE USE DURING PREGNANCY ........ 10
  2.1  DRUGS .................................................................................................. 10
      2.1.1  Amphetamines ............................................................................ 10
      2.1.2  Benzodiazepines ........................................................................ 11
      2.1.3  Cannabinoids ............................................................................. 12
      2.1.4  Cocaine ....................................................................................... 13
      2.1.5  Opioids ....................................................................................... 15
  2.2  ALCOHOL ............................................................................................. 16
      2.2.1  Fetal Alcohol Spectrum Disorder ............................................. 17
  2.3  SUMMARY ............................................................................................ 22

3  PREVIOUS STUDIES INVESTIGATING PREVALENCE AND TRENDS OF SUBSTANCE USE DURING PREGNANCY ......................................................... 22
  3.1  DRUGS .................................................................................................. 22
  3.2  ALCOHOL ............................................................................................. 23
  3.3  SUMMARY ............................................................................................ 25

4  BIOMARKERS OF SUBSTANCES OF ABUSE ........................................... 25
  4.1  DRUGS .................................................................................................. 26
      4.1.1  Amphetamines ............................................................................ 26
      4.1.2  Cannabinoids ............................................................................. 27
      4.1.3  Cocaine ....................................................................................... 27
      4.1.4  Opioids ....................................................................................... 28
CHAPTER 2 THESIS SCOPE .............................................................................................................. 37
1  OVERALL AIM ............................................................................................................................... 37
2  OBJECTIVES AND HYPOTHESES ............................................................................................. 38

CHAPTER 3 METHODS ..................................................................................................................... 41
1  RATES OF FETAL POLYDRUG EXPOSURES IN METHADONE-MAINTAINED PREGNANCIES FROM A HIGH RISK POPULATION ................................................................. 41
   1.1  SAMPLE COLLECTION AND ANALYSIS ................................................................................. 41
   1.2  DATA ANALYSIS ..................................................................................................................... 43
2  PREVALENCE OF DRUG USE DURING PREGNANCY IN MIRAMICHI, NB: ANALYSIS OF A ROUTINE URINE DRUG SCREEN IN THE OBSTETRIC UNIT ................................................................. 44
   2.1  DATA COLLECTION .................................................................................................................. 44
   2.2  DATA ANALYSIS ..................................................................................................................... 45
3  PREVALENCE OF HEAVY FETAL ALCOHOL EXPOSURE IN CANADA: A MULTI-CENTER MECONIUM STUDY .................................................................................................................. 46
   3.1  SAMPLE AND DATA COLLECTION ......................................................................................... 46
   3.2  SAMPLE ANALYSIS .................................................................................................................. 49
   3.3  DATA ANALYSIS ..................................................................................................................... 51

CHAPTER 4 RESULTS ........................................................................................................................ 53
1  RATES OF FETAL POLYDRUG EXPOSURES IN METHADONE-MAINTAINED PREGNANCIES FROM A HIGH RISK POPULATION ...................................................................................... 53
2  PREVALENCE OF DRUG USE DURING PREGNANCY IN MIRAMICHI, NB: ANALYSIS OF A ROUTINE URINE DRUG SCREEN IN THE OBSTETRIC UNIT ...................................................................................... 55
3  PREVALENCE OF HEAVY FETAL ALCOHOL EXPOSURE IN CANADA: A MULTI-CENTER MECONIUM STUDY .................................................................................................................. 60

CHAPTER 5 DISCUSSION .................................................................................................................... 70
1  SUMMARY AND SIGNIFICANCE OF RESEARCH FINDINGS .......................................................... 70
   1.1  RATES OF FETAL POLYDRUG EXPOSURES IN METHADONE-MAINTAINED PREGNANCIES FROM A HIGH RISK POPULATION ...................................................................................... 70
1.2 Prevalence of drug use during pregnancy in Miramichi, NB: Analysis of a routine urine drug screen in the obstetric unit ................................................................. 73
1.3 Prevalence of heavy fetal alcohol exposure in Canada: A multi-center meconium study ....................................................................................................................... 75
1.4 Biomarkers as an indirect marker of the child’s environment ....................... 78

2 STRENGTHS, LIMITATIONS AND FUTURE DIRECTIONS ........................................ 82

3 CONCLUSIONS ............................................................................................................. 84

REFERENCES .................................................................................................................. 85

APPENDICES .................................................................................................................. 105

COPYRIGHT ACKNOWLEDGEMENTS ........................................................................... 114
List of Tables

Table 1.1. Institute of Medicine Diagnostic Guidelines for Fetal Alcohol Spectrum Disorder.

Table 1.2. Select studies utilizing FAEE measurement in meconium to assess the prevalence of heavy fetal alcohol exposure.

Table 1.3. Window of detection of common substances of abuse in urine.

Table 3.1. Quantification and qualifier ions for all analytes and their respective internal standard for the analysis of drugs of abuse by GC-MS.

Table 3.2. Qualifier and quantifier ions for the 4 FAEEs analyzed in meconium by GC-MS.

Table 3.3. Limits of detection and quantification for the 4 FAEEs analyzed in meconium by GC-MS.

Table 4.1. Rates of positivity for six drug classes.

Table 4.2. Rates of positivity for individual compounds.

Table 4.3. Rate of positivity for overall and specific drug use.

Table 4.4. Significant maternal characteristics and neonatal outcomes through Chi Square and Fisher Exact Test analyses.

Table 4.5. Significant maternal characteristics and neonatal outcomes through Mann Whitney U test analyses.

Table 4.6. Logistic regression of predictors of maternal substance use during pregnancy. Cox & Snell R square = 0.301.

Table 4.7. FAEE meconium analysis results and overall prevalence of heavy fetal alcohol exposure in Canada.

Table 4.8. FAEE meconium analysis results and maternal alcohol consumption self-report data.

Table 4.9. Neonatal birth outcomes for neonates with or without positive FAEE meconium results.
List of Figures

Figure 3.1. MIREC questionnaire and biospecimen collection details.

Figure 4.1. Rate of overall drug use between 2006 and 2012.

Figure 4.2. Median FAEE concentration (nmol/g) of women who reported any or no alcohol consumption in the 32-35 Week Gestation questionnaire.

Figure 4.3. Comparison of self-report of any alcohol consumption in past three months at both Baseline and 32-35 Week Gestation questionnaires for individuals with positive and negative FAEE meconium analyses.

Figure 4.4. Correlation between self-reported ethanol consumption (g/week) and FAEE meconium analysis result.

Figure 4.5. Changes in median ethanol consumption (g/week) in MIREC questionnaires.

Figure 4.6. Self-report of current smoking in baseline and 32-35 week gestation questionnaires.

Figure 4.7. Education level and annual household income of the MIREC Study population.
List of Appendices

Appendix A. Research Ethics Board Approval

Appendix B. Data Collection Form for Miramichi Regional Hospital

Appendix C. Questionnaire data collected from MIREC Study

Appendix D. GC-MS Parameters of FAEE Detection in Meconium Method
List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-OH-THC</td>
<td>11-hydroxy-Δ⁹-tetrahydrocannabinol</td>
</tr>
<tr>
<td>6-MAM</td>
<td>6-monoacetylmorphine</td>
</tr>
<tr>
<td>ADD</td>
<td>Attention deficit disorder</td>
</tr>
<tr>
<td>ADHD</td>
<td>Attention deficit hyperactivity disorder</td>
</tr>
<tr>
<td>ARBD</td>
<td>Alcohol related birth defects</td>
</tr>
<tr>
<td>ARND</td>
<td>Alcohol related neurodevelopmental disorder</td>
</tr>
<tr>
<td>BAC</td>
<td>Blood alcohol concentration</td>
</tr>
<tr>
<td>BSTFA</td>
<td>N,O-Bistrifluoroacetamide</td>
</tr>
<tr>
<td>CEDIA</td>
<td>Cloned enzyme donor immunoassay</td>
</tr>
<tr>
<td>CYP</td>
<td>Cytochrome P450</td>
</tr>
<tr>
<td>d5</td>
<td>Deuterated internal standard</td>
</tr>
<tr>
<td>EDDP</td>
<td>2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme linked immunosorbent assay</td>
</tr>
<tr>
<td>EMDP</td>
<td>2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline</td>
</tr>
<tr>
<td>EtG</td>
<td>Ethyl glucuronide</td>
</tr>
<tr>
<td>EtS</td>
<td>Ethyl sulfate</td>
</tr>
<tr>
<td>FAEE</td>
<td>Fatty acid ethyl ester</td>
</tr>
<tr>
<td>FAS</td>
<td>Fetal alcohol syndrome</td>
</tr>
<tr>
<td>FASD</td>
<td>Fetal alcohol spectrum disorder</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Gas chromatography-mass spectrometry</td>
</tr>
<tr>
<td>hCE-1</td>
<td>Hepatic carboxylesterase-1</td>
</tr>
<tr>
<td>hCE-2</td>
<td>Hepatic carboxylesterase-2</td>
</tr>
<tr>
<td>HSC</td>
<td>The Hospital for Sick Children</td>
</tr>
<tr>
<td>IDEAL</td>
<td>Infant Development, Environment, and Lifestyle Study</td>
</tr>
<tr>
<td>IUGR</td>
<td>Intra-uterine growth restriction</td>
</tr>
<tr>
<td>LGA</td>
<td>Large for gestational age</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>LOQ</td>
<td>Limit of quantification</td>
</tr>
<tr>
<td>MDMA</td>
<td>3,4-methylenedioxy-methamphetamine</td>
</tr>
<tr>
<td>MIREC</td>
<td>Maternal-Infant Research on Environmental Chemicals</td>
</tr>
<tr>
<td>MMT</td>
<td>Methadone maintenance treatment</td>
</tr>
<tr>
<td>MRH</td>
<td>Miramichi Regional Hospital</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>Acronym</td>
<td>Definition</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------------------------------------------------------------</td>
</tr>
<tr>
<td>MSTFA</td>
<td>N-methyl-n-(trimethylsilyl)trifluoroacetamide</td>
</tr>
<tr>
<td>NAS</td>
<td>Neonatal abstinence syndrome</td>
</tr>
<tr>
<td>NICU</td>
<td>Neonatal intensive care unit</td>
</tr>
<tr>
<td>NSAID</td>
<td>Non-steroidal anti-inflammatory drug</td>
</tr>
<tr>
<td>NSQ</td>
<td>Non-sufficient quantity</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffer solution</td>
</tr>
<tr>
<td>PCP</td>
<td>Phencyclidine</td>
</tr>
<tr>
<td>pFAS</td>
<td>Partial fetal alcohol syndrome</td>
</tr>
<tr>
<td>pOHMAMP</td>
<td>p-hydroxymethamphetamine</td>
</tr>
<tr>
<td>QC</td>
<td>Quality control</td>
</tr>
<tr>
<td>SES</td>
<td>Socioeconomic status</td>
</tr>
<tr>
<td>SGA</td>
<td>Small for gestational age</td>
</tr>
<tr>
<td>SIDS</td>
<td>Sudden infant death syndrome</td>
</tr>
<tr>
<td>SPE</td>
<td>Solid phase extraction</td>
</tr>
<tr>
<td>SPME</td>
<td>Solid phase microextraction</td>
</tr>
<tr>
<td>THC</td>
<td>$\Delta^9$-tetrahydrocannabinol</td>
</tr>
<tr>
<td>THC-COOH</td>
<td>11-nor-$\Delta^9$-tetrahydrocannabinol-9-carboxylic acid</td>
</tr>
<tr>
<td>UDS</td>
<td>Urine drug screening</td>
</tr>
<tr>
<td>UGT</td>
<td>Uridine 5'-diphospho-glucuronosyltransferase</td>
</tr>
</tbody>
</table>
Chapter 1
Introduction

1 Substance Use

1.1 Substance use by women

Overall, women report lower rates of substance use when compared to men. However, women report higher rates of prescription drug misuse than men, which include pain relievers, antidepressants, sleeping pills and opioid analgesics. (Wong et al., 2011) In contrast, men report higher rates of alcohol consumption in general, as well as chronically exceeding low-risk drinking guidelines in Canada. (Health Canada, 2013)

In Canada, 7% of females reported using cannabis in the past year and 35.5% reporting any use during their lifetime. (Health Canada, 2013) In terms of other illicit drugs, including cocaine, amphetamines (“speed”), 3,4-methylenedioxy-methamphetamine (MDMA) (“ecstasy”), hallucinogens, and heroin, 1% and 11.4% of females reported using in the past year and in their lifetimes, respectively. (Health Canada, 2013) A small proportion, 1.4%, reported abuse of a pharmaceutical agent, which includes sedatives, stimulants and pain relievers. (Health Canada, 2013)

Similar rates of illicit drug use by women have been reported in other countries. In the United Kingdom, approximately 8% of women 16–59 years of age had used any illicit drug within the past year between 2004 and 2005. (Crome and Kumar, 2007) Higher rates, between 20 and 30%, have been found in younger women (12–25 years) in the United States. (Crome and Kumar, 2007) The incidence of marijuana abuse in the United States increased by 35% in reproductive aged women between 1998 and 2003. (Cox et al., 2007; Mark et al., 2015)

Abstention from alcohol is reported at higher rates in women when compared to men. (Crome and Kumar, 2007) However, alcohol consumption, especially ingestion beyond safe limits, in
young women has been found to be increasing. (Crome and Kumar, 2007) In Canada, 89.3% of women report using alcohol during their lifetime, with 74.4% reporting past year use and 57.2% reporting past 30-day use. (Health Canada, 2013) Among female alcohol users, 15.9% report chronically exceeding low-risk drinking guidelines of 10 drinks per week, with no more than 2 drinks each day. (P Butt et al., 2011; Health Canada, 2013) Alcohol dependence in women, worldwide, varies between 0 and 5.7%. (Crome and Kumar, 2007)

1.2 Substance use during pregnancy

Lower rates of substance use are seen when comparing pregnant and non-pregnant women. (Substance Abuse and Mental Health Services Administration, 2009; Substance Abuse and Mental Health Services Administration, 2011) The highest rate of substance use occurs during the first trimester and then decreases throughout the pregnancy. (Ko et al., 2015) Specifically looking at alcohol consumption, the pattern of use during pregnancy has been found to become less frequent after pregnancy recognition with a modest increase throughout the third trimester. (Murphy et al., 2014; Lundsberg et al., 2015)

The prevalence of substance use during pregnancy varies depending on the study population, survey methodology, and definitions of substance use utilized in a particular study. The 2006–2007 Maternity Experience Survey reported that 10.5% of women aged 15 years and older who had a pregnancy prior to the 2006 Canadian Census had consumed alcohol during that pregnancy, and 1% had used illicit drugs. (Public Health Agency of Canada, 2009) Through the 2008 Canadian Perinatal Health Report, it was found that 11% of pregnant women consumed alcohol, while 5% reported illicit drug use in the previous month. (Public Health Agency of Canada, 2008) Through the American National Pregnancy Health Survey, 5.5% of pregnant women reported using any illicit drug during their pregnancy, with similar rates reported through birth certificate reports in the U.S., and the National Drug Strategy Household Survey in
Overall, in the United States, substance use by pregnant women has increased over the past 30 years. (Keegan et al., 2010)

Worldwide, approximately 30% of pregnant women consume alcohol during pregnancy. (Vall et al., 2015) The majority of these women spontaneously stop consuming alcohol during their pregnancy, typically upon pregnancy recognition. (Crome and Kumar, 2007) In Canada, 14% of women reported using alcohol during their last pregnancy and 5.8% report consuming alcohol during their current pregnancy. (Wong et al., 2011) (Thanh and Jonsson, 2010) In the United States, approximately 1 in 8 women report consuming alcohol during pregnancy and 2% report binge drinking which is defined as the consumption of 5 or more drinks in a single occasion. (Heller and Burd, 2014) In terms of binge drinking pattern and frequency during pregnancy, pregnant women had similar reports as non-pregnant women, averaging 3 binge episodes of 6 drinks per month. (Centers for Disease Control and Prevention, 2012)

Polydrug use is a common practice for drug users. (Passey et al., 2014) Kreitinger et al. (2015) found that 91.4% of individuals used two or more substances in addition to their opioid maintenance treatment, with heroin, non-maintenance opioids and marijuana being the most commonly used substances. They further report that alcohol use ranged between 5% and 28% in pregnant women in substance abuse treatment facilities. (Kreitinger et al., 2015)

Pattern and intensity of substance use during pregnancy can differ between alcohol and drugs. In a study by Harrison and Sidebottom (2009), pregnant users reported their frequency of drug use as weekly or daily, while reporting alcohol use as monthly or rarely, demonstrating the inherent difficulty of drug use cessation. (Harrison and Sidebottom, 2009)

1.3 Risk factors of substance use during pregnancy

A variety of environmental factors and maternal characteristics can be involved in determining if a woman will use substances during pregnancy.
Education level has been found to be a major factor in the risk of substance use during pregnancy. The majority of studies have found that lower education levels are associated with substance use during pregnancy. (Beijers et al., 2014; Mark et al., 2015) Individuals with lower education tend to have higher rates of unemployment and using substances during pregnancy is also associated with employment status. (Mark et al., 2015) Less education may also be associated with multi-drug use; in one study, individuals with less than a grade 10 education were found to use two or more substances concurrently. (Passey et al., 2014) However, some studies have found that individuals with a higher income level are at a higher risk of using alcohol during pregnancy. (Kitsantas et al., 2014) Women of higher socioeconomic status (SES), specifically, have been found to increase their alcohol intake, and had more frequent low-level intake during late pregnancy. (Pfinder et al., 2014) This is thought to occur due to alcohol intake being a more integral part of high SES women’s lifestyles. (Bloomfield et al., 2006) Women of high SES are most likely to miss being identified by physicians for alcohol consumption during pregnancy, as this is not a commonly known risk factor. (Sarkar et al., 2009)

Smoking is another major predictor for the continuation of substance use during pregnancy. (Kennare et al., 2005; Mark et al., 2015; Ko et al., 2015) In a recent Canadian study, it was found that women who smoked daily or occasionally during pregnancy were 2.54 and 2.71 more likely, respectively, to consume alcohol during their pregnancy. A previous history of smoking was also found to be associated with a two-fold increase in consuming alcohol during pregnancy. (Lange et al., 2015) Nonsmokers are more likely to abstain from alcohol consumption during pregnancy than smokers. (Perreira and Cortes, 2006) If women are heavy smokers prior to their pregnancy, they are at a higher risk to continue their concurrent alcohol use and smoking throughout their pregnancy, and are less likely to reduce their binge drinking. (Ebrahim et al., 1998; Powers and Oltmanns, 2012) Risk factors for concurrent use of tobacco
and substances include low educational level, single status, having previous pregnancies, and having high psychological stress early in the first trimester. (Beijers et al., 2014)

The individual’s substance use prior to pregnancy can also predict her use during pregnancy. A large proportion of women who drink in early pregnancy, after recognition, continue to drink late in pregnancy. (Pfinder et al., 2014) Women who continued to drink throughout their entire pregnancies or during the third trimester were more likely to have histories of illicit drug use in a prospective cohort study conducted in Ireland. (Murphy et al., 2014) The inverse relationship has also been studied, with a history of illicit drug use, along with partner violence, being associated with alcohol use, even at a low level (between 1 and 10 drinks) during pregnancy. (Flynn and Chermack, 2008) Higher levels of substance use prior to pregnancy increase the risk of continuation of use during the current pregnancy, and this has also been found with respect to tobacco use. (Harrison and Sidebottom, 2009) In terms of intensity of use, individuals who binge drink and/or heavy drink on a weekly basis are also more likely to continue drinking during pregnancy. These individuals are also less likely to reduce their binge drinking than their weekly drinking habits during their pregnancy. (Anderson et al., 2014) Some risk factors associated specifically with binge drinking during pregnancy include low education level, nulliparity, smoking and binge drinking in year prior, and not actively trying to become pregnant. (McDonald et al., 2014) Previous patterns of substance use can also influence use during a current pregnancy. Women who consumed alcohol during a previous pregnancy are likely to repeat this behaviour in subsequent pregnancies. (Burd and Hofer, 2008; Paintner et al., 2012; Burd, 2014)

Stress events during pregnancy and the severity of these events have been found to be associated with alcohol consumption, and likely drug use, during pregnancy. The sources of stress can include conflict with loved ones, crime-related and pregnancy-specific stress. Women who abuse substances during pregnancy are more likely to experience (or have
experienced) domestic violence. (Hans, 1999) The risk of substance use during pregnancy following domestic violence may be disproportionally present in lower income women, speaking to the complex relationship between these risk factors. (Alhusen et al., 2013) History of abuse in general, including emotional, sexual and physical, is associated with higher rates of substance use during pregnancy as well as high prevalence of posttraumatic stress disorder diagnoses. (Harrison and Sidebottom, 2009; Wong et al., 2011; Mark et al., 2015) Substance use serves as a form of self-medication and a coping mechanism for these individuals, which can significantly complicate substance abuse treatment. (O’Brien, 2012; Beijers et al., 2014)

Stress is not the sole mental health factor that can affect substance use during pregnancy: anxiety and depression have also been found to be related to substance use in pregnancy. (Kitsantas et al., 2014; Mark et al., 2015) Women who had depression or clinical depressive symptoms were twice as likely to have a substance use disorder as non-depressed controls. (Forray et al., 2014) Depression scores, measured by the Edinburgh Postnatal Depression Scale, are positively associated with drug and/or alcohol use during pregnancy. (Holden et al., 2012) Individuals with low dispositional optimism or a psychologically uncomfortable pregnancy are at higher risk of binge drinking during pregnancy. (O’Brien, 2012; McDonald et al., 2014) Kashiwagi et al. report that 88% of opiate-dependent pregnant women were under psychological support for diagnoses including depression and bulimia, while 22% of healthy controls were also receiving this type of support. (Kashiwagi et al., 2009) Depression, substance use during pregnancy, and intimate partner violence have been found to be interconnected, with women who abuse substances during pregnancy having higher rates of depression and intimate partner violence. (Holden et al., 2012)

Family history of alcohol consumption can also affect substance use during pregnancy. Alati et al. found that drinking levels increased between mother and daughter dyads, and daughters were more likely to drink at higher levels than mothers. (Alati et al., 2014) While not related
directly to alcohol consumption during pregnancy, there is an increased risk of alcohol consumption and early initiation in 14-year-olds if their mothers consumed alcohol before, during, and after pregnancy. (Alati et al., 2008) As well, children exposed to alcohol prenatally have a three-fold higher risk of becoming alcohol-dependent by the age of 21 relative to unexposed children. (Baer et al., 2003) Women who abuse alcohol are more likely to have a partner who also abuses alcohol, which may exacerbate other previously discussed factors such as stressful and abusive environments. (Vanyukov et al., 1996; Dierker et al., 1999)

Intention of pregnancy may also determine if a woman continues to use substances during pregnancy. Women with unwanted pregnancies are less able to modify harmful behaviours once pregnancy recognition occurs. (Altfeld et al., 1997; Cheng et al., 2009; Chisolm et al., 2014) These women have an increased risk of binge drinking during pregnancy when compared to women with intended or mistimed pregnancies. Subsequent unintended pregnancies were found to increase this risk even further. (Terplan et al., 2014)

No singular risk factor can be attributed to all cases of women using substances during pregnancy. Rather the interplay between multiple factors, the genetic makeup of a woman as well as her environment, determines whether she will continue to use substances during pregnancy. These risk factors are meant to help guide clinicians in identifying at-risk women so that a safe and open dialogue can be created between patient and physician.

1.4 Treatment of substance use during pregnancy

Typically, individuals who are younger, have higher levels of education and income, are nonsmokers, and use smaller amounts of substances are more likely to cease using substances during their pregnancy. (Harrison and Sidebottom, 2009) However, women typically engage in substance use treatment less frequently than men, with less than 20% of women who need treatment actually receiving it in a given year. (Greenfield et al., 2007; Terplan et al., 2012) Low rates of treatment engagement by women may be due to several factors such as
social stigma, lack of childcare, and lack of finances. (Greenfield et al., 2007) Once in
treatment, however, women and men have similar rates of response, so lack of access is
clearly a major limiting factor in successful treatment for women with substance use issues.
(McHugh et al., 2014)

Methadone, a long acting µ-opioid agonist, is currently the first line of treatment for opioid
dependence for both pregnant and non-pregnant individuals. (Mactier, 2013) Due to the long
half-life of methadone (mean of 22 hours, but can range from 5-130 hours), methadone
maintenance treatment (MMT) provides more stable blood concentrations relative to most
forms of illicit opiate use, and this results in decreased withdrawal symptoms. (Eap et al., 2002;
Minozzi et al., 2013) Decreased withdrawal symptoms can aid in reducing illicit use of other
opioids and facilitate addiction treatment interventions. (Delano et al., 2013) Pregnant women
in MMT programs have been found to receive better prenatal care, have better social
stabilization, and are less likely to relapse during pregnancy, when compared with drug-using
women not enrolled in an MMT program. (Kandall et al., 1977; Binder and Vavrinková, 2008) In
a large prospective study, methadone was found to be potentially effective in treating abuse of
other substances, e.g., cocaine and marijuana; however, this is not the primary indication for
MMT. (Fairbank et al., 1993) Buprenorphine, a partial µ-opioid agonist, is an alternate
prescription drug for the treatment of opioid dependency. The use of buprenorphine during
pregnancy has been found to decrease the length of hospital stay when compared to
methadone treatment. (Jones et al., 2005) No relationship has been found between maternal
methadone or buprenorphine dose and the subsequent intensity of NAS or the number of
infants requiring treatment for NAS after birth. (Kacinko et al., 2008; Cleary et al., 2010)
Overall, the continued exposure to methadone or buprenorphine treatment during pregnancy
carries lower risk than continued illicit opioid use, and is associated with better prenatal care
and outcomes for mother and baby.
Treatment interventions for illicit drugs other than opioids are very limited and potentially viable options do not have significant data available regarding their effectiveness. (Wong et al., 2011) Cocaine withdrawal symptoms can be managed with benzodiazepines, but no pharmacological treatment for dependence has been found to be effective. (Day and George, 2005) Similarly, benzodiazepine dependence is only managed through low dose maintenance or gradual reduction to minimize withdrawal symptoms, with no treatment available for the dependence itself. (Day and George, 2005) For alcohol withdrawal during pregnancy, thiamine and diazepam are commonly used, along with folic acid supplementation at times. For withdrawal occurring during labour, lorazepam is most commonly used, while also monitoring hydration and electrolyte levels. (Wong et al., 2011)

“Therapeutic communities” or comprehensive treatment centers have been found to be beneficial for substance using women, as these act as ‘one-stop’ centres including addiction treatment, child care, parenting education, medical services, life skills development, counseling and employment services. These centers break down many barriers, including lack of transportation and economic burdens, which may otherwise prevent women from initiating and continuing substance use treatment. (Niccols et al., 2012) Women engaged with these treatment centers have reported decreases in their substance use, improvements in their health and better access to community resources. (Ordean and Kahan, 2011)

1.5 Summary

In general, pregnant women have lower rates of substance use than non-pregnant women with use during pregnancy being the highest prior to pregnancy recognition. Women who are of low SES, smoke, have a history of abuse, used substances prior to their pregnancy and have a family history of substances use are at the highest risk of continuing to use substances during pregnancy. Pharmacological treatment options are available to individuals with opioid dependency with treatments for other illicit drugs being very limited. Treatment centres are a
promising treatment approach and combine both pharmacological and non-pharmacological interventions.

2 Adverse Outcomes of Substance Use During Pregnancy

2.1 Drugs

2.1.1 Amphetamines

No consistent teratological effects have been found following amphetamine exposure in utero. (Wright et al., 2015) However, amphetamine use during pregnancy is associated with other complications during pregnancy such as maternal hypertension, placental abruption, anemia, preterm delivery, and meconium-stained amniotic fluid. (Stewart and Meeker, 1997; Phupong and Darojn, 2007) Maternal hypertension can result from the effects of amphetamines on heart rate and vascular constriction, and in turn can cause placental abruption and preterm delivery. (Stewart and Meeker, 1997) Continuous methamphetamine use during pregnancy is associated with a 3.5-fold increase in preterm delivery. (Wright et al., 2015) In addition, elevated risks of preeclampsia and fetal demise have been associated with methamphetamine use during pregnancy. (Gorman et al., 2014)

In terms of neonatal outcomes, higher rates of small for gestational age (SGA, defined as birth weight below the 10th percentile for gestational age) births have been found in amphetamine-exposed pregnancies, as well as smaller birth measurements in general, but these findings may also be secondary to anemia or poor nutrition. (Phupong and Darojn, 2007; Liles et al., 2012; Gorman et al., 2014) Through the large, prospective Infant Development, Environment, and Lifestyle (IDEAL) study, similar findings of higher rates of SGA were detected as well as decreased birth length. (Nguyen et al., 2010; Zabaneh et al., 2012) This study also found that neonates exposed to methamphetamine displayed poor quality of movement, low arousal and signs of increased stress during the neonatal period. (Smith et al., 2008) Singer et al. found that 4-month-old infants exposed to MDMA showed poorer motor quality and that a dose-
response relationship was present with higher maternal MDMA use. (Singer et al., 2012) Case reports have reported congenital abnormalities, including heart defects, cataracts, and cleft palate, in infants exposed to amphetamines during the first trimester. (McElhatton et al., 1999; Clarke et al., 2009; Keegan et al., 2010) However, as previously stated, no consistent or characteristic malformations have been associated with maternal use of amphetamines during pregnancy.

All of the amphetamine compounds have been associated with similar impacts on the behaviour and cognition of exposed children including lower IQ scores, difficulties with advancement in school, and physical fitness activities. (Lester and Lagasse, 2010) The IDEAL study detected differences in externalizing behaviour and attention-deficit/hyperactivity order (ADHD) issues at 5 years of age. When comparing the children at 3 and 5 years, the 5-year-olds showed less aggressive behaviour overall, but scored higher on internalizing, anxiety, depression and withdrawn scales than the 3-year-olds. (LaGasse et al., 2012) Also through the IDEAL study, it was discovered that children exposed to methamphetamine were more likely to score higher on the cognitive problems scale portion of the Conners’ Parent Rating Scales – Revised: Short Form at 7.5 years. (Diaz et al., 2014) This subscale focuses on learning problems (e.g., problems organizing work and speed of learning) and inattention. (Conners et al., 1998) By age 4 years, amphetamine-exposed children had lower IQ scores than controls, and by 14 years of age, decreased school performance was noted, especially in math, language, and physical fitness subject areas. (Cernerud et al., 1996)

2.1.2 Benzodiazepines

There is conflicting evidence regarding the teratogenicity of benzodiazepine use in pregnancy. Most studies conclude that benzodiazepines are non-teratogenic, or have low teratogenic potential, but some cases of high-dose use have been associated with cleft lip and cleft palate. (Day and George, 2005; Bellantuono et al., 2013)
“Floppy infant syndrome” has been documented in cases of benzodiazepine exposure, specifically with high doses. This syndrome occurs in the context of high doses of benzodiazepines with long half-lives (e.g., nitrazepam and diazepam) and poor metabolism by neonates, leading to bioaccumulation. Symptoms of floppy infant syndrome include low muscle tone, muscle weakness, and lethargy; these symptoms develop immediately after birth and persist for hours to days. (Kieviet et al., 2013)

Limited research has been conducted on the neurobehavioural effects of benzodiazepine exposure in utero, especially when compared to other substances of abuse. The majority of findings are through animal studies, where exposure to benzodiazepines resulted in mild and reversible anomalies in neurobehavioural development, including the reduction of locomotor activity and an increase in maternal aggression. (Bignami et al., 1992) Inconsistent findings in human studies have been reported, with some reports finding delays in locomotor and social behaviour at 10 and 18 months of age, but these results were not reproducible in a U.S. cohort. (Mantovani and Calamandrei, 2001) Retrospective studies focusing on preschool-aged children found no developmental delays following benzodiazepine exposure, even with high-dose exposures. (Gentile, 2010)

2.1.3 Cannabinoids

Marijuana use during pregnancy is associated with preterm labour, low birth weight, stillbirth and NICU admissions. (Ko et al., 2015; Metz and Stickrath, 2015) Growth restriction and increased tremors have been found in neonates following maternal cannabis use. (Higuera-Matas et al., 2015) The largest deficits in growth are seen in neonates exposed throughout pregnancy. (Huizink, 2014) However, differences in preterm labour rates and birth weight can be eliminated if proper prenatal care is provided throughout pregnancy. (Mark et al., 2015) Increased risk of neonatal morbidity was found for exposed neonates; however, after adjusting for confounding factors, the increased risk was no longer significant. (Conner et al., 2015)
Inconsistent findings have been found regarding marijuana use during pregnancy and the risk of congenital anomalies. Considering the low quality of the studies that did find an associated risk of congenital anomalies, there is no evidence currently that marijuana exposure is linked to any specific birth defects. (Metz and Stickrath, 2015)

One-month-old infants with higher prenatal marijuana exposure showed signs of poorer attention as well as behavioural regulation. (Singer et al., 2012) By the age of 4 years, visual perception and language deficits were detected in exposed children. (Fried, 1995) Reading and math scores were found to be significantly lower in a cohort of 14-year-old adolescents who were exposed as fetuses to at least 1 joint per day during the first trimester. However, in this same cohort second and third trimester exposures were not found to be associated with any deficits. (Goldschmidt et al., 2012) Problem solving skill deficits have been noted in cannabinoid-exposed children, especially those involving visual memory and sustained attention. (Behnke and Smith, 2013)

In terms of behavioural problems following prenatal cannabinoid exposure, increased aggression and inattention have been documented, as have poor executive functioning and self-control. (Higuera-Matas et al., 2015) Exposed children exhibit higher rates of depressive symptoms at the age of 10 than do nonexposed children. (Gray et al., 2005)

2.1.4 Cocaine

Cocaine use causes vasoconstriction and during pregnancy can reduce blood flow to the fetus, causing numerous adverse maternal and neonatal outcomes. (Day and George, 2005) Maternal outcomes associated with cocaine use during pregnancy include placental abruption, hemorrhage, uterine rupture and seizures. (Ogunyemi and Hernández-Loera, 2004)

Neonatal outcomes following in utero cocaine exposure include prematurity, intrauterine growth restriction and lower birth weight. (Bandstra et al., 2001) Tremors, hemorrhage and necrotizing
enterocolitis have also been documented in exposed neonates. (Singer et al., 1994; Ogunyemi and Hernández-Loera, 2004; Bauer et al., 2005) Motor development following cocaine exposure may be slightly affected, but predominately in cases where heavy cocaine or second trimester exposures occurred. (Frank et al., 2002; Richardson et al., 2008) Conflicting evidence has been presented on the teratogenicity of cocaine. Limb defects, cardiac malformations, and genitourinary anomalies have been documented in exposed neonates but there is no consistent pattern of these malformations. (Buehler et al., 1996; Bauer et al., 2005)

Behavioural and cognitive deficits have been noted in children exposed to cocaine in utero. Visual attention was found to be diminished in young children between the ages of 8 months and 3.5 years when compared to unexposed children of similar age. (Heffelfinger et al., 1997) Deficits in language functioning have also been noted in 7-year-old children prenatally exposed to cocaine with expressive language being most affected. (Bandstra et al., 2011) These language deficits generally improve over time with general improvements noted by the age of 17. (Betancourt et al., 2011) Poor executive functioning and inability to sustain attention have also been documented in exposed children. (Sithisarn et al., 2012)

Both internalizing and externalizing behaviours can be affected by prenatal cocaine exposure with exposed 7-year-olds demonstrating these problematic behaviours and continuing these behaviours at 10 and 15 years as well. (Richardson et al., 2013; Min et al., 2014) Delinquent behaviour (e.g., theft and vandalism) has been self-reported at higher rates in 15-year-olds exposed to cocaine in the first trimester. (Richardson et al., 2015) However, delinquent behaviour in prenatally exposed children predominately occurs in low socioeconomic status populations. (Lambert and Bauer, 2012) Attention deficit hyperactivity disorder, depression and anxiety later in life have been associated with prenatal cocaine exposure, although this may be an indirect effect due to other confounding factors. (Lambert and Bauer, 2012)
2.1.5 Opioids

Opioid use during pregnancy is associated with increased risk of maternal cardiac arrest, placental abruption, stillbirth, PROM and preterm labour. (Maeda et al., 2014; Desai et al., 2015) Placental abruption may in fact occur due to poor nutrition and other lifestyle factors rather than opioid use itself. (Pinto et al., 2010) Slightly increased rates of postpartum hemorrhage have also been found in opioid-using mothers when compared to controls. (Nezvalová-Henriksen et al., 2011) Higher rates of SIDS have been found in neonates exposed to methadone; however, this increased rate may be present for all substances of abuse. (Cohen et al., 2015) IUGR and prematurity are associated with opioid use during pregnancy, along with lower birth measurements. (Bandstra et al., 2010) However, these effects can be confounded by maternal lifestyle, including poor nutrition, drug contaminants and intoxication-withdrawal cycle. (Day and George, 2005) Thus far, there is no evidence that opioid use during pregnancy is associated with congenital malformations. (Nezvalová-Henriksen et al., 2011)

In utero exposure to methadone can lead to NAS but improved birth measurements, especially birth weight, are seen when compared to children exposed to heroin. (Kandall et al., 1976; Dashe et al., 2002) NAS symptoms typically appear within 2-3 days after birth and occur in 60-80% of neonates exposed to methadone, heroin or other opioids. Some of the hallmark symptoms of NAS include seizures, tremors, increased muscle tone, high-pitched crying and jitteriness. (Kaltenbach et al., 1998) The risk of NAS increases following longer duration of exposure as well as higher cumulative dose throughout pregnancy. Additional substance use and smoking increase the absolute risk of NAS. (Desai et al., 2015) Neonates, even those not diagnosed with NAS, demonstrate high levels of irritability, sleep disturbances and hyperactivity during the first few weeks of life. (Day and George, 2005)

Inattention and hyperactivity have been noted in toddlers exposed prenatally to opioids with older children experiencing memory and perceptual problems. (Rosen and Johnson, 1985;
Poorer scores were achieved on general cognitive scales as well as perceptual, quantitative, and memory subscales of the McCarthy Scales. (Hans, 1996) Deficits in reading and arithmetic skills have been documented in exposed children aged 5-12 years. (Ornoy et al., 2001) Externalizing and internalizing behaviours, and poor mental development are seen at higher rates in exposed children. (Sithisarn et al., 2012) Increased risks of anxiety and aggression have been reported for children exposed to methadone, with the mothers of these children reporting more behaviour problems in general. (de Cubas and Field, 1993)

### 2.2 Alcohol

The specific mechanism of alcohol teratogenesis is currently unknown; however several possible mechanisms are hypothesized and include oxidative stress, altered cell cycle, disrupted cell-cell interactions and interference with growth factor signaling. (Goodlett et al., 2005)

Alcohol consumption during pregnancy is associated with preterm delivery, spontaneous abortion, and reduced birth weight. (Beijers et al., 2014) Heavy alcohol consumption in particular is associated with a marked increase in the risk of low birth weight, preterm birth and small for gestational age status. (Patra et al., 2011) The rate of spontaneous abortion in heavy drinking women is three fold higher than the general pregnant population, (Hannigan and Armant, 2000) and a six-fold increase in the rate of stillbirth pregnancies has been documented for women who consumed alcohol during pregnancy. (Comman-Homonoff et al., 2012) Maternal alcohol abuse has been found to increase the risk for both sudden infant death syndrome (SIDS) and non-SIDS infant deaths. (Iyasu et al., 2002; O’Leary et al., 2013a) Binge drinking has been found to increase the risk of adverse outcomes such as birth defects, growth restrictions and fetal mortality. (Anderson et al., 2014)
The brain regions most affected by ethanol teratogenesis include but are not limited to the corpus callosum, frontal lobe, thalamus, cerebellum and basal ganglia. (Bookstein et al., 2002; McGee and Riley, 2006; Lebel et al., 2011) Alterations in neurotransmitter systems have been noted in animals and are expected to be present in humans. (Carneiro et al., 2005)

No consensus has been reached regarding the effects of low to moderate levels of alcohol consumption during pregnancy. Numerous studies have been conducted on this matter with support for both arguments. O’Leary et al. reported that consumption of as little as 70 g of alcohol per week increases the risk of childhood behavioural problems. (O’Leary et al., 2010) Various behavioural characteristics have been detected in children with low to moderate prenatal alcohol exposure, such as aggression, externalizing behaviour and learning disabilities, some extending into adulthood. (Olson et al., 1997; Sood et al., 2001; Sayal et al., 2007; Day et al., 2013) Several studies have not found any increase in risk of adverse neonatal outcomes following low-to-moderate alcohol exposure in utero. (O’Keeffe et al., 2014; Lundsberg et al., 2015) Maternal and neonatal outcomes were not found to be different in women who reported low levels of alcohol consumptions when compared to women who abstained. (Han et al., 2012) A recent systematic review and a Danish follow-up study found that there was no increased risk of executive functioning or speech and language delays after low to moderate drinking during pregnancy. (Skogerbø et al., 2012; O’Keeffe et al., 2014) However, methodological weaknesses have been noted in many of the studies that did not find an association between low-to-moderate alcohol consumption during pregnancy and poor neonatal outcomes. (Henderson et al., 2007)

2.2.1 Fetal Alcohol Spectrum Disorder

Fetal alcohol spectrum disorder (FASD) is an umbrella term that describes the range of fetal alcohol effects, and is the leading preventable cause of mental impairment. (O’Leary, 2004) Included in this spectrum are fetal alcohol syndrome (FAS), partial FAS (pFAS), alcohol-related
neurodevelopmental disorders (ARND), and alcohol-related birth defects (ARBD). (Stratton et al., 1996) The current prevalence rates of FASD in young school-aged children in the United States are between 2-5%. (Heller and Burd, 2014) The global prevalence of FASD varies substantially due to variable alcohol intake patterns and differences in diagnostic methods and questionnaires for self-reports. (Vall et al., 2015)

Due to the range of symptoms and deficits seen in children affected by FASD, accurate diagnosis can be extremely challenging. As well, some forms of FASD lack any physical signs, creating further diagnostic difficulties. The exceptions to this are FAS and pFAS, where characteristic facial features have been associated with these syndromes. The specific pattern of facial dysmorphology, identified independently by Dr. Paul Lemoine and Drs. Kenneth Jones and David Smith, includes short palpebral fissures, thin vermilion border of the upper lip, smooth philtrum, epicanthal folds, flattened medial midface and maxillary hypoplasia. (Jones and Smith, 1973; Lemoine et al., 2003) Diagnostic criteria from the Institute of Medicine are summarized in Table 1.1. The 4-Digit Diagnostic Code provides diagnostic categories, which are used to classify patients in a more accurate, reproducible manner. The ranks range from 1 to 4, with 1 indicating the mildest form and 4 indicating the more severe form of the diagnostic category. For example, in the diagnostic category of FAS Facial Phenotype, 1 indicates absence of the phenotype, 2 indicates the presence of one of the three key features, 3 indicates the presence of two to three of the features, and 4 indicates the presence of all three features in the most severe form. (Astley and Clarren, 2000)
### Table 1.1. Institute of Medicine Diagnostic Guidelines for Fetal Alcohol Spectrum Disorder. (Stratton et al., 1996)

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>IOM Diagnostic Guidelines</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAS with confirmed maternal alcohol exposure</td>
<td>• Confirmed maternal alcohol exposure&lt;br&gt;• Characteristic facial anomalies&lt;br&gt;• Growth retardation&lt;br&gt;• CNS neurodevelopmental abnormalities</td>
</tr>
<tr>
<td>FAS without confirmed maternal alcohol exposure</td>
<td>• Characteristic facial anomalies&lt;br&gt;• Growth retardation&lt;br&gt;• CNS neurodevelopmental abnormalities</td>
</tr>
<tr>
<td>pFAS with confirmed maternal alcohol exposure</td>
<td>• Confirmed maternal alcohol exposure&lt;br&gt;• Some characteristic facial anomalies&lt;br&gt;AND&lt;br&gt;• Growth retardation or CNS neurodevelopmental abnormalities or inconsistent behavioural or cognitive abnormalities, not explained by other factors</td>
</tr>
<tr>
<td>ARND</td>
<td>• Confirmed maternal alcohol exposure&lt;br&gt;AND&lt;br&gt;• CNS neurodevelopmental abnormalities or inconsistent behavioural or cognitive abnormalities, not explained by other factors</td>
</tr>
<tr>
<td>ARBD</td>
<td>• Confirmed maternal alcohol exposure&lt;br&gt;• Congenital anomalies (incl. malformations and dysplasia)</td>
</tr>
</tbody>
</table>

The profile of FASD can vary substantially among children, which could be due to three confounding factors. First, more severe birth defects correlate with advanced maternal age, history of alcohol dependence, and patterns of alcohol use during pregnancy. The highest risk of giving birth to a child with FASD occurs with mothers who report drinking during all trimesters; this risk decreases with earlier abstention from drinking during pregnancy. (May et al., 2013) Generally, exposures in the first 20 weeks of gestation affect the formation of the neural crest and organogenesis, while exposures later in gestation affect brain development.
Second, deleterious social conditions, such as poverty, smoking, substance use and poor prenatal care, can increase the risk of FASD. These environmental factors may have a larger effect on the behavioural problems associated with FASD than the prenatal alcohol exposure itself. A significant difference has been found in the rate of FASD diagnosis between low and high SES (71% vs. 4.6%), similar to the disparity of ADD diagnosis between different SES levels. In general, children of parents with mental health disorders are at a higher risk of exhibiting behavioural problems when compared to those born to mentally healthy parents. However, if these environmental factors are controlled for, adolescents with prenatal alcohol exposure are no longer at an increased risk of delinquent behaviour. Third, genetics can affect the risk for FASD. Individuals with the genetic variant ADH2*3 have a decreased risk of having a child with FASD (specifically FAS), as this variant codes for a more efficient alcohol dehydrogenase enzyme. One third of children with FASD have congenital cardiac problems. As well, craniofacial anomalies (e.g., microcephaly) and limb defects are also common health conditions found at higher rates in this population. Cases of orofacial clefts have been documented following prenatal alcohol exposure; however, a recent meta-analysis did not detect an increased risk for these congenital anomalies. Children with FASD are at an increased risk to have deficiencies in gross motor skills, including balance, coordination, and ball-handling skills; fine motor skill development is also delayed in these children. These deficits are also seen in children who were exposed to moderate or heavy amounts of alcohol prenatally but did not meet the criteria for FASD. Behavioural problems associated with FASD include, but are not limited to, conduct problems (disobedience), externalizing behaviour (stealing, cruelty, physical aggression), maladaptive
behaviour (avoidance of school or work, substance use), antisocial behaviour and disruptive behaviour (impulsivity, hyperactivity). (Malone and Koren, 2012) Binge drinking in early and late pregnancy has been found to be associated with higher scores for externalizing behaviours in 7-year-old children. (Niclasen et al., 2014) Lower educational achievements were attained between the ages of 8 and 9 years in a cohort of children exposed to heavy maternal alcohol consumption during the first trimester. (O’Leary et al., 2013b) Through numerous studies, all domains of neurocognition have been found to be affected after prenatal alcohol exposure, including attention, language, visuospatial functioning, verbal memory, and learning. With regard to attention and memory in particular, pattern of drinking and number of drinks per occasion appear to have a greater effect on producing deficits than timing of drinking during pregnancy or the number of drinking occasions. (Irner, 2012)

These behavioural deficits contribute to a series of secondary disabilities in FASD. (Kully-Martens et al., 2012) Individuals with FASD have a lifetime prevalence of legal difficulties of 60%, and approximately half will be imprisoned during their lifetime. (Malone and Koren, 2012) Youths with FASD are at an increased risk of being incarcerated when compared to youths without FASD, and the number of incarcerated individuals with undiagnosed FASD is suspected to be high. (Popova et al., 2011) Inappropriate sexual behaviours, as well as alcohol or drug problems and mental health disorders (e.g., anxiety, depression) are also common amongst individuals diagnosed with FASD. (Stade et al., 2006; Malone and Koren, 2012) These cognitive and behavioural deficits affect the futures of these children, both socially and in terms of occupation, with some individuals requiring lifelong social assistance. (Irner, 2012)

Children with FASD have significantly lower quality of life and health outcomes later in life when compared to the general population (Sarkar et al., 2009), which in turn negatively affects the health care system and economy. It is estimated that the cost to the Canadian health care
system is approximately $14,000 per year for each child with FASD, totaling over $300 million for all individuals with FASD aged 1 to 21 years. (Stade et al., 2006; Stade et al., 2007)

2.3 Summary

Substance use during pregnancy is associated with numerous adverse outcomes for both mother and child. With the exception of alcohol, no consistent patterns of malformations have been documented for any substance of abuse. Behavioural deficits are associated with substance use during pregnancy, with the possible exception of benzodiazepines.

3 Previous Studies Investigating Prevalence and Trends of Substance Use During Pregnancy

3.1 Drugs

Numerous studies have been conducted to determine the prevalence of drug use during pregnancy, the majority utilizing self-report or urine drug screen analysis. Depending on the population studied, prevalence rates and trends of substance use during pregnancy vary. These results cannot be generalized due to the differences in the populations studied, but still can provide invaluable information for local communities on the rates of drug use. A few key studies that demonstrate unique methodology and/or more generalizable results will be reviewed.

Mark and colleagues (2015) discuss the findings of a routine urine drug screen program at a University of Maryland-affiliated hospital. Over the year that was retrospectively studied, 29.3% of patients screened tested positive for marijuana, either through urine toxicology or self-report. This hospital universally screens all patients starting prenatal care through self-report and urine toxicology. Subsequent urine screens are performed each trimester as well as at the time of delivery. Individuals who used marijuana during their pregnancy had lower levels of education, and higher rates of concurrent smoking, alcohol use, psychiatric diagnosis and history of physical or sexual abuse. (Mark et al., 2015) This study highlights the utility of routine urine
drug screen programs, which are relatively unique and rarely reported on in the literature. Wexelblatt et al. (2015) also detail the results of a universal maternal urine drug testing program which involves referral to hospital-based social services where resources for addiction treatment can be provided if appropriate. This study found a 5.4% prevalence rate of positive urine tests, with 20% of opioid-positive tests coming from women who would have been missed if typical screening risk factors were used. (Wexelblatt et al., 2015) This type of drug screening minimizes selection bias by physicians and captures both low- and high-risk individuals. Results from a routine screen allow for a relatively accurate picture of substance use within a specified population.

Multicentre studies may provide more generalizable results as larger populations can be recruited, and any selection bias present at a given hospital due to its’ geographical location can be minimized. The Maternal Lifestyle Study is an exemplary multicenter study which, by utilizing meconium analysis and ELISA screening, determined the prevalence of cannabinoid, cocaine and opioid use in pregnancy to be 7.2%, 9.5% and 2.3%, respectively. (Lester et al., 2001) Four sites in different geographical regions of the United States were involved in this study and the results can be seen as more generalizable than a single site study.

### 3.2 Alcohol

Like drugs of abuse, there have been numerous studies focusing on the prevalence of alcohol consumption during pregnancy. Again, the prevalence rates detected in these studies vary immensely depending on the population and context studied. Some of the key prevalence studies on heavy fetal alcohol exposure utilizing meconium and fatty acid ethyl esters (FAEEs) are summarized in Table 1.2.
In a recent meta-analysis, the prevalence rates determined by meconium analysis and self-report were compared to determine if meconium analysis does in fact perform better than self-report. This study found that the prevalence rates determined through meconium analysis were 4.26 times higher than the prevalence measured by self-report. (Lange et al., 2014) This finding could potentially also be generalized to studies focusing on drug use. Underreporting of substance use during pregnancy may occur due to recall bias, but the major factor remains the social stigmas associated with substance use in general, and during pregnancy in particular. (Sayal, 2007) To obtain objective and more accurate prevalence rates, methodologies similar to the ones previously mentioned, namely biomarker analysis, should be utilized rather than maternal self-report alone.
3.3 Summary

The prevalence of substance use during pregnancy has been widely studied previously with self-report or questionnaire being the most common form of data collection. Multicentre studies, routine urine drug screening programs and meconium analysis are alternative methods, which help provide accurate and reliable information about the prevalence and trends of substance use during pregnancy.

4 Biomarkers of Substances of Abuse

“There are multiple definitions of a biomarker, specific to how the biomarker is used. The official National Institute of Health definition is: “a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.”(Biomarkers Definitions Working Group, 2001) In the context of detecting external toxins after exposure during intrauterine life, biomarkers are critical, as many chemicals may not persist in the blood or urine of the neonate. Consequently, without the availability of appropriate biomarkers, even potentially toxic intrauterine exposures may be missed. Therefore, biomarkers must be able to generate relevant preclinical or clinical interpretations. (Goodsaid et al., 2008) The sensitivity (the ability to correctly identify true positives) and specificity (the ability to correctly identify true negatives) of a biomarker are extremely important, as biomarkers too sensitive or non-specific may not detect clinically relevant exposures or effects. (Timbrell, 1998)

A biomarker of internal dose is a subtype of biomarkers of exposure, which indicate the occurrence and extent of exposure to a compound and/or its metabolite(s). (Timbrell, 1998) Measuring the actual amount of the compound and/or metabolite in a matrix allows for a more accurate and objective estimation of the actual exposure. (Timbrell, 1998) By using biomarkers of both the compound and metabolite(s), more information concerning the exposure can be gathered and more accurate clinical interpretations can be made. When available, measuring
multiple biomarkers simultaneously may provide more clinically relevant information about the exposure.” (Delano and Koren, 2012; page 1059-1060)

4.1 Drugs

4.1.1 Amphetamines

Methamphetamine is biotransformed into its major metabolite, amphetamine, by CYP2D6 via N-demethylation. Another metabolite, p-hydroxymethamphetamine (pOHMAMP), has recently been identified as an additional biomarker of methamphetamine use, particularly for continued use into the third trimester. (Gray et al., 2009) “Methamphetamine is able to cross the placenta at a very rapid rate and does so even at low levels on the fetal side; it persists due to slower elimination, resulting in prolonged fetal exposure. (Gray et al., 2009; White et al., 2011) The fetal elimination rate of amphetamine is also reduced, to a greater extent than that of methamphetamine. (White et al., 2011) The prolonged elimination of both methamphetamine and amphetamine can cause accumulation of both compounds on the fetal side if the mother is using these compounds on a consistent basis.” (Delano and Koren, 2012; page 1066)

All of the above mentioned amphetamine derivatives could be tested for, to assess exposure. “Interpretation of samples positive for amphetamines can become very complex depending on which compounds are detected. If either methamphetamine or amphetamine is detected alone, this indicates exposure or use of the respective compound. Samples positive for both methamphetamine and amphetamine can be interpreted in three ways. First, the amphetamine could be a product of methamphetamine metabolism, indicating methamphetamine use or exposure. Second, seized illicit methamphetamine contains amphetamine as well, indicating methamphetamine use and unintentional amphetamine exposure. Third, it can suggest both methamphetamine and amphetamine were used during the tested time frame. Clearly, it is necessary to test for all amphetamine compounds if exposure is suspected, as the
interpretation of positive amphetamine results can be complex.” (Delano and Koren, 2012; page 1066)

4.1.2 Cannabinoids

“Cannabis sativa, marijuana, produces the class of cannabinoids, with over 60 unique compounds. (Huestis, 2007) Δ⁹-tetrahydrocannabinol (THC) is the primary compound of the cannabinoids and is metabolized to the active compound 11-hydroxy-Δ⁹-tetrahydrocannabinol (11-OH-THC) and subsequently 11-nor-Δ⁹-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) via CYP2C9 and CYP3A4 respectively. (Watanabe et al., 2007)” (Delano and Koren, 2012; page 1066) Over 100 different metabolites are formed following THC biotransformation but the previously mentioned two metabolites are produced in the largest amounts. (Huestis, 2007) THC readily crosses into the placenta while 11-COOH-THC does not, indicating that the latter functions best as a confirmatory or secondary biomarker. (Bailey et al., 1987; Huestis et al., 1996) THC is highly lipophilic (log P = 6.9–7.6) and has been found to accumulate in adipose tissue. This accumulation lends itself to a longer detection window, especially in chronic users where positive urine screens can be detected days and possibly weeks after the last use. (Huestis, 2007) It is recommended that samples be analyzed for THC, 11-OH-THC and THC-COOH to determine prenatal cannabis exposure.

4.1.3 Cocaine

Cocaine is metabolized into several metabolites, including benzoylecgonine (via hepatic carboxylesterase-1 (hCE-1)), norcocaine (via CYP3A4) and cocaethylene (via hCE-1 in the presence of ethanol). (Maurer et al., 2006) Due to the short elimination half-life of cocaine (50 minutes), detection in blood or urine can be limited to a few days after last use. (Taguchi et al., 2007; Delano and Koren, 2012) However, cocaine and its’ metabolites are able to accumulate into alternative matrices, such as hair and meconium.
Cocaine crosses the placenta via passive diffusion and is almost always found with at least one of its metabolites in meconium samples. (Gareri et al., 2006) The site of metabolite production, either fetal or maternal, is currently undetermined. (Oyler et al., 1996) The most common cocaine metabolite tested for and found in biological matrices is benzoylecgonine. Other metabolites, including norcocaine and cocaethylene, are also used in sample analysis for cocaine exposure. (Oyler et al., 1996) “Cocaethylene is an emerging biomarker that can provide additional information regarding exposure. This metabolite is formed when cocaine and ethanol are present concurrently. (Natekar and Koren, 2011) By using cocaethylene as a biomarker, alcohol use can be detected without assessing alcohol specific biomarkers. This has great advantages for detecting a risk of fetal alcohol syndrome in neonates who are positive for this metabolite.” (Delano and Koren, 2012; page 1065)

4.1.4 Opioids

“Heroin is rapidly deacetylated to the active metabolite 6-monoacetylmorphine (6-MAM) and morphine by hCE-1, hepatic carboxylesterase-2 (hCE-2) and plasma-borne pseudocholinesterase; readily crosses the placenta and is incorporated into fetal tissues within one hour of maternal administration. (Maurer et al., 2006; Keegan et al., 2010) As 6-MAM persists in the system for a longer period of time than heroin, it is used as a biomarker to detect heroin use. Along with heroin metabolism to 6-MAM, detectable levels of morphine can also be produced. (Lee et al., 2009) In addition, illicit heroin usually contains acetylcodene and users commonly supplement their heroin with codeine before injection. (Lee et al., 2009) This may complicate the interpretation of test results, as the exact source of codeine and morphine may be unknown in 6-MAM-positive samples.

Codeine and morphine are commonly tested together as morphine is a metabolite of codeine through CYP2D6 metabolism via O-demethylation. (Kirchheiner et al., 2007) Morphine is widely distributed in fetal tissues and its level in meconium has been found to correlate with maternal
dose, time, and duration of gestational exposure. (Gareri et al., 2006) Similar to codeine and morphine testing, oxycodone and its metabolite oxymorphone (via CYP2D6 metabolism) are commonly tested together. Hydrocodone is tested with one of its metabolites, hydromorphone (formed via CYP2D6 metabolism), which is also available as a separate analgesic. (Smith, 2009)” (Delano and Koren, 2012; page 1065) Finally, methadone is biotransformed into its inactive metabolites 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and 2-ethyl-5-methyl-3,3-diphenyl-1-pyrrolidine (EMDP) by CYP3A4 and CYP2B6, but predominantly methadone and EDDP are used as biomarkers. (Maurer et al., 2006) “It is best to determine which drug was prescribed prior to result interpretation, as each of the metabolites is also a prescription drug. As opioids are not contraindicated during pregnancy and can be used during labor, it is necessary to try to identify all medications administered to the mother to properly assess opioid levels.” (Delano and Koren, 2012; page 1065)

4.2 Alcohol

4.2.1 Fatty acid ethyl ethers (FAEEs)

“FAEEs are non-oxidative metabolites of ethanol, formed through the esterification of ethanol with endogenous fatty acids or fatty acyl-CoA. (Caprara et al., 2006) These reactions are catalyzed by FAEE synthase or microsomal acyl-CoA:ethanol O-acetyltransferase. (Kulaga et al., 2010) FAEE synthase, in particular, is present in almost all human tissues, with detectable activity in the heart, liver, lungs, adipose tissue, gall bladder, and pancreas. (Caprara et al., 2006) Depending on the carbon chain length and the locations of double bonds, different species of FAEEs can be formed and over 20 different FAEE species are known. (Koren et al., 2008; Cabarcos et al., 2014)” (Delano and Koren, 2012; page 1063) They are lipophilic molecules, easily distributed throughout the body and stable at neutral pH. (Cabarcos et al., 2015) “Most FAEEs are transported by albumin within the blood, and once free are readily broken down by cellular structures in the blood, liver and pancreas. (Caprara et al., 2006) Unlike ethanol, FAEEs persist in the body for more than a day after significant alcohol
consumption, and are able to accumulate in various matrices. (Brien et al., 2006) Also in contrast to ethanol, FAEEs are readily metabolized by the placenta and thus do not cross through it. (Chan et al., 2004d) This indicates that FAEE levels present in meconium represent fetal ethanol metabolism and thus actual fetal exposure to ethanol. (Chan et al., 2004a; Gareri et al., 2008)” (Delano and Koren, 2012; page 1063)

“Several individual FAEEs are analyzed in samples to detect alcohol exposure or use. Inter-individual variation in specific FAEEs formed can result from genetic variations in the enzymes responsible for FAEE formation, the amounts of specific fatty acids in different diets, the degree of alcohol exposure or consumption and FAEE synthase enzyme kinetics. (Gareri et al., 2006; Brien et al., 2006; Koren et al., 2008) Ethyl palmitate, oleate, stearate, and linoleate are the predominant FAEEs found in meconium of ethanol-exposed neonates. (Caprara et al., 2006) The cumulative level of select FAEEs is measured as this provides a redundancy system resulting in higher efficiency, sensitivity, and specificity. (Chan et al., 2004b; Gareri et al., 2006)” (Delano and Koren, 2012; page 1063)

“Several studies looking at baseline FAEE levels found that infants born to women who did not drink alcohol during pregnancy had low FAEE levels. (Chan et al., 2003) Of note, because the body produces some ethanol during normal metabolism, FAEEs are detected in nondrinking individuals, thus requiring a clear, matrix-specific cutoff value for differentiation between drinking and nondrinking individuals. (Caprara et al., 2005)” (Delano and Koren, 2012; page 1063) This may be why it is difficult to establish a clear dose-response relationship between self-reported ethanol intake and the concentration of FAEEs detected in meconium. However, limitations of the self-report itself may have a larger effect on the lack of relationship. (Derauf et al., 2003) Kwak et al. (2014) found a weak association between drinks per week and FAEE concentration in meconium, but this association only explained 28% of the variability in FAEE concentrations once an outlier was excluded from regression analysis. (Kwak et al., 2014)
With respect to meconium, the positive cutoff of cumulative FAEE levels (ethyl palmitate, oleate, stearate, and linoleate) was established at 2 nmol/g (~600 ng/g) meconium. (Chan et al., 2003) However, this cutoff value does not allow for differentiating between neonates born to nondrinkers and social drinkers. (Chan et al., 2004b) Timing of light alcohol exposure, in either second or third trimester, did not affect FAEE levels in meconium either. (Kwak et al., 2014) Other cutoffs have been proposed and used in FAEE analysis. Moore et al. (2003) suggested a total FAEE concentration (ethyl palmitate, linoleate, oleate, stearate, palmitoleate, linoleate) positive cutoff of 10,000 ng/g (approximately 33 nmol/g); however, this cutoff may only identify very significant alcohol exposure and for some analysis method. Other proposed cutoffs involve up to nine FAEE species and range from 200 ng/g to 600 ng/g. The sensitivity and specificity of these cutoffs varies between 52%-100% and 45.1%-98.4% respectively. (Chan et al., 2004c; Himes et al., 2015) The important factor in cutoff determination is that multiple FAEE species are used for a cumulative concentration, as it has been confirmed that this is more appropriate for interpretation than a single FAEE. (Bakdash et al., 2010)

"An important limitation of FAEE meconium testing is that samples excreted later in the postpartum period have higher levels of FAEEs than those collected earlier from the same infant due to de novo fermentative production of alcohol from carbohydrates in the meconium. This could lead to false-positive FAEE results, and it is recommended that meconium samples be collected within 24 hours to ensure FAEE results will properly reflect in utero ethanol exposure. (Zelner et al., 2012a)" (Delano and Koren, 2012; page 1063)

4.2.2 Other biomarkers
Ethyl glucuronide (EtG) is a minor metabolite of ethanol and is formed through glucuronidation by UDP-glucuronosyl transferases (UGT). (Tavakoli et al., 2011) EtG is detectable only if alcohol has been consumed and can be detected in urine for approximately 90 hours. (Cabarcos et al., 2013; Bryanton et al., 2014) Several studies have reported sensitivities and
specificities of approximately 90% for EtG. (Tavakoli et al., 2011) Through the comparison of FAEEs and EtG in the same meconium samples, it is recommended that these two biomarkers be used in conjunction for confirmatory purposes. (Cabarcos et al., 2014)

EtG and another alcohol biomarker, ethyl sulfate (EtS) have been found to be potentially elevated in urine samples following less than one drink per day but have a detection window of less than 5 days. (Bakhireva and Savage, 2011) EtS and EtG can be used in conjunction as well to detect recent alcohol use. (Junghanns et al., 2009)

4.3 Matrices

4.3.1 Urine

Urine drug screening (UDS) is one of the most frequently used methods of detection due to its convenience, cost, and accessibility. (Moeller et al., 2008) Sample collection is easy and non-invasive, but can be adulterated if unsupervised. Immunoassay and chromatographic confirmatory testing has become the most popular screening method for UDSs. The immunoassay can be based on competitive or non-competitive binding, relatively inexpensive, and easy to perform. (Brahm et al., 2010) If a sample is found to be positive through the immunoassay screening step, it can then be run through a confirmatory chromatographic analysis. However, these rapid screening methods lack sensitivity and specificity and may result in false-positive results. (Nelson et al., 2015) Some examples of drugs that can yield false-positive immunoassay results due to cross-reactivity include: bupropion for amphetamine and methamphetamine assays, ibuprofen for barbiturate assays, and rifampin for opiate assays. (Casey et al., 2011; Rollins et al., 1990; de Paula et al., 1998)

The temporal window of detection for most analytes ranges between 24 and 72 hours from time of use, which may require multiple samples to be collected if monitoring substance use over the long term is the ultimate goal. Table 1.3 summarizes the window of detection of common substances of abuse.
Table 1.3. Window of detection of common substances of abuse in urine. (Adapted from Nelson et al. 2015)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Window of Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amphetamines</strong></td>
<td></td>
</tr>
<tr>
<td>Amphetamine</td>
<td>3 days</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>3 days</td>
</tr>
<tr>
<td>MDMA</td>
<td>2 days</td>
</tr>
<tr>
<td><strong>Barbiturates</strong></td>
<td></td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>15 days</td>
</tr>
<tr>
<td>Pentobarbital</td>
<td>3 days</td>
</tr>
<tr>
<td><strong>Benzodiazepines</strong></td>
<td></td>
</tr>
<tr>
<td>Diazepam/Nordiazepam (metabolite)</td>
<td>10 days</td>
</tr>
<tr>
<td>Lorazepam</td>
<td>5 days</td>
</tr>
<tr>
<td>Midazolam</td>
<td>2 days</td>
</tr>
<tr>
<td><strong>Cocaine</strong></td>
<td></td>
</tr>
<tr>
<td>Cocaine</td>
<td>&lt; 1 day</td>
</tr>
<tr>
<td>Benzoylecgonine (metabolite)</td>
<td>5 days</td>
</tr>
<tr>
<td><strong>Marijuana (THC)</strong></td>
<td></td>
</tr>
<tr>
<td>Single Use</td>
<td>3 days</td>
</tr>
<tr>
<td>Heavy Use</td>
<td>10 days</td>
</tr>
<tr>
<td>Chronic Heavy Use</td>
<td>30 days</td>
</tr>
<tr>
<td><strong>Opioids</strong></td>
<td></td>
</tr>
<tr>
<td>Codeine</td>
<td>3 days</td>
</tr>
<tr>
<td>Morphine</td>
<td>3 days</td>
</tr>
<tr>
<td>Hydrocodone</td>
<td>3 days</td>
</tr>
<tr>
<td>Oxycodone</td>
<td>3 days</td>
</tr>
<tr>
<td>Methadone/EDDP (metabolite)</td>
<td>1-7 days</td>
</tr>
</tbody>
</table>

Despite the limited window of detection and risk of false positive results, when paired with maternal interview, urine drug testing can increase the detection of problematic substance use during pregnancy. (Wong et al., 2011) UDS still remains the most widely used methodology, especially in high throughput screening and in smaller hospital settings where chromatography techniques are too expensive and have longer turnaround times.

4.3.2 Meconium

“Meconium is comprised of the first few bowel movements of a neonate. (Kwong and Ryan, 1997) This highly complex matrix begins to form approximately during the 12th week of gestation and consists of water, gastrointestinal tract epithelial cells, bile acids and salts, enzymes, sugars, lipids, intestinal secretions and swallowed amniotic fluid. (Righetti et al., 2003) Fetal swallowing of amniotic fluid is the mechanism thought to concentrate compounds within meconium, as fetal urine is deposited into the amniotic fluid and is subject to swallowing.
again. (Gareri et al., 2006) Deposition directly into meconium can also occur for substances that reach fetal circulation, which are then excreted in fetal bile. (Ostrea, 1999) Determining factors of drugs incorporation into meconium and the extent of their concentration are mainly determined by the ability of the drug to cross the placenta. Many drugs are able to cross the placenta, and the rate of transfer is determined by molecular size, ionization state, lipophilicity, and protein binding. (Gareri et al., 2006) Because most drugs are small enough in size to transfer via passive diffusion, the major limiting factor, in terms of drug transport to the fetus, is placental blood flow. (Szeto, 1993)" (Delano and Koren, 2012; page 1061-1062)

“Once meconium is formed in the fetal intestine, it is considered a physically static matrix, becoming a record of fetal exposure to the drugs in question during the second and third trimesters of pregnancy. (Gareri et al., 2006)" (Delano and Koren, 2012; page 1062) Recently, however, research has found that meconium likely represents only third trimester exposures. Gray et al. studied pregnant women enrolled in drug treatment programs and found that positive meconium results for cocaine and opioids agreed best with third trimester-positive urine drug screen results. Opioid-positive meconium samples were associated with third trimester and/or continued use for the previous 2 months, while cocaine-positive meconium samples were associated with third trimester and/or continued use for the previous 3 months. (Gray et al., 2010) A positive meconium test would then indicate intrauterine exposure during the third, and possibly second, trimesters, and is unable to show more specific timing or patterns of use. (Chan et al., 2004a)

“Dose-response relationships are difficult to determine using meconium samples, mainly due to urine contamination. If fetal exposure occurs close to term and the compound is incorporated into the urine, contamination of meconium can occur once urine is evacuated into a soiled diaper. (Gareri et al., 2006) This would increase the sensitivity of meconium testing due to the increased compound levels in the sample. However, it could affect the ratio of drugs and metabolites in the sample, and the development of dose-response relationships.” (Delano and...
Koren, 2012; page 1062) Additionally, a disproportionate ratio of parent drug to metabolite concentrations have been found in meconium, potentially due to the longer half-lives of the metabolites and the accumulation of these compounds throughout the third, and possibly second, trimester. (Gray et al., 2009; de Castro et al., 2011)

“Collection of meconium specimens is easy and non-invasive. As it is discarded material and there is usually sufficient quantity for analysis, this matrix is practical and useful. (Moller and Koren, 2010)” (Delano and Koren, 2012; page 1062) It has been proposed that complete collection of meconium is the least biased collection method. Complete collection entails collecting all meconium passed by the neonate until they begin producing postnatal feces, and then homogenizing all serial samples collected. (Park and Lee, 2014) “Ninety nine percent of infants pass their first meconium within 48 hours, giving this matrix a wider window of detection than blood or urine. (Verma and Dhanireddy, 1993) However, once 24–48 hours have passed, it is necessary to evaluate the texture and odour of the sample to determine whether it is still meconium or has transitioned to postnatal feces.” (Delano and Koren, 2012; page 1062) With regard to drug analysis, drug concentrations gradually decrease over time and are undetectable by the third day when serial meconium samples are collected from exposed neonates. (López et al., 2009) In terms of alcohol analysis, collection of meconium within the first 24 hours is crucial to minimize the risk of false positive results, as previously mentioned. “The time allowed for sample collection may be seen as limited, but if the neonate is at high risk for drug or alcohol in utero exposure and remains in hospital care, obtaining a viable sample is not problematic.” (Delano and Koren, 2012; page 1062)

4.4 Summary

Biomarkers of substance use can provide objective information on in utero exposures when measured in matrices like urine and meconium. Biomarkers can be both the parent drug and/or metabolites of the compound of interest, depending on its’ pharmacokinetic properties. While
urine and meconium both have limitations, these two matrices have numerous advantages when utilized in the correct setting and collected properly.
Chapter 2
Thesis Scope

1 Overall Aim

Substance use, both during pregnancy and not, is a growing concern in communities across Canada. While numerous studies have previously been conducted to determine the prevalence of substance use during pregnancy, many of these rely solely on maternal self-report collected through surveys and/or questionnaires. Self-reporting provides a simple and cost-effective way to assess substance use during pregnancy, but is subject to major limitations in reliability and accuracy. Women have been found to refrain from providing an accurate self-report of substance use due to the embarrassment, guilt, fear, and stigma attached to this type of behaviour during pregnancy. Biological markers, or biomarkers, have been utilized for decades to detect substance use during pregnancy and provide more objective and reliable results to clinicians and researchers.

Despite the increased use of biomarkers in both research and clinical settings, prevalence rates of substance use during pregnancy are still predominantly assessed through maternal self-report, and several knowledge gaps are present within this field. Firstly, polydrug use is a common practice for many substance users but very few studies have focused on this pattern of use within the pregnant, substance-using population, even less so in the methadone-using subpopulation. A better understanding of polydrug use within this highly specific population can provide insight into the overall effectiveness of methadone maintenance treatment (MMT) during pregnancy to decrease this pattern of use. Secondly, routine urine drug screens within the obstetric unit have been developed in very few regional hospitals, mainly in the United States, with little to no data published on the rates of substance use in the population following implementation of the program. To determine if this type of screening method is effective in identifying substance use during pregnancy, and possibly decreasing this rate over time, a detailed look at the rates and trends of substance use over time must be investigated. Lastly,
the majority of prevalence studies focus on a single population or region, with the results stemming from this research not generalizable to larger, national populations. With FASD being the leading, most preventable cause of neurodevelopmental delays in North America, a more accurate estimate of the number of neonates at risk within Canada can inform clinicians and researchers about what is truly ongoing in the country, and subsequently help shape public initiatives and resource allocation in the future.

Thus, the overall aim of this thesis is to objectively assess ongoing substance use prevalence and trends of substance use during pregnancy in Canada, so that the key knowledge gaps previously mentioned can be addressed.

2 Objectives and Hypotheses

OVERALL OBJECTIVE:

To utilize biomarkers of substances of abuse to objectively assess the prevalence and trends of substance use during pregnancy in Canada in a variety of populations.

PRIMARY OBJECTIVES AND HYPOTHESES:

I. To determine the rate of polydrug use in a high-risk population of women using methadone while pregnant and involved with social services.

Hypothesis: Pregnant women using methadone during pregnancy will have lower rates of polydrug use, in particular opioid use, than a control group stemming from the same high risk population.

Rationale: The involvement in MMT is paralleled with the expectation of reduction or abstinence from additional opioid use. (Mactier, 2013) As methadone is a long acting opioid receptor agonist, the physiological stability it provides for patients should aid these individuals in reducing their illicit opioid use and additional drug-seeking behaviour. (Minozzi et al., 2013) If methadone
is performing as effectively as intended, individuals using methadone should have lower rates of drug use, especially opioids, while engaging in MMT when compared to a cohort not using methadone.

II. **To assess the prevalence of drug use during pregnancy in a regional medical center which utilizes a routine urine drug screen program within the obstetric unit, and the maternal and neonatal risks that are associated with substance use during pregnancy.**

*Hypothesis:* The rate of drug use within the Miramichi, New Brunswick, population will be higher than the national average, with opioids and marijuana being the most commonly used substances, and poorer maternal and neonatal outcomes being associated with maternal substance use during pregnancy.

*Rationale:* As the routine urine drug screen in Miramichi Regional Hospital was introduced due to suspected higher-than-average drug use within the pregnant population, the prevalence rates, especially those early on in the program, should be higher than the national average, which is approximately 5%. (Public Health Agency of Canada, 2008) Regardless of the prevalence of substance use within this population, similar nationwide trends for commonly used substances should also be detected within this population, with marijuana and opioids being detected at the highest rates. (Health Canada, 2013) Countless adverse outcomes have been documented following substance use during pregnancy and several of these poor outcomes will be noted in the case cohort.

III. **To estimate the prevalence of heavy fetal alcohol exposure in Canada through the analysis of FAEEs in meconium samples collected from volunteers in multiple study sites across the country**
**Hypothesis:** The prevalence of heavy fetal alcohol exposure in Canada will be in excess of 3%, with FAEE meconium analysis identifying higher rates of heavy alcohol consumption during pregnancy than maternal self-report.

**Rationale:** Currently, approximately 1% of live births in North America are affected by FASD, with some research indicating rates between 2 and 5% both nationwide and for high risk populations. However, the current prevalence of FASD is thought to only represent 40-50% of heavily exposed neonates, guiding the hypothesis that the prevalence rate is actually in excess of 3%, to address the large proportion of undiagnosed children. Several previous studies have documented higher rates of heavy alcohol consumption identified through meconium analysis of FAEEs over maternal self-report. This disproportionate finding may be due to the fear, stigma, embarrassment and guilt perceived by pregnant women, ultimately deterring them from accurately reporting their alcohol consumption during pregnancy.
Chapter 3
Methods

1 Rates of fetal polydrug exposures in methadone-maintained pregnancies from a high risk population

The following section of this thesis has been previously peer reviewed and published, with minor additions to provide a more detailed methodology:


[KD helped with study design, performed all data analysis, and prepared manuscript for submission]

1.1 Sample Collection and Analysis

This study is a retrospective, observational assessment of meconium toxicology performed on samples referred by physicians, primarily at the request of social services, between July 2010 and December 2012. Frozen meconium samples were shipped from sites of collection to the Motherisk Laboratory at the Hospital for Sick Children and stored at -80°C until analysis. Research ethics board approval was granted from The Hospital for Sick Children and is included in Appendix A.

Briefly, 300 mg of meconium was thawed to room temperature, homogenized and transferred to a 13 mL test tube (Sarstedt; Montreal, QC). One mL of methanol was added to each sample, vortexed for 30 seconds then centrifuged for 15 min at 3,500 rpm at room temperature. The supernatant for each sample was then decanted into a 5 mL test tube. Samples were then screened by ELISA (Immunalysis, Pomona CA) for the following drugs and metabolites: cocaine, benzoylecgonine, opiates (including codeine, morphine, 6-monoacetylmorphine, hydrocodone and hydromorphone), oxycodone (with cross-reactivity to oxymorphone), amphetamine, methamphetamine, THC, benzodiazepines, barbiturates, meperidine, and methadone. One hundred µL aliquots of the methanol extract were used for each individual ELISA test and 400 µL of phosphate buffered saline (PBS) was added. Samples were then added to the ELISA plate, 100 µL of enzyme conjugate added, then incubated for 60 min at
room temperature in the dark. Wells were then washed with ddH₂O, and 100 µL of substrate reagent was added to each well and reincubated for 30 minutes at room temperature, in the dark. Stop solution was added and absorbance was measured at a dual wavelength of 450 nm and 650 nm using a SUNRISE Absorbance Reader (Tecan Systems Inc., San Jose CA). Samples were deemed positive or negative, if the absorbance is below or above the cut-off standard respectively.

THC analysis was performed by ELISA only; all other screen-positive ELISA samples were subsequently confirmed through GC-MS analysis using previously published methods for drugs of abuse and metabolites. (Aleksa et al., 2012) The drugs of abuse GC-MS method is capable of simultaneously detecting and quantifying cocaine, benzoylecgonine, norcocaine, cocaethylene, 6-monoacetylmorphine, morphine, codeine, oxycodone, oxymorphine, hydrocodone, hydromorphone, meperidine, methadone, amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine, and methylenedioxyamphetamine. Deuterated internal standards of the 17 analytes and 20% methoxylamine solution were added to an aliquot of the previously described methanol extract from each sample, vortexed and incubated at room temperature for 1 hour. Two mL of 0.1 M phosphate solution was added to each sample, vortexed and incubated for 15 minutes at room temperature. Samples then underwent automated SPE extraction (Gilson ASPEC GX-274 Automated SPE extractor; Middleton, WI, USA), transferred to a 10 mL SPME vial (Supelco; Bellefonte, PA) and dried under nitrogen at 35°C. Once dried, 20 µL of derivatizing mixture (BSTFA and MSTFA 3:1) was added, and samples were analyzed by GC-MS on a Shimadzu QP2010 Plus (Shimadzu Scientific Inc.; Columbia, MD). The limit of detection (LOD), quantifying and qualifying ions for each analyte is summarized in Table 3.1.
Table 3.1. Quantification and qualifier ions for all analytes and their respective internal standard for the analysis of drugs of abuse by GC-MS.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Limit of Detection (ng/mg)</th>
<th>Ions (m/z)</th>
<th>Analyte IS</th>
<th>Ions (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine</td>
<td>0.2</td>
<td>116</td>
<td>Amphetamine-d₅</td>
<td>120</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>0.2</td>
<td>130</td>
<td>Methamphetamine-d₅</td>
<td>135</td>
</tr>
<tr>
<td>MDA</td>
<td>0.2</td>
<td>116</td>
<td>MDA-d₅</td>
<td>120</td>
</tr>
<tr>
<td>MDMA</td>
<td>0.2</td>
<td>130</td>
<td>MDMA-d₅</td>
<td>135</td>
</tr>
<tr>
<td>Codeine</td>
<td>0.2</td>
<td>371, 146, 178</td>
<td>Codeine-d₃</td>
<td>374, 149, 181</td>
</tr>
<tr>
<td>Hydrocodone</td>
<td>0.2</td>
<td>328, 297, 120</td>
<td>Hydrocodone-d₃</td>
<td>331, 300</td>
</tr>
<tr>
<td>Oxycodone</td>
<td>0.2</td>
<td>416, 230, 401</td>
<td>Oxycodone-d₃</td>
<td>419, 233, 404</td>
</tr>
<tr>
<td>Hydromorphone</td>
<td>0.2</td>
<td>386, 355</td>
<td>Hydromorphone-d₃</td>
<td>389, 358</td>
</tr>
<tr>
<td>Oxymorphone</td>
<td>0.2</td>
<td>474, 287</td>
<td>Oxymorphone-d₃</td>
<td>477, 290</td>
</tr>
<tr>
<td>6-AM</td>
<td>0.2</td>
<td>399, 340, 287</td>
<td>6-AM-d₃</td>
<td>402, 343, 290</td>
</tr>
<tr>
<td>Morphine</td>
<td>0.2</td>
<td>429, 196, 236</td>
<td>Morphine-d₃</td>
<td>432, 199, 239</td>
</tr>
<tr>
<td>Cocaine</td>
<td>0.13</td>
<td>182, 82, 303</td>
<td>Cocaine-d₃</td>
<td>185, 85, 306</td>
</tr>
<tr>
<td>Benzoylecognine</td>
<td>0.13</td>
<td>240, 82, 361</td>
<td>Benzoylecognine-d₃</td>
<td>243, 85, 364</td>
</tr>
<tr>
<td>Cocaethylene</td>
<td>0.13</td>
<td>196, 82, 317</td>
<td>Cocaethylene-d₃</td>
<td>199, 82, 320</td>
</tr>
<tr>
<td>Norcocaine</td>
<td>0.13</td>
<td>240, 346</td>
<td>Norcocaine-d₃</td>
<td>243, 349</td>
</tr>
<tr>
<td>Methadone</td>
<td>0.13</td>
<td>296</td>
<td>Methadone-d₃</td>
<td>299</td>
</tr>
<tr>
<td>Meperidine</td>
<td>0.2</td>
<td>247</td>
<td>Meperidine-d₄</td>
<td>251</td>
</tr>
</tbody>
</table>

NOTE: The quantifier ion is underlined, while the qualifier ions are not.

For quality control (QC) procedures, QC samples were analyzed after every 20 patient samples. These QC samples are blank meconium specimens, which were spiked with a known amount of the tested compounds. Any discordance between ELISA and GC-MS analyses resulted in re-analysis of the sample.

1.2 Data Analysis

Meconium results were dichotomized into two groups, positive and negative for methadone. Number and proportions of positive drug classes (cocaine, amphetamines, opioids, cannabinoids, benzodiazepines and alcohol) were calculated. As some of the compounds tested are both metabolites of a drug of abuse and drugs of abuse themselves, the source of these compounds cannot be determined, thus requiring the calculation of minimum and maximum number of positive drugs. For example, morphine is itself a drug of abuse as well as a metabolite of both codeine and heroin; hydromorphone and oxymorphone are drugs of abuse but also metabolites of hydrocodone and morphine, and oxycodone respectively; finally, amphetamine is both a metabolite of methamphetamine and a drug of abuse itself. This
apparent overlap must be taken into consideration when determining the number of positive
drugs detected in the meconium samples.

For statistical analyses, Chi Square and Fisher’s exact tests were performed, where
appropriate, to compare the rates of positivity of individual drugs and drug classes between
methadone-positive and methadone-negative samples. T test was performed to compare the
mean number of positive drugs overall and mean number of positive opioids between the two
groups. All statistical tests were performed using IBM SPSS Statistics 20.0.

2 Prevalence of drug use during pregnancy in Miramichi, NB:
Analysis of a routine urine drug screen in the obstetric unit

2.1 Data Collection

The urine drug screen at Miramichi Regional Hospital (MRH) began in 2006 and screens all
women who deliver in MRH for common drugs of abuse including cocaine, amphetamines,
opioids (including oxycodone and methadone), cannabinoids/marijuana (THC), barbiturates,
benzodiazepines, and phencyclidine (PCP). Urine samples are centrifuged then analyzed by
CEDIA with a Beckman UniCel DxC 600 chemical analyzer to determine qualitatively if a
sample is positive or negative for the tested compounds. The following are the positive cutoffs
for each drug class tested for: amphetamines (e.g. amphetamine and methamphetamine) 1000
ng/mL, benzodiazepines (e.g. diazepam and oxazepam) 200 ng/mL, cocaine 300 ng/mL,
methadone 300 ng/mL, opiates (e.g. codeine, morphine, and 6-MAM) 300 ng/mL, oxycodone
100 ng/mL, and THC 50 ng/mL. If a sample tests positive for drugs of abuse, the attending
physician begins a dialogue with the patient to discuss her drug usage along with the risks
associated with drug use in general and during pregnancy. These individuals are also referred
to the Department of Social Work in MRH to gain access to resources to aid with their
substance use problems.
All deliveries at MRH between April 2006 and January 2013 were identified and their urine screen results accessed. Women with positive urine screen results during pregnancy and/or at the time of delivery were categorized as cases and randomly paired with a control (a woman with negative urine screens during pregnancy).

A chart review was conducted for both cases and controls to collect the following information: all results of urine drug screens performed during the pregnancy, maternal age, weight, height, education level, gravidity, parity, spontaneous abortions, maternal self-report of alcohol, drug and tobacco use, maternal health conditions (including arthritis, asthma, diabetes, epilepsy, hypertension, hypothyroidism, learning disabilities, placental abruption, psychiatric disorders, group B streptococcal and urinary tract infections), and neonatal information (including sex, gestational age at birth, birth weight, length, head circumference, Apgar scores, complications at birth, birth defects and any subsequently diagnosed health conditions). The data collection form used for the chart review is provided in Appendix B.

Research ethics board approvals were granted from both Horizon Health Network and The Hospital for Sick Children, and are provided in Appendix A.

2.2 Data Analysis

Rates of positivity for each year were calculated for each drug, as was the overall rate of drug use in the population. Mann-Whitney U test was used to compare continuous variables whereas Chi Square or Fisher exact tests were used to compare all dichotomous variables. Correlations were employed to determine the variables associated with maternal drug use during pregnancy. A Spearman correlation was conducted and all relevant, significant variables were used in a logistic regression. All significant variables from the maternal characteristics were used in subsequent regressions, both linear and logistic, for other outcomes, in addition to any other relevant variables including any drug use and specific drug use (e.g., stimulant, opioid, marijuana use). All statistical analyses were performed using SPSS Version 20.0.
Prevalence of heavy fetal alcohol exposure in Canada: A multi-center meconium study

3.1 Sample and Data Collection

This study was conducted in collaboration with the Maternal-Infant Research on Environmental Chemicals (MIREC) Study Group, which has been previously described. (Arbuckle et al., 2013)

The MIREC study aims to assess the role environmental chemicals may play on the health of pregnant women and their children. The key environmental chemicals that the MIREC group is studying include heavy metals, such as lead, phthalates, brominated flame retardants and bisphenol A (BPA). Secondary objectives of the study include biomonitoring smoking behaviour and exposure to tobacco, measurement of beneficial compounds (e.g. vitamins and minerals), and assessment of immunoprotective end points (e.g., lysozyme, prolactin).

Ten cities across Canada were included in this study (Vancouver, BC; Edmonton, AB; Winnipeg, MB; Sudbury, ON; Toronto, ON; Hamilton, ON; Kingston, ON; Ottawa, ON; Montreal, QC; and Halifax, NS) and recruited 2,000 volunteering women attending prenatal clinics during the first trimester of pregnancy at the participating sites between 2008 and 2011. Eligibility criteria for enrollment into the MIREC study included: age 18 years or older; <14 weeks gestation; ability to communicate in English or French; plan to deliver at a local hospital; and willingness to provide a cord blood sample. Women were excluded from the study if they had known fetal abnormalities, chromosomal or major malformations in the current pregnancy, and/or a history of medical complications including epilepsy, hepatitis, cancer, hematological disorder, threatened spontaneous abortion, illicit drug use, or disease of the major organs (heart, kidney, liver, lungs).

Upon enrollment into the MIREC study, women were contacted in each trimester, at delivery, and up to 10 weeks post delivery. Trained research staff administered questionnaires during each trimester, and conducted post-delivery interviews. Chart reviews were conducted in the first and second trimesters, as well as post-delivery. All participant data were de-identified and
each participant was assigned a coded ID number. In addition to the questionnaires, numerous biospecimens were collected throughout the pregnancy. Details of biospecimen collection and timing of collection are summarized in Figure 3.1. Remaining biospecimens were catalogued in a Biobank at the Institut National de Santé Publique du Québec (INSPQ) when proper consent was obtained from participating women. Access to the Biobank is granted to future researchers studying the health of pregnant women and their children.

**Figure 3.1. MIREC questionnaire biospecimen collection details.**

<table>
<thead>
<tr>
<th>Visit Type</th>
<th>n</th>
<th>Sample Collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prenatal Visit #1</td>
<td>1983</td>
<td>Questionnaire and Medical Record Review, Maternal Urine and Blood</td>
</tr>
<tr>
<td>Prenatal Visit #2</td>
<td>1831</td>
<td>Questionnaire and Medical Record Review, Maternal Urine and Blood</td>
</tr>
<tr>
<td>Prenatal Visit #3</td>
<td>1733</td>
<td>Questionnaire and Medical Record Review, Maternal Urine and Blood</td>
</tr>
<tr>
<td>Delivery</td>
<td>1727</td>
<td>Questionnaire and Medical Record Review, Maternal Urine and Blood, Cord Blood (n = 1516)</td>
</tr>
<tr>
<td>Post Partum Visit #1</td>
<td>1727</td>
<td>Questionnaire and Medical Chart Review, Meconium (n = 1591)</td>
</tr>
<tr>
<td>Post Partum Visit #2</td>
<td>1385</td>
<td>Questionnaire, Maternal Hair (n = 1490), Breast Milk (n = 1017)</td>
</tr>
</tbody>
</table>
In regard to this specific collaboration, meconium samples were collected from 1,591 neonates, with 1,436 available for collection from the MIREC Biobank. Approximately 500 mg of meconium was collected from each Biobank sample and transported to The Hospital for Sick Children (HSC). Meconium samples were stored in -80°C until analysis. Select questionnaire and health chart review data of all women who consented to their data being used in future studies were also sent to HSC, and are summarized in Appendix C. Maternal self-report of alcohol consumption during pregnancy was obtained from the third trimester (between 32 and 35 weeks gestation) questionnaire. Participants were asked questions regarding their alcohol consumption in the previous 3 months. Questions included:

- During the past 3 months, have you had a drink of beer, wine, liquor or any other alcoholic beverage? If yes, how much did you drink of the following?
  - White wine (1 glass = 4 oz.)
  - Red wine (1 glass = 4 oz.)
  - Beer (1 glass = 8 oz.)
  - Liquor (1 drink = 1 oz.)

- Thinking back over the past 3 months, did you ever consume 5 or more alcoholic drinks on one occasion? If yes, how many times?

In terms of frequency and amount consumed, participants were asked to provide the number of drinks consumed of each alcohol subtype and the frequency of consumption (per day, week, month, or 3 months). Frequency of binge drinking (consuming 5 or more alcoholic drinks on one occasion) was also collected per week, month, and 3 months. Self-report of alcohol consumption was also collected in the first trimester (between 6 and 14 weeks gestation) and post-delivery (up to 10 weeks after delivery). However, these self-reports do not exclusively include the pregnancy period, and were not used for any comparisons with meconium results, but rather were used to analyze any changes in alcohol consumption behaviour throughout pregnancy.

Research ethics board approval was granted from Health Canada, CHU St. Justine Hospital, and The Hospital for Sick Children; documentation is provided in Appendix A.
3.2 Sample Analysis

For meconium analysis of FAEEs, a previously published method was used and is comprised of a liquid-liquid extraction followed by headspace solid-phase microextraction, coupled with gas chromatography-mass spectrometry. (Hutson et al., 2011) Blank meconium was pooled and confirmed to have a total FAEE concentration of less than 0.4 nmol/g. This meconium was then used for calibration standards and quality control (QC) samples. Initially, a five-point calibration curve was used for FAEE concentration quantification (0, 50, 100, 200, 400, and 1,000 ng). After repeating this curve in triplicate with consistent results of an $r^2$ greater than 0.98, a single point calibration curve (400 ng) was used for concentration calculations, as previously validated by Hutson et al. (2011). Samples were thawed at room temperature and weighed in 15 ml disposable glass centrifuge tubes (Fisher Scientific; Toronto, ON). A minimum of 100 mg of meconium is required for analysis with 500 mg being the recommended and optimal weight.

Calibration standards and QC samples were prepared using a FAEE mix (10 µg/mL) and a deuterated (d5) FAEE mix (10 µg/mL) of the 4 FAEEs measured (ethyl palmitate, ethyl oleate, ethyl linoleate and ethyl stearate). Forty microliters of the d5-FAEE mix was pipetted into each standard or QC to serve as the internal standard. Forty microliters of the FAEE mix were pipetted into duplicate calibration standards (one-point calibration curve) as well as high QC samples. Ten microliters of the FAEE mix were pipetted into low QC samples. Quality control samples were analyzed after every 25 research samples, alternating between low and high concentrations.

Research samples, like the calibration standards and QC samples, were spiked with 40 µl of the d5-FAEE mix. All research samples, calibrators and QC samples were extracted using 5 mL of a 5:2 heptane:acetone solution. Tubes were vortexed for 1 minute then centrifuged at 3,500 rpm for 15 minutes at 4°C. Upon completion of centrifugation, the supernatant (heptane layer) was removed using a glass Pasteur pipette and placed in a 10 mL SPME vial (Supelco;
Bellefonte, PA). The heptane layer was then dried under nitrogen flow at approximately 10 psi at 37°C. Once completely dried, the sample was then reconstituted using 1 mL of pH 7.6 phosphate buffer.

Extracted samples were then analyzed using headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry (SPME GC-MS). In terms of SPME, the pre-incubation time was 5 minutes followed by an incubation period of 40 minutes at 90°C and a desorption period of 12 minutes. The GC-MS analysis parameters are included in Appendix D. Chromatography results were analyzed using GCMSsolutions Software. Both retention time and qualifier and quantifier ions were used to identify peaks of the specified analytes. The retention time of the analyte and its calibrator differ by 0.2 minutes, and the qualifying and quantifier ions for each FAEE are listed in Table 3.2. Once the analyte and calibrator peaks were identified, manual integration was performed to calculate the peak area counts. The ratio of FAEE/d5-FAEE peak area is calculated for each FAEE and then converted to nmol. The 4 FAEEs were then added together to achieve a cumulative sum, then divided by the sample’s weight to determine the total concentration of FAEEs. The LOD and limit of quantification (LOQ) of each individual FAEE and the total cumulative sum are listed in Table 3.3. The cutoff of 2 nmol/g, established by Chan et al. (2003) was used to determine if a sample was positive or negative. (Chan et al., 2003) The inter-day and intra-day variability was 6.51% and 5.41%, and 6.45% and 5.16%, for low and high QC samples respectively.

Table 3.2. Qualifier and quantifier ions for the 4 FAEEs analyzed in meconium by GC-MS.

<table>
<thead>
<tr>
<th>FAEE</th>
<th>Qualifier Ions (m/z)</th>
<th>Quantifier Ions (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl palmitic</td>
<td>88, 101, 157</td>
<td>284</td>
</tr>
<tr>
<td>IS -Ethyl palmitic</td>
<td>93, 106, 162</td>
<td>289</td>
</tr>
<tr>
<td>Ethyl linoleic</td>
<td>81, 95</td>
<td>308</td>
</tr>
<tr>
<td>IS -Ethyl linoleic</td>
<td>87, 100, 114, 272</td>
<td>313</td>
</tr>
<tr>
<td>Ethyl oleic</td>
<td>88, 101</td>
<td>310</td>
</tr>
<tr>
<td>IS-Ethyl oleic</td>
<td>93, 106</td>
<td>315</td>
</tr>
<tr>
<td>Ethyl stearic</td>
<td>88, 101, 157</td>
<td>312</td>
</tr>
<tr>
<td>IS-Ethyl stearic</td>
<td>93, 106, 162</td>
<td>317</td>
</tr>
</tbody>
</table>
Table 3.3. Limits of detection and quantification for the 4 FAEEs analyzed in meconium by GC-MS.

<table>
<thead>
<tr>
<th>Individual FAEE</th>
<th>LOD (ng/vial)</th>
<th>LOQ (ng/vial)</th>
<th>Upper limit of quantification (nmol/g meconium)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic</td>
<td>5.902</td>
<td>17.706</td>
<td></td>
</tr>
<tr>
<td>Linoleic</td>
<td>5.961</td>
<td>17.882</td>
<td></td>
</tr>
<tr>
<td>Oleate</td>
<td>3.839</td>
<td>11.517</td>
<td></td>
</tr>
<tr>
<td>Stearate</td>
<td>3.127</td>
<td>9.380</td>
<td></td>
</tr>
</tbody>
</table>

If a transitional sample (i.e., a sample that is a combination of both meconium and stool) was found to be positive, it was deemed inconclusive to avoid the elevated risk of a false positive result. (Zelner et al., 2012a) Transitional samples were identified either through visual inspection of the sample or by the characteristic low peak area counts of the d5-FAEE analytes. Any samples that had poor chromatography (i.e. poor peak separation, interfering background levels), results close to the 2 nmol/g cutoff, or were lost during sample preparation were re-analyzed and the second result was used for data analysis.

All meconium sample results were compiled with the corresponding questionnaire data from the MIREC Study Group. Meconium collection information was also obtained from the MIREC group and compiled within a database.

3.3 Data Analysis

Prevalence of heavy in utero exposure to alcohol was calculated for the entire study population. To account for meconium collection time, findings from previous research were utilized to adjust for the elevated risk of false positive results, as follows. In Zelner et al. (2012), after serial meconium collection the false positive rate was approximately 20%, 40% and 60% after 24, 48 and 72 hours, respectively. The following equation was used to calculate the prevalence rates:
Meconium FAEE analysis results were compared to maternal alcohol consumption self-report data from the third trimester (between 32 and 35 weeks gestation) to determine any disparities. As well, meconium results (both cumulative and individual FAEE concentrations) were correlated, using Spearman correlation; with maternal self-report of alcohol consumption (grams of ethanol consumed/week) to determine if a dose-response was present. Individual FAEE concentrations were also analyzed to determine if any single FAEE was prevalent more frequently or at higher concentrations in meconium samples overall, or in positive or transitional samples. Median FAEE concentrations were also compared between positive and negative samples, as well as between twin neonate results, using Mann-Whitney U statistical analysis.

Maternal characteristics (e.g., education level, income, age, smoking status, etc.) and neonatal outcomes (e.g., birth measurements, gestational age, Apgar scores, complications, etc.) of neonates with positive meconium samples were compared to neonates with a negative meconium result, using Chi Square, Fisher Exact and Mann-Whitney U tests where appropriate, to determine if any of these variables are associated with alcohol consumption during pregnancy. Neonatal birth measurements (i.e., birth weight, length and head circumference) were also correlated, using Spearman correlation, with meconium FAEE concentration to determine if any relationships are present.

Maternal smoking status and alcohol consumption during pregnancy were compared for each questionnaire, using Chi Square and Mann-Whitney U tests where appropriate, to determine if behavioural changes occurred during pregnancy. All statistical tests were performed using IBM SPSS Statistics 20.0.
Chapter 4
Results

1 Rates of fetal polydrug exposures in methadone-maintained pregnancies from a high risk population

The following section of this thesis has been previously peer reviewed and published:


[KD helped with study design, performed all data analysis, and prepared the manuscript for submission]

Of the 273 meconium samples analyzed for methadone, 164 were found to be positive and 109 were negative. Fifty-eight (35.37%) of methadone-positive samples were positive, at minimum, for one additional opioid and 18 (10.98%) were positive for 2 additional opioids. In regards to maximum number of positive opioids, 39 (23.78%), 19 (11.59%), 14 (8.54%), 3 (1.83%) and 1 (0.61%) were positive for 1, 2, 3, 4 and 5 additional opioids respectively.

No statistically significant differences were found with regards to proportions of illicit drug use between the methadone-positive and negative groups for any drug class (Table 4.1); although a trend towards higher rates of amphetamine (drug) and benzodiazepine (class) use was noted (Table 4.2). As well, no statistical difference was found for mean minimum number of positive drugs (methadone positive 1.293, SD = 0.997; methadone negative 1.431, SD= 1.141; p=0.477). Also, no statistical difference was found for mean maximum number of positive drugs (methadone positive 1.567, SD = 1.348; methadone negative 1.716, SD = 1.441; p=0.451)
Table 4.1. Rates of positivity for six drug classes.

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Rate of Positivity (%) (n)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methadone Positive (N=164)</td>
<td>Methadone Negative (N=109)</td>
</tr>
<tr>
<td>Amphetamines</td>
<td>4.27 (7)</td>
<td>2.75 (3)</td>
</tr>
<tr>
<td>Cocaine</td>
<td>30.49 (50)</td>
<td>26.61 (29)</td>
</tr>
<tr>
<td>Opioids</td>
<td>46.34 (76)</td>
<td>46.79 (51)</td>
</tr>
<tr>
<td>Cannabinoids</td>
<td>55.10 (54)</td>
<td>52.17 (48)</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>14.29 (4)</td>
<td>3.23 (2)</td>
</tr>
<tr>
<td>Alcohol</td>
<td>5.88 (4)</td>
<td>6.67 (4)</td>
</tr>
</tbody>
</table>

* Pearson Chi-square test performed; † Fisher’s Exact test performed; * Methadone positive n=98, Methadone negative n=92; † Methadone positive n=28, Methadone negative n=62; § Methadone positive n=68, Methadone negative n=60.

When looking specifically at opioids, no statistical difference was found for either mean minimum or maximum number of positive opioids (min: methadone positive 0.580, SD = 0.685; methadone negative 0.602, SD = 0.723; p=0.907; max: methadone positive 0.840, SD=1.114; methadone negative 0.880, SD=1.174; p=0.913).

Table 4.2. Rates of positivity for individual compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rate of Positivity (%) (n)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine*</td>
<td>5.31 (6)</td>
<td>1.09 (1)</td>
</tr>
<tr>
<td>Methamphetamine#</td>
<td>3.57 (4)</td>
<td>2.36 (3)</td>
</tr>
<tr>
<td>MDMA§</td>
<td>0</td>
<td>2.17 (2)</td>
</tr>
<tr>
<td>Meperidine*</td>
<td>4.55 (1)</td>
<td>1.09 (1)</td>
</tr>
<tr>
<td>Cocaine*</td>
<td>23.46 (38)</td>
<td>21.30 (23)</td>
</tr>
<tr>
<td>Ecstasy*</td>
<td>2.47 (4)</td>
<td>0.94 (1)</td>
</tr>
<tr>
<td>Benzoylecgonine</td>
<td>30.86 (50)</td>
<td>27.78 (30)</td>
</tr>
<tr>
<td>Norcocaine*</td>
<td>12.30 (21)</td>
<td>14.81 (16)</td>
</tr>
<tr>
<td>Codeine*</td>
<td>11.73 (19)</td>
<td>16.67 (18)</td>
</tr>
<tr>
<td>Morphine*</td>
<td>29.63 (48)</td>
<td>20.37 (22)</td>
</tr>
<tr>
<td>6-MAM§</td>
<td>0.62 (1)</td>
<td>0.93 (1)</td>
</tr>
<tr>
<td>Hydromorphone*</td>
<td>11.73 (19)</td>
<td>11.11 (12)</td>
</tr>
<tr>
<td>Oxycodone*</td>
<td>17.28 (28)</td>
<td>22.22 (24)</td>
</tr>
<tr>
<td>Oxymorphone*</td>
<td>12.96 (21)</td>
<td>16.67 (18)</td>
</tr>
</tbody>
</table>

* Pearson Chi-square test performed; † Fisher’s Exact test performed; * Methadone positive n=113, Methadone negative n=92; † Methadone positive n=112, Methadone negative n=92; § Methadone positive n=111, Methadone negative n=92; † Methadone positive n=22, Methadone negative n=66; § Methadone positive n=162, Methadone negative n=108; * Methadone positive n=162, Methadone negative n=106; † Methadone positive n=162, Methadone negative n=108.

When looking specifically at opioids, no statistical difference was found for either mean minimum or maximum number of positive opioids (min: methadone positive 0.580, SD = 0.685; methadone negative 0.602, SD = 0.723; p=0.907; max: methadone positive 0.840, SD=1.114; methadone negative 0.880, SD=1.174; p=0.913).
Prevalence of drug use during pregnancy in Miramichi, NB: Analysis of a routine urine drug screen in the obstetric unit

Since April 2006, there were 2678 deliveries at Miramichi Regional Hospital, of which all women but 163 (6.01%) had urine samples collected at the time of delivery. Three hundred and seventy-eight cases (three of whom delivered twins) were identified with positive urine drug screens either during pregnancy or at time of delivery. This yields a 15.03% positivity rate over the 82-month period. Across all years, THC was the most commonly detected compound, while opiates and oxycodone were the next most prevalent compounds for the majority of years (Table 4.3). Thirty-one percent of cases were positive for more than one substance while only 20.6% of women accurately self-reported their substance use during pregnancy. Opiates plus marijuana was the most common drug combination in this population. An increase in overall drug use is noted, from 12.35% in 2006 to 17.63% in 2012, and shown in Figure 4.1. Table 4.3 highlights any changes in overall or specific drug positivity rates.

Table 4.3. Rate of positivity for overall and specific drug use.

<table>
<thead>
<tr>
<th>Rate of Positivity</th>
<th>2006 (n=324)</th>
<th>2007 (n=403)</th>
<th>2008 (n=375)</th>
<th>2009 (n=364)</th>
<th>2010 (n=392)</th>
<th>2011 (n=400)</th>
<th>2012 (n=397)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Substance Use*</td>
<td>12.35%</td>
<td>12.41%</td>
<td>12.00%</td>
<td>12.91%</td>
<td>14.80%</td>
<td>16.00%</td>
<td>17.63%</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>1.23%</td>
<td>0.74%</td>
<td>0.53%</td>
<td>1.10%</td>
<td>0.77%</td>
<td>0.00%</td>
<td>3.02%</td>
</tr>
<tr>
<td>Barbiturates#</td>
<td>0.93%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.27%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>3.09%</td>
<td>2.73%</td>
<td>1.07%</td>
<td>2.20%</td>
<td>2.81%</td>
<td>3.00%</td>
<td>2.02%</td>
</tr>
<tr>
<td>Opiates</td>
<td>4.32%</td>
<td>2.23%</td>
<td>3.20%</td>
<td>4.12%</td>
<td>6.63%</td>
<td>3.75%#</td>
<td>4.53%</td>
</tr>
<tr>
<td>Oxycodone*</td>
<td>0.31%</td>
<td>0.74%</td>
<td>3.73%#</td>
<td>3.02%</td>
<td>2.55%</td>
<td>3.25%</td>
<td>2.32%</td>
</tr>
<tr>
<td>PCP</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.25%</td>
</tr>
<tr>
<td>Cocaine</td>
<td>0.93%</td>
<td>0.99%</td>
<td>0.27%</td>
<td>1.65%#</td>
<td>0.51%</td>
<td>0.50%</td>
<td>0.76%</td>
</tr>
<tr>
<td>THC</td>
<td>7.10%</td>
<td>8.93%</td>
<td>6.93%</td>
<td>7.97%</td>
<td>6.63%</td>
<td>10.75%#</td>
<td>9.32%</td>
</tr>
<tr>
<td>Methadone</td>
<td>2.47%</td>
<td>0.99%</td>
<td>1.87%</td>
<td>4.40%#</td>
<td>1.53%#</td>
<td>3.00%</td>
<td>2.52%</td>
</tr>
</tbody>
</table>

*Significant difference in rate of positivity between 2006 and 2012
#Significant difference (p < 0.05) in rate of positivity compared to previous year
Non-significant trend (0.05 < p > 0.10): if indicated beside variable this trend is between 2006 and 2012
Table 4.4 and Table 4.5 outline the maternal characteristics and neonatal outcomes that were found to be statistically significant between cases and controls. Case women tended to have lower education, more psychiatric disorders and higher rates of smoking and alcohol use than controls. Neonates born to case women had more complications in general, including jitteriness and respiratory distress, smaller measurements of weight, length and head circumference, and longer stay in hospital.
Table 4.4. Significant maternal characteristics and neonatal outcomes through Chi Square and Fisher Exact Test analyses.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases, n (%)</th>
<th>Controls, n (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maternal Characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education – Elementary School*</td>
<td>138 (44.1)</td>
<td>75 (19.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Education – Post Secondary</td>
<td>50 (16.0)</td>
<td>127 (33.8)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Involved with Children's Aid*</td>
<td>10 (2.6)</td>
<td>0 (0.0)</td>
<td>0.002</td>
</tr>
<tr>
<td>Psychiatric Disorders</td>
<td>90 (23.6)</td>
<td>46 (12.1)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Self report - Alcohol</td>
<td>27 (7.1)</td>
<td>11 (2.9)</td>
<td>0.008</td>
</tr>
<tr>
<td>Self report - Smoking</td>
<td>246 (64.6)</td>
<td>72 (18.9)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Neonatal Outcomes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any complication at birth</td>
<td>222 (58.4)</td>
<td>148 (39.5)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Congested*</td>
<td>6 (1.6)</td>
<td>0 (0.0)</td>
<td>0.03</td>
</tr>
<tr>
<td>Cord around neck</td>
<td>25 (6.6)</td>
<td>11 (2.9)</td>
<td>0.02</td>
</tr>
<tr>
<td>High pitched crying*</td>
<td>10 (2.6)</td>
<td>0 (0.0)</td>
<td>0.002</td>
</tr>
<tr>
<td>Increased muscle tone*</td>
<td>14 (3.7)</td>
<td>0 (0.0)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Indrawing of abdomen*</td>
<td>14 (3.7)</td>
<td>1 (0.3)</td>
<td>0.001</td>
</tr>
<tr>
<td>Infant required morphine*</td>
<td>17 (4.5)</td>
<td>0 (0.0)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Irritable*</td>
<td>13 (3.4)</td>
<td>0 (0.0)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>IUGR*</td>
<td>8 (2.1)</td>
<td>1 (0.3)</td>
<td>0.04</td>
</tr>
<tr>
<td>Jittery*</td>
<td>47 (12.3)</td>
<td>4 (1.0)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Large Anterior Fontanelle*</td>
<td>8 (2.1)</td>
<td>0 (0.0)</td>
<td>0.008</td>
</tr>
<tr>
<td>LGA – Birth Weight</td>
<td>31 (8.3)</td>
<td>57 (15.0)</td>
<td>0.004</td>
</tr>
<tr>
<td>LGA – Head Circumference</td>
<td>48 (13.4)</td>
<td>80 (22.0)</td>
<td>0.002</td>
</tr>
<tr>
<td>LGA – Length</td>
<td>64 (17.8)</td>
<td>93 (25.6)</td>
<td>0.01</td>
</tr>
<tr>
<td>Meconium stained amniotic fluid</td>
<td>15 (3.9)</td>
<td>5 (1.3)</td>
<td>0.02</td>
</tr>
<tr>
<td>Mucousy</td>
<td>32 (8.4)</td>
<td>7 (1.8)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>NAS*</td>
<td>72 (18.9)</td>
<td>0 (0.0)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Respiratory Distress</td>
<td>23 (6.0)</td>
<td>9 (2.4)</td>
<td>0.01</td>
</tr>
<tr>
<td>Resuscitation required*</td>
<td>6 (1.6)</td>
<td>0 (0.0)</td>
<td>0.03</td>
</tr>
<tr>
<td>SGA – Birth Weight</td>
<td>48 (12.8)</td>
<td>17 (4.5)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SGA – Length</td>
<td>25 (7.0)</td>
<td>13 (3.6)</td>
<td>0.04</td>
</tr>
<tr>
<td>Tachypnea*</td>
<td>13 (2.0)</td>
<td>2 (0.5)</td>
<td>0.007</td>
</tr>
<tr>
<td>Transferred to Another Hospital*</td>
<td>13 (3.4)</td>
<td>3 (0.8)</td>
<td>0.02</td>
</tr>
<tr>
<td>Tremors – disturbed*</td>
<td>14 (3.7)</td>
<td>0 (0.0)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Two vessel cord*</td>
<td>7 (1.8)</td>
<td>0 (0.0)</td>
<td>0.06</td>
</tr>
<tr>
<td>Withdrawal*</td>
<td>10 (2.6)</td>
<td>0 (0.0)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

* Fisher Exact test; N= 381, except for variables noted with * where cases n= 313 and controls n=376.
LGA = large for gestational age; SGA = small for gestational age.
Table 4.5. Significant maternal characteristics and neonatal outcomes through Mann Whitney U test analyses.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases Median (IQR Range) [n]</th>
<th>Controls Median (IQR Range) [n]</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal BMI</td>
<td>29.53 (25.76-34.37) [361]</td>
<td>31.84 (27.61-36.73) [368]</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Parity</td>
<td>1 (0-2) [380]</td>
<td>1 (0-1) [381]</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Spontaneous Abortions</td>
<td>0 (0-1) [380]</td>
<td>0 (0-0) [381]</td>
<td>0.01</td>
</tr>
<tr>
<td>Cigarettes per day</td>
<td>4 (0-12) [372]</td>
<td>0 (0-0) [378]</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Gestational Age (d)</td>
<td>273 (267-280) [378]</td>
<td>274 (269-282) [381]</td>
<td>0.01</td>
</tr>
<tr>
<td>Birth Weight (g)</td>
<td>3252 (2903-3537) [381]</td>
<td>3493 (3206-3775) [381]</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>50 (48.5-52) [361]</td>
<td>51.5 (50-53) [364]</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>34.5 (33.5-35.5) [363]</td>
<td>35 (34-36) [365]</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Hospital Stay (d)</td>
<td>3 (2.5-4.69) [374]</td>
<td>2.75 (2-3) [367]</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Logistic regression revealed that gravidity, smoking status, the number of cigarettes smoked per day and psychiatric disorders were positively associated with maternal drug use while BMI, parity, and spontaneous abortions were negatively associated (Table 4.6).
Table 4.6. Logistic regression of predictors of maternal substance use during pregnancy. Cox & Snell R square = 0.301.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>p</th>
<th>Odds ratio</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>0.20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Age</td>
<td>0.59</td>
<td>0.99</td>
<td>0.95 – 1.03</td>
</tr>
<tr>
<td>BMI</td>
<td>0.001</td>
<td>0.95</td>
<td>0.93 – 0.98</td>
</tr>
<tr>
<td>Elementary School</td>
<td>0.91</td>
<td>1.11</td>
<td>0.19 – 6.47</td>
</tr>
<tr>
<td>High School</td>
<td>0.67</td>
<td>0.69</td>
<td>0.12 – 3.86</td>
</tr>
<tr>
<td>Post Secondary School</td>
<td>0.50</td>
<td>0.56</td>
<td>0.10 – 3.11</td>
</tr>
<tr>
<td>Gravidity</td>
<td>0.001</td>
<td>12.66</td>
<td>2.65 – 60.43</td>
</tr>
<tr>
<td>Parity</td>
<td>0.002</td>
<td>0.08</td>
<td>0.02 – 0.38</td>
</tr>
<tr>
<td>Spontaneous Abortions</td>
<td>0.008</td>
<td>0.12</td>
<td>0.03 – 0.57</td>
</tr>
<tr>
<td>Alcohol Self Report</td>
<td>0.37</td>
<td>1.56</td>
<td>0.59 – 4.14</td>
</tr>
<tr>
<td>Smoking Status</td>
<td>&lt; 0.001</td>
<td>3.19</td>
<td>1.79 – 5.70</td>
</tr>
<tr>
<td>Cigarettes per day</td>
<td>0.001</td>
<td>1.10</td>
<td>1.04 – 1.17</td>
</tr>
<tr>
<td>Placental abruption</td>
<td>0.58</td>
<td>1.76</td>
<td>0.23 – 13.34</td>
</tr>
<tr>
<td>Psychiatric Disorders</td>
<td>0.02</td>
<td>1.79</td>
<td>1.08 – 2.96</td>
</tr>
</tbody>
</table>

A series of regression analyses were performed to determine if any maternal variables or drug use, general or specific, were associated with maternal and neonatal outcomes. Drug use in general was associated with jitteriness (OR 5.91, p = 0.006, R² = 0.08) and low birth weight (β = -0.18, p = 0.006, R² = 0.138). Opioid and benzodiazepine use were associated with neonatal abstinence syndrome (OR 6.27, p < 0.001 and OR 2.16, p = 0.04 respectively; R² = 0.203).

Opioid and stimulant (cocaine and amphetamine) use were associated with longer neonatal hospital stay (β = 0.27, p < 0.001 and β = 0.09, p = 0.02 respectively; R² = 0.156).

Benzodiazepine use was also associated with lower birth length (β = -0.09, p = 0.03; R² = 0.092). Smoking status and the number of cigarettes per day were included in all regressions to determine whether outcomes were solely due to drug use or in combination with smoking.

Smoking status or the number of cigarettes per day was associated with neonatal abstinence syndrome (smoking status: OR 3.01, p = 0.02), jitteriness (cigarettes per day: OR 1.08, p = 0.007), birth weight, (cigarettes per day: β = -0.11, p = 0.04) and birth length (smoking status: β = -0.16, p = 0.007).
3 Prevalence of heavy fetal alcohol exposure in Canada: A multi-center meconium study

Overall, 1,440 meconium samples were provided from the MIREC Biobank, with 1,315 eligible for FAEE analysis and 125 either having not sufficient quantity (NSQ) for analysis or were identified to be postnatal stool. The meconium analysis results were divided by result type and meconium collection time, and are summarized in Table 4.7. By applying the equation previously provided to all the samples that were analyzed for FAEEs, the prevalence of heavy fetal alcohol exposure is 1.16%. This equation took meconium collection time into consideration, to account for the increased risk of false positive results with delayed sample collection. Considering only the samples collected within the first 24 hours, the prevalence rate would increase to 2.40%.

Table 4.7. FAEE meconium analysis results and overall prevalence of heavy fetal alcohol exposure in Canada.

<table>
<thead>
<tr>
<th>Meconium Result</th>
<th>Meconium Collection Time</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;24 h</td>
<td>24-48 h</td>
</tr>
<tr>
<td>Positive</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>Negative</td>
<td>157</td>
<td>478</td>
</tr>
<tr>
<td>Inconclusive</td>
<td>6</td>
<td>63</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>RATE</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Numbers represent meconium samples analyzed for FAEEs. Equation used to calculate rate of 1.16% is Prevalence = [(# of positives <24 h + (# of positives 24-48 h/2) + (# of positives >48 h/3))/Total Samples] * 100
Comparing the meconium results to their respective maternal self-reports generated from the 32–35 Week Gestation questionnaire, the majority of women reported no alcohol consumption during the previous 3 months (Table 4.8). A small percentage (0.24%) reported higher than social level drinking on this questionnaire, which was defined as more than 2 standard drinks per week. The majority of women (82.39%) reported no alcohol consumption during pregnancy, with the remaining 17.37% reporting social level drinking (less than 2 standard drinks per week). Of the 32 samples found to be positive for FAEEs, only 4 of the women reported social level drinking and none reported higher levels of drinking. If only self-report was used to determine the rate of heavy alcohol consumption during pregnancy, the prevalence rate would be 0.23%, which is approximately five times lower than the conservative prevalence rate determined with all meconium results, and approximately ten-and-a-half times lower than the prevalence rate determined by the meconium results collected within the optimal time frame.
Table 4.8. FAEE meconium analysis results and maternal alcohol consumption self-report data.

<table>
<thead>
<tr>
<th>Meconium Result</th>
<th>&lt; 24 h</th>
<th>24 - 48 h</th>
<th>&gt; 48 h</th>
<th>N/A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ND</td>
<td>SD</td>
<td>HD</td>
<td>ND</td>
</tr>
<tr>
<td>Positive</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Negative</td>
<td>130</td>
<td>21</td>
<td>1</td>
<td>373</td>
</tr>
<tr>
<td>Inconclusive</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>49</td>
</tr>
</tbody>
</table>

ND = no drinking, SD = < 27.2g/week, HD = > 27.2 g/week; 27.2 g/week is the equivalent of 2 standard drinks.
To determine if there were any differences in meconium FAEE concentration between women who did and did not report any alcohol consumption in the 32-35 Week Gestation questionnaire, both the median FAEE concentration of the “Any Alcohol Consumption” and “No Alcohol Consumption” groups, as well as the percent of self-reported alcohol consumption of the “Positive” and “Negative”, were compared. There were no significant differences found for any of these analyses (Figure 4.2 and 4.3). The only difference detected was between the percent of self-reported alcohol consumption between the Baseline and 32-35 Week Gestation questionnaires for the “Negative” group (p < 0.001). These results indicate that there is no difference in self-reported alcohol consumption between individuals with positive or negative meconium results, nor was there a difference in FAEE concentration between those who did and did not report alcohol consumption. A correlation was performed between self-reported alcohol consumption (g/week) and FAEE concentration, which was not found to be significant ($R^2 = 0.0007$, p = 0.753) (Figure 4.4). This result indicates that no dose-response relationship could be established in this study, likely due to the inaccuracy of maternal self-reports.
Figure 4.2. Median FAEE concentration (nmol/g) of women who reported any or no alcohol consumption in the 32-35 Week Gestation questionnaire. Any alcohol consumption n=221, no alcohol consumption n=1,030. $p = 0.767$ where the bolded line indicates the median, the top of box plot indicates the 75th percentile and whiskers indicate the 90th percentile.
Figure 4.3. Comparison of self-report of any alcohol consumption in past three months at both Baseline (12 week) and 32-35 Week Gestation questionnaires for individuals with positive and negative FAEE meconium analyses. ** indicates p < 0.001 (Baseline: Positive n = 32, Negative n = 1,112; 32-35 week: Positive n = 29, Negative n = 1,069)
Figure 4.4. Correlation between self-reported ethanol consumption (g/week) and FAEE meconium analysis result. $R^2 = 0.0007$, $p = 0.753$.

Looking further into the self-reported alcohol consumption in this study cohort, median ethanol consumption (g/week) was compared for all 3 questionnaires administered for the entire study cohort (Figure 4.5). Statistical differences were found between each questionnaire, with alcohol consumption appearing to decrease throughout the pregnancy, then increasing upon delivery of the child. Despite the medians of both the Baseline and 32-35 Week Gestation questionnaires being zero, the statistical difference is due to the difference in distribution of ethanol consumption per week (g), indicating more variability in reported alcohol consumption in the Baseline questionnaire than the 32-35 Week questionnaire. In terms of binge drinking, three women reported binge episodes (>5 or more drinks on one occasion) in the 32-35 Week Gestation questionnaire. The corresponding FAEE meconium analysis results for these 3 individuals were all negative (< 2 nmol/g).
When looking at neonatal outcomes, focus was placed on birth measurements and gestational age. These outcomes were compared between neonates with and without positive FAEE meconium results. Positive neonates were found to have a slightly higher median gestational age than negative neonates ($p = 0.038$) while the other outcomes were not found to differ between the two groups (Table 4.9). Also, positive and negative groups did not differ.
significantly with the presence of congenital anomalies (3.13\% vs. 2.48\%; \(p = 0.559\)), with one such neonate identified in the positive group and 29 neonates in the negative group.

**Table 4.9.** Neonatal birth outcomes for neonates with or without positive FAEE meconium results.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Positive</th>
<th>Negative</th>
<th>(p) value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (IQR Range)</td>
<td>Median (IQR Range)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>[n]</td>
<td>[n]</td>
<td></td>
</tr>
<tr>
<td>Gestational Age (week)</td>
<td>40 (38.25-41)</td>
<td>39 (38-40)</td>
<td>0.038</td>
</tr>
<tr>
<td></td>
<td>[32]</td>
<td>[1269]</td>
<td></td>
</tr>
<tr>
<td>Birth Weight (g)</td>
<td>3495 (2922.5-3947)</td>
<td>3415 (3110-3727)</td>
<td>0.612</td>
</tr>
<tr>
<td></td>
<td>[32]</td>
<td>[1269]</td>
<td></td>
</tr>
<tr>
<td>Length (cm)</td>
<td>51 (49-52.75)</td>
<td>51 (50-53)</td>
<td>0.557</td>
</tr>
<tr>
<td></td>
<td>[28]</td>
<td>[1140]</td>
<td></td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>35 (34-36)</td>
<td>35 (34-36)</td>
<td>0.720</td>
</tr>
<tr>
<td></td>
<td>[30]</td>
<td>[1219]</td>
<td></td>
</tr>
</tbody>
</table>

Looking at other maternal characteristics of this study population, it was found that over 10\% of the population continued smoking into the third trimester of pregnancy. A slight decrease in the proportion of smokers was noted between the Baseline and 32-35 Week Gestation questionnaires; however, this was not found to be statistically significant (\(p = 0.086\)) (Figure 4.6). Additionally, maternal educational level and annual household income were analyzed (Figure 4.7). The median annual household income for this population was found to be in the $80,000 to $90,000 bracket, while approximately 62\% had either an undergraduate or graduate school degree. The key maternal characteristics, year of birth, education level and income, were compared between positive and negative FAEE result groups and none were found to be significantly different (\(p = 0.809, 0.457\) and 0.233 respectively).
Figure 4.6. Self-report of current smoking in baseline and 32-35 week gestation questionnaires. $p = 0.086$

Figure 4.7. Education level and annual household income of the MIREC Study population.
Chapter 5
Discussion

1 Summary and Significance of Research Findings

1.1 Rates of fetal polydrug exposures in methadone-maintained pregnancies from a high risk population

The following section of this thesis has been previously peer reviewed and published, with minor changes for editorial purposes:


[KD helped with study design, performed all data analysis, and prepared manuscript for submission]

Individuals are expected to be engaged in MMT with the clear clinical goal of replacing the need for other opioids. While in most programs, routine urine tests are conducted to verify that this is indeed the case; sample tempering is a major issue. (Jaffee et al., 2007; Clancy et al., 2013) Our results, based on meconium analysis, which yields an integral of fetal exposure to drugs over at least the last trimester of pregnancy, indicate that at least one third of pregnant women on MMT continue to use at least one additional opioid narcotic. When comparing their meconium to meconium with no detected methadone, indicating that the mother was not on MMT, no significant difference was found in the proportion of opioid or other illicit drug use between the methadone-positive and -negative meconium samples, indicating similar patterns of substance use during late pregnancy. This alarming finding of an apparent lack of decrease in opioid use for those positive for methadone indicates ineffective MMT for a large proportion of enrolled patients, and increased burden of illicit drug exposure to the fetus. Similar results have been reported from an obstetrical unit in Glasgow, Scotland with approximately half of the women studied having positive results for polysubstance use in addition to MMT. (McGlone et al., 2013)

With such a large proportion of the methadone positive meconium samples also testing positive for at least one other opioid, the efficacy and effectiveness of methadone therapy is brought
into question. Changes in clearance rate and volume of distribution during pregnancy may affect the efficacy of methadone. Wolff et al. (2005) found that weight-adjusted methadone clearance increased between the first and third trimesters (Wolff et al., 2005). Dickmann and Isoherranen (2012) observed increased CYP2B6 activity in human hepatocytes due to estradiol induction, which would increase methadone metabolism via CYP2B6 (Dickmann and Isoherranen, 2013). Additionally, Jarvis et al (2008) determined, in a population of methadone dependent pregnant females, that the half-life of methadone was almost one half that of non-pregnant methadone users. (Jarvis et al., 1999) Absorption of methadone may also be affected during pregnancy, as gastric acid secretions decrease, inhibiting gastrointestinal absorption. (Jarvis et al., 1999) Overall, these pharmacokinetic changes may render the prescribed dose of methadone subtherapeutic. The unstable exposure to opioids in utero may induce fetal stress, increasing the risk of spontaneous abortion and preterm birth. (McCarthy, 2012)

Polydrug use was found in both methadone positive and negative cohorts with cannabinoids, opioids and cocaine being the most common drug classes detected in the samples for both groups. This highlights the numerous compounds a fetus can be exposed to, and the increased risk of fetal stress and adverse outcomes. Polydrug use is commonly reported in those who tested positive for certain drugs. In terms of opioid dependent women, benzodiazepines, cocaine, and marijuana are the most common comorbid drugs. (Abrahams et al., 2012) This was confirmed in a previous study, which demonstrated that meconium samples positive for opioids were likely to also be positive for cocaine, benzodiazepines, methadone and FAEEs. (Moller et al., 2010) As well, it was found that stimulant use (amphetamines and cocaine) by the mother was a potential risk factor for alcohol use and subsequent fetal alcohol exposure. (Kulaga et al., 2010) Knowing which drugs are most often used concomitantly can allow clinicians to potentially diagnose drug exposures in children that otherwise would have gone undetected.
The population assessed in this study is high-risk; almost all women whose neonates were tested are actively involved with social service organizations and demonstrate ongoing child protection concerns. This study assumes that methadone present in meconium is the result of MMT; however, methadone is used illicitly as well and it must be considered that a proportion of the methadone-positive group might not be actively engaged in MMT. While the characteristics of the study population limit the generalizability of these findings to the MMT population at large, the lack of effectiveness of MMT in this particular population underscores current limitations in the delivery of effective addiction treatment services to this highly vulnerable group. Despite substantial mobilization of government resources to this population, they remain under-served in meeting their addiction treatment needs. While comprehensive strategies for addressing substance addiction in pregnancy have been extensively reported in the literature (Finnegan et al., 1971; Poole, 2000; Pepler et al., 2002; Loudenburg and Leonardson, 2003); these data demonstrate that the current model of MMT delivery does not address the addiction treatment needs of women who have already been identified as high-needs.

This study demonstrates a need for closer clinical monitoring of pregnant women in MMT to ensure that treatment is effective in preventing maternal or neonatal withdrawal symptoms, continued opioid or polydrug use, and adverse neonatal outcomes. The high rate of polydrug use in the methadone-positive cohort demonstrates that the current level of care provided to these women is ineffective and should be augmented with consideration given to: i) therapeutic drug monitoring of methadone to evaluate necessity for dose increases due to altered pharmacokinetics in pregnancy, ii) alternative therapies, such as buprenorphine, that may provide a more stable maintenance treatment regime and result in potentially better neonatal outcomes (Welle-Strand et al., 2013), iii) ensuring accessibility of complementary non-pharmacological interventions (e.g. cognitive behavioural therapy) that are an established requirement to successful addiction treatment.
1.2 Prevalence of drug use during pregnancy in Miramichi, NB: Analysis of a routine urine drug screen in the obstetric unit

This study found that approximately 15% of women in the Miramichi, NB region used substances of abuse during pregnancy. This rate is almost triple the Canadian national average of 5.9%. (Health Canada, 2013) Similar to numerous previous studies, marijuana was the most commonly used substance in this population. (Westfall et al., 2006; Gerardin et al., 2011) Despite the introduction of this routine screen, the rate of drug use in the population did not decrease but rather increased over the 6-year period.

Women who used substances during pregnancy tended to have less education, be smokers and suffer from psychiatric disorders, such as depression and anxiety. These characteristics have been found to be associated with substance use both in pregnancy and general in several studies. (Torchalla et al., 2011; Casper and Arbour, 2013) Typically, younger women tend to have higher rates of substance use during pregnancy, but while there was a trend of this in the Miramichi population, this trend did not reach statistical significance (0.06). (Substance Abuse and Mental Health Services Administration, 2011)

Drug use and tobacco smoking commonly coincide and this adds to the complexity of evaluating the risk of pregnancy and neonatal outcomes. These exposures during pregnancy share some of the same outcomes, including lower birth weight, restricted growth and sudden infant death syndrome. (Bauer et al., 2005; Hendryx et al., 2014) To address the fact that these are co-factors in many measured outcomes, smoking status and number of cigarettes smoked per day were included in all regressions. Smoking status and/or number of cigarettes smoked per day were associated with almost all adverse outcomes that were also associated with drug use, either general or specific. Despite having both drug use and smoking variables associated with measured outcomes, in general drug use had a greater effect on the outcome than did smoking. A recent study analyzed the combined effect of drugs, alcohol and smoking on neonatal outcomes and found that polysubstance use has the greatest effects on birth weight.
(Janisse et al., 2014) Hence, even though drug use had a greater effect on most outcomes focused on in this study, polysubstance use is the most concerning and potentially debilitating factor for neonatal outcomes.

Many of the neonatal outcomes found to be more prevalent in case neonates have previously been found to be associated with specific drug use. For example, it is well known that neonatal abstinence syndrome can be caused by in utero exposure to opioids. (Gaalema et al., 2013) Our study also found this association with opioids and benzodiazepine use. Benzodiazepines have previously been associated with NAS occurrence as well as with prolonging the duration of treatment needed when concomitantly taken with methadone. (Seligman et al., 2008) In the present study, numerous symptoms associated with NAS were found to be more common in case neonates including high pitched crying, irritability, jitteriness, and increased muscle tone. Despite the absence of significant differences between case and control women and their rate of diabetes, the four control neonates that suffered from jitteriness were born to diabetic women.

While this study has several strengths of population based design, access to and thorough review of medical charts and large sample size, there are also several limitations that must be acknowledged. First, all detection of drug use occurs through the analysis of urine samples collected mostly at time of delivery. Urine analysis for drugs of abuse has a very limited window of detection, typically being able to detect use only up to 72 hours prior to sample collection. (Moeller et al., 2008) A proportion of patients did have multiple urine analyses during pregnancy, and this allowed for a larger time frame to be examined, but multiple test results were not available for all individuals. Also, some of the substances under investigation are both substances of abuse and prescription medications. With no access to patients’ prescription data, there was no method to determine whether or not patients had prescriptions for drugs such as oxycodone. Furthermore, some medical charts reviewed were missing data in terms of variables collected. Finally, nurses were not blinded to lab test results, and were thus aware of
which women had positive urine drug screen results; this may have resulted in more detailed attention to neonatal symptoms in such cases.

This study took an in-depth look at a unique program in Eastern Canada, which provides clinicians with critical information about substance use during pregnancy. The program is regarded as being very successful, as it provides attending physicians the opportunity to intervene to support mothers and infants at risk. However, as the rate of substance use in the population did not decrease over time, the program may be successful in identifying substance use, but not with treatment of substance use afterwards. Other hospitals may consider adopting routine maternal drug screening programs to both identify rates of substance use and begin a dialogue between patients and health care providers to improve maternal and child health, especially if high rates of substance use are expected. The key to a successful program is the incorporation of proper and accessible non-pharmacological treatment to address the addiction issues in this population.

1.3 Prevalence of heavy fetal alcohol exposure in Canada: A multi-center meconium study

This is the first study of its kind to estimate the prevalence of heavy in utero alcohol exposure through the use of a multicentre site cohort, which spans across a nation. Through FAEE meconium analysis, it can now be estimated that the rate of heavy in utero alcohol exposure in Canada ranges from 1.16 to 2.40%. When comparing the self-reported alcohol consumption and the respective meconium results, the meconium analysis identified a much larger proportion of heavy alcohol consumption during pregnancy (0.23% vs. 1.16%). This significant gap between data collection methods speaks to the limitations of self-report that have been documented numerous times throughout the literature. (Russell et al., 1996; Süsse et al., 2010; Zelner et al., 2012b)

The upper part of this estimated range is similar to previously reported prevalence rates in studies conducted in Canada. (Chan et al., 2004b; Gareri et al., 2008; Zelner et al., 2012b;
Bryanton et al., 2014) This prevalence rate should be considered an estimate of the true rate of heavy \textit{in utero} alcohol exposure, as these results may not be generalizable to the entire population of Canada. As seen in a previous study focusing on a high-risk obstetric unit, the prevalence rate in this type of population is significantly higher than the general population. (Goh et al., 2010) As the MIREC study cohort was found to have a higher level of education and annual household income than the Canadian average, due to the volunteering nature of the recruitment, this estimate most likely reflects the higher SES portion of the Canadian population. (Statistics Canada, 2011; Statistics Canada, 2014b) It is reasonable to speculate that if lower SES individuals were better represented in this cohort that the prevalence rate would be higher than what was found. Lower SES individuals are at higher risk of substance use in general and in pregnancy, and to truly calculate a prevalence of heavy alcohol \textit{in utero} exposure, it is imperative to include these individuals into all prevalence studies. (Mark et al., 2015)

There were no differences found between neonates with positive or negative FAEE results, with the exception of gestational age. As well, no differences were found in key maternal characteristics that have been previously found to be risk factors for substance use during pregnancy. (Beijers et al., 2014) Due to the small number of positive samples identified, this may be the reason that subtle differences between the positive and negative groups were not detected.

Approximately 32\% of women reported alcohol consumption in the previous 3 months in the 32-35 Week Gestation questionnaire. While the majority of alcohol consumption was at a social level, this is still a surprising finding and may further support previous findings that higher SES women continue drinking throughout their pregnancy as it is a normal part of their social lives. (Pfinder et al., 2014) The few individuals who did report above social level drinking had negative FAEE results, most likely due to the fact that the level of drinking was not high enough to be detected by this biomarker. It was found that a larger proportion of individuals with a
negative FAEE result discontinued alcohol consumption between 12 and 32 weeks gestation. As women who drink alcohol, especially at higher levels, prior to pregnancy can have a more difficult time altering their behaviours once they learn about the pregnancy, the lack of significant change in the positive group may reflect these difficulties. (Altfeld et al., 1997; Cheng et al., 2009; Chisolm et al., 2014) The level of self-reported alcohol consumption may speak to the fact that these outcomes were not the primary ones studied by the MIREC group, and thus there was a lack of consequence perceived by the women. Removing the associated fear and stigma, to a degree, may have allowed the women to provide more accurate self-reports, and could provide insight into general drinking habits during pregnancy in Canada. However, as stated earlier, there still remains a large difference between self-reported alcohol consumption and positivity of meconium samples, indicating that despite the secondary nature of this study, self-report remains inaccurate.

While no dose-response relationship was found between reported alcohol consumption and FAEE concentration in meconium, it has been found that increasing levels of FAEEs in meconium samples are associated with poorer psychomotor and mental outcomes in children up to the age of 2 years. (Peterson et al., 2008) As well, increasing doses of alcohol during pregnancy have been found to result in more pronounced effects on the fetus. (Feldman et al., 2012) Binge drinking is the pattern of alcohol consumption associated with the highest levels of alcohol intake, as well as higher blood alcohol concentrations (BAC). Higher peak BAC has been found to be more highly associated with adverse outcomes than total amount of alcohol consumed. (Maier and West, 2001) In previous studies focusing on drinking behaviours and the prevalence of FASD, it has been found that populations with higher rates of binge drinking also have the highest prevalence of FASD, especially the most severe forms. (Viljoen et al., 2005; May et al., 2007; Urban et al., 2008)

While the multicentre nature of this study helps provide a more accurate prevalence rate by including a larger population, as previously stated, these results may not be generalizable to
the population of Canada but rather to its higher SES strata. Another limitation of this study is
the meconium collection time: while collection time for meconium is not crucial for the
measurements of other biomarkers, it is a very important factor when measuring alcohol
biomarkers, including FAEEs. Delayed meconium collection carries an increased risk of false
positive results. (Zelner et al., 2012a) Attempts were made to minimize this risk by adjusting the
prevalence rate equation to reflect the increasing risk over time. Future large, prospective
nationwide studies with optimally-timed meconium collection are necessary to ensure that no
samples are lost due to being transitional.

Overall, it was found that the estimated rate of heavy in utero alcohol exposure in Canada
ranges between 1.16 and 2.40%. This prevalence rate provides a better estimate at what the
current trends of alcohol consumption during pregnancy are in Canada. While the self-reported
alcohol consumption data is not the most accurate source, it also informs researchers and
clinicians of the current alcohol consumption rates and trends in Canada, with a small
proportion of women continuing to drink during pregnancy at a low level. The literature
regarding the effects of alcohol at low to moderate levels are inconsistent, with some studies
finding no effect on neonatal birth outcomes nor behavioural outcomes later in life. (Skogerbø
et al., 2012; O’Keeffe et al., 2014) However, due to the complex nature of ethanol
teratogenicity, it is generally recommended that pregnant women abstain from alcohol
consumption, and that no safe amount of alcohol has been determined. (Feldman et al., 2012)
The most conservative prevalence rate calculated in this study (1.16%) is the equivalent of
approximately 1,800 new cases of FASD each year in Canada. (Statistics Canada, 2014a)
While prevalence rates provide insight into substance use in a population, it is imperative to
provide resources to both the women using substances and their exposed children.

1.4 Biomarkers as an indirect marker of the child’s environment

“Substance abuse, prenatal or not, is an ongoing public health concern, impacting not only the
users themselves but also their families, and the health system economically. Beyond being
biomarkers for physical and neurological well being of exposed children, hair and meconium measurements identify women who continued to use drugs of abuse or alcohol despite being aware of their pregnancy. Children reared by addicted mothers have increased risk for neglect, and abuse. (Koren et al., 2008)” (Delano and Koren, 2012; page 1067) Behavioural problems associated with exposure to drugs of abuse can begin with these in utero exposures, but the quality of the postnatal environment can also modify the child’s behaviour and development. (Lester and Lagasse, 2010) As well, children of addicted mothers have been found to exhibit more behavioral problems in general. (Lester and Lagasse, 2010) Hence, these biomarkers constitute strong predictors of environmental risk for the child, and a need for close follow up of child safety and wellbeing.

Women who abuse substances have been found to have more difficulties in providing a stable environment for themselves and their children. They have difficult life circumstances, including economic and social problems, which further impede providing this optimal environment. (Kelley, 1998) Poorer quality of home has been found to be associated with higher rates of attention problems and aggressive behaviour in children aged 3 and 5 years. (LaGasse et al., 2012) Children being raised in these environments are at a greater risk for poor development, growth, and cognitive functioning. They also have an increased risk of psychiatric disorders, behavioural problems as well as substance use. (Barnard and McKeeganey, 2004) They may also experience less supervision and more disciplinary forms of parenting. (Day and George, 2005) Gerteis and colleagues (2011) found that exposure to violence during childhood was actually significantly associated with delinquent behaviour, and not prenatal cocaine exposure itself. This further supports the theory that drug exposures may not be the underlying cause of various behavioural deficits, but rather the post-natal environment in which the child is raised. (Gerteis et al., 2011) Parenting deficits of substance using women have also been studied with deficiencies found in all areas, including skills (e.g., ability to maintain sleeping and eating routines), knowledge (e.g., knowledge of typical child development), attitudes (e.g., empathy),
and capacity (e.g., lack of social services involvement). These deficits may be due to the woman’s abilities despite the substance use, but may also be exacerbated by the woman’s need to satisfy her addiction. (Niccols et al., 2012)

Parental psychopathology has been found to be the strongest predictor of child behavioural outcomes, with children born to parents with psychiatric disorders exhibiting higher rates of these poor outcomes (e.g., anxiety, depression, and poor attention) when compared to control children. (Staroselsky et al., 2009) Behavioural problems are seen at higher rates in children with parents who suffer from depression. This could be due to multiple reasons but researchers believe that mentally healthier women may be more effective parents, demonstrating to their children the abilities of emotional regulation and adaptive behaviour. Also, there might be a genetic predisposition for mental health problems that is inherited by children from their mothers. (Beck, 1999; Todorow et al., 2010) However, women with mental health problems are more likely to over-report behavioural issues with their children, introducing a bias into these studies. (Najman et al., 2000) As an association between psychiatric disorders and higher rates of substance use has previously been found, the environmental effect of parental psychopathology on child behavioural outcomes must be carefully considered.

Prenatal exposures may potentially be associated with an increased risk of substance use and misuse by the child later in life. For example, prenatal exposure to alcohol was found to be associated with an increased risk of problems with alcohol by the age of 21 in an American cohort, but these findings could not be duplicated when studying a similar Australian cohort. (Baer et al., 2003; Alati et al., 2005) While some of the specific findings about risk of substance use are contradictory, in general, children who were prenatally exposed to substances initiate substance use at an earlier age than children who were not exposed. (Frank et al., 2014)

Interventions for substance use and substance exposure are available, and range from accessible prenatal care to methadone treatment to comprehensive treatment centers. Some
birth outcomes can improve following 4 or more prenatal care visits, demonstrating the confounding effect poor neonatal care can have on neonatal birth outcomes. (Racine et al., 1993) Interventions for children that include social skill development have been found to improve problematic behaviours not only immediately following treatment but months after. Children’s Friendship Training is an example of intervention available to children prenatally exposed to alcohol. This program involves a 90-minute session once a week for 12 weeks with teaching of social skills and parental coaching. The control group that did not receive this intervention during the study was able to participate once the study concluded and also showed improvements in social skills and problematic behaviours. This demonstrates that access to the intervention is a critical factor, but not necessarily its timing. (O’Connor et al., 2006; Kully-Martens et al., 2012)

Through studies focusing on the role that environment plays on the cognitive and linguistic development of substance-exposed children, it was found that children raised in non-kin foster care or adoptive homes had better development in these domains when compared to exposed children who remained with their mothers or relatives. (Singer et al., 2004; Singer et al., 2008) While a primary objective should always be to provide interventions that assist the mother and her child, if necessary, foster care is a viable option for a small subset of this population.

The interplay of drug exposure and the child’s postnatal environment is critical in determining the behavioural outcomes of children exposed to substances of abuse. These factors are never mutually exclusive and therefore should not be treated in that way for research purposes. While prevalence studies provide a glimpse into the substance use of a population, the more clinically relevant question is what the child is exposed to postnatally, whether it is additional drug exposure or other environmental factors. Taking such environmental factors into consideration is imperative when conducting behavioural outcome studies in children exposed to substances in utero. The research area of substance use, during pregnancy or not, is a complex matter and these intricacies must be considered moving forward to ensure that knowledge gaps are
appropriately addressed by researchers and that useful information is obtained to develop and provide better treatment services for addicted women and their children.

2 Strengths, Limitations and Future Directions

Biomarkers can provide an objective assessment of substance use during pregnancy and are more reliable than maternal self-report. The three studies within this thesis have utilized biomarkers in an effective manner to address key knowledge gaps in the literature. Firstly, having access to all laboratory results provided the possibility to study polydrug use in a high-risk population, which can be relatively hard to obtain. Polydrug use is understudied within the literature, especially in regards to additional drug use in a methadone-using population. Secondly, the routine maternal urine drug screen established in Miramichi, NB is an extremely unique program in Canada, with invaluable information available to clinicians and researchers. This program allowed for a population based study to be conducted in a thorough manner and provided insight into a population with higher than average drug use. In addition, without this program, the Miramichi population would most likely not have been focused on, as it is not an urban centre. The findings of this study may represent what is occurring in other populations with similar demographics. Lastly, the MIREC Cohort was a comprehensive multi-centre study, which covers the major urban centres in Canada. The findings of this study are the most accurate estimate, to date, on the rates of heavy alcohol consumption during pregnancy in the higher socioeconomic status portion of the population.

However, there are a few key limitations to the utilization of these biological markers, predominantly concerning the matrices in which they are measured. Meconium is a cumulative matrix that begins forming in the second trimester, but is believed to accumulate mainly throughout the third trimester. (Burd and Hofer, 2008) While this matrix provides a larger window of detection than blood or urine, prenatal exposures during the first and possibly second trimesters will not be detected. Another limitation with meconium analysis, in particular for alcohol, is the collection time window. In general, this window is approximately 72 hours
post-delivery, but the ideal collection window for alcohol analysis is within 24 hours. (Zelner et al., 2012a) Premature neonates can have delayed passage of meconium, and it is unknown whether this delay can result in contamination with postnatal stool and an increased risk of false positive results. (Kumar and Dhanireddy, 1995) Both of these limitations of meconium analysis should be studied further to better understand the accumulation and window of detection of meconium, as well as the impact of prematurity and other unique circumstances on meconium results.

Urine analysis of biomarkers for substance use is a commonly used technique as it is non-invasive, easy to collect, and has cost-effective analytical methods. The window of detection is narrow, only spanning 24-72 hours, depending on the compound. (Markway and Baker, 2011) This short detection window means that only very recent use will be detected in a maternal urine screen, requiring multiple urine screens throughout pregnancy to capture all exposures. In addition to the short detection window for urine samples, some of the analytical methods used, like ELISA and CEDIA, have increased risks of false positive results due to cross-reactivity with other compounds present in the urine. (Moeller et al., 2008) For example, dextromethorphan, an antitussive, can cause a false-positive phencyclidine result, while ranitidine, a histamine H$_2$ receptor antagonist, can cause a false-positive amphetamine result. (Brahm et al., 2010)

As previously mentioned, while prevalence rates are crucial to understanding the rates and trends of substance use within a population or community, this is not where efforts by clinicians and researchers should end. With a growing understanding of the effects of a child’s environment on their development, future research should investigate the interplay between in utero drug exposures and the environment, in addition to therapeutic interventions for both mother and child.
3 Conclusions

The overall aim of this thesis was to objectively assess ongoing substance use prevalence and trends of substance use during pregnancy in Canada. Through the use of biomarkers, new knowledge has been presented on the prevalence and trends of substance use in Canada in this thesis. First, this research found that in a high risk population of women involved with social services, polydrug use is a common practice for individuals taking methadone and that this form of treatment may not be meeting the needs of pregnant women. Second, this research showed that routine urine drug screens in the obstetric unit can be effective in detecting substance use during pregnancy, and better understanding the rates and trends within a community population, but may not be effective in initiating the provision of resources and treatment options to these women so that rates can decrease over time. Lastly, this research found that the estimated prevalence of heavy in utero alcohol exposure in Canada ranges between 1.16% and 2.40%. This study is the first of its kind, utilizing a multicentre approach spanning across the country. This prevalence rate is equal to at least 1,800 new cases of FASD each year in Canada. In summary, the findings presented in this thesis address numerous knowledge gaps pertaining to the rates and trends of substance use during pregnancy in Canada, and highlight the complex nature of the substance-using pregnant population and the dire need for more effective and accessible treatment programs and interventions for exposed children.
References


Statistics Canada (2014b) Median total income, by family type, by province and territory (All census families). CANSIM Table No. 111-0009. Statistics Canada: Ottawa, Ontario


Substance Abuse and Mental Health Services Administration (2009) The NSDUH Report: Substance use among women during pregnancy and following childbirth. SAMHSA: Rockville, MD
Substance Abuse and Mental Health Services Administration (2011) Results from the 2010 National Survey on Drug Use and Health: summary of national findings, NSDUH Series H-41, HHS Publication No.(SMA) 11–4658. SAMHSA: Rockville, MD


Appendices

Appendix A. Research Ethics Board Approval

RESEARCH ETHICS BOARD

September 14, 2012

Dr. Gideon Koren
Clinical Pharmacology & Toxicology
The Hospital for Sick Children

Dear Dr. Koren:

Your study “Presence of Drugs of Abuse in Hair and Meconium: A Database based study.”

REB File No.: 1000006229

On behalf of the REB, I am writing to confirm that the above noted study was re-approved by the REB for one year ending in September 2013. The REB approved continuing review at level 1A. As necessary, the Clinical Research Office will be contacting you to arrange follow-up.

Please note that, in accordance with the Personal Health Information Protection Act of Ontario, you are responsible for adhering to all conditions and restrictions imposed by the REB governing the use, security, disclosure, return and disposal of the research subjects’ personal health information. You are also responsible for reporting immediately any privacy breaches to the REB Chair and to Janice Campbell, the Sick Kids privacy officer.

Yours truly,

Richard Sugarman
Chair, Research Ethics Board

Co-Investigator(s): Facundo Garcia Bournissen
September 14, 2012

Dr. Michael Dickinson
Principal Investigator
Miramichi Regional Hospital
500 Water Street
Miramichi, NB E1V 3G5

Dear Dr. Dickinson:

Re: “Pregnancy exposure to drugs of abuse in Miramichi Regional Hospital.”

Our File #: 2012-1766

The above noted proposal has been reviewed and approved via the delegated review process of the Horizon Health Network REB.

APPROVED:
- Research Study Application: signed and dated August 14, 2012 and

Also received and on file:
- Your CV and Medical License

The Research Ethics Board for the Horizon Health Network is organized and operates according to the principles of the ICH Harmonized Tripartite Guidelines: Good Clinical Practice, Tri-Council Policy Statement and Division 5 of the Food and Drug Regulations. Re-approval should be initiated two months prior to the due date of September 14, 2013.

Please do not hesitate to contact the office, if we can be of further assistance. Best wishes as you proceed.

With kind regards,

Jeff Jennings, MSc,
Chair, Research Ethics Board
Horizon Health Network

Ji/as

Cc: Jenn Tuttle
Le 27 novembre 2012

Docteur William Fraser
5757 Decelles
suite:120

OBJET: Titre du projet: Estimer l’incidence de l’exposition fœtale à l’alcool au Canada par la mesure des esters d’éthanol et acides gras (EEAG) dans les spécimens de meconium disponibles auprès de MIREC
No. de dossier: 3508

Cher Docteur,

Votre projet cité en rubrique a été approuvé par le comité d'éthique de la recherche en date du 26 novembre 2012. Vous trouverez ci-joint la liste des documents approuvés. Aucun formulaire d'information et de consentement n'a été estampillé puisque vous utilisez ceux du projet #2462 – Recherche en périnatalité sur les produits chimiques de l'environnement: Une enquête nationale sur l'exposition aux contaminants de l'environnement pendant la grossesse et l'allaitement / Maternal-Infant Research on Environmental Chemicals (MIREC): A National Profile of In Utero and Lactational Exposure to Environmental Contaminants. (Notez que pour une collaboration avec un (ou plusieurs) tiers (institutions ou entreprises privées) impliquant des transferts de fonds et/ou données et/ou matériel biologique, une entente (contrat) doit être conclue avec le Bureau des ententes de recherche (BER).

Tous les projets de recherche impliquant des sujets humains doivent être réexaminés annuellement et la durée de l'approbation de votre projet sera effective jusqu'au 26 novembre 2013. Notez qu'il est de votre responsabilité de soumettre une demande au comité pour que votre projet soit renouvelé avant la date d'expiration mentionnée. Il est également de votre responsabilité d'aviser le comité dans les plus brefs délais de toute modification au projet ainsi que de tout effet secondaire survenu dans le cadre de la présente étude.

Nous vous souhaitons bonne chance dans la réalisation de votre projet et vous prions de recevoir nos meilleures salutations.

Geneviève Cardinal, juriste
Vice-Présidente du Comité d'éthique de la recherche
GC/mhl
c.c. : BER
Liste des documents approuvés par le CÉR

Titre du projet:
Estimer l'incidence de l'exposition foetale à l'alcool au Canada par la mesure des esters d'éthanol et acides gras (EEAG) dans les spécimens de meconium disponibles auprès de MIREC

No. de dossier: 3508

Date d'approbation: lundi 26 novembre 2012

Responsables du projet: FRASER WILLIAM M.D., chercheur responsable au CHU Sainte-Justine. Chercheur principal: Gideon Koren, Hospital for Sick Children

Liste:

- Protocole de recherche non daté
Good afternoon

The following 2 retrospective study applications have received Research Ethics Board approval on June 25, 2015.

“Prevalence of drug use during pregnancy in Miramichi, NB: Analysis of a routine urine drug screen in the obstetric unit”.

And

‘Estimating the incidence of fetal alcohol exposure in Canada by measuring meconium fatty ester in MIREC samples”

Please do not hesitate to contact me if you have any questions

Kind regards

Rose

Rose Gaitere, RN, BScN, MSN
Research Ethics Board, Vice Chair
And
Critical Care Response Team, RN Lead
Department of Critical Care Medicine
The Hospital for Sick Children | 555 University Ave | Toronto, Ont, M5G 1X8
Tel.: 416-813-8301
e-mail: rose.gaitere@sickkids.ca
Appendix B. Data Collection Form for Miramichi Regional Hospital

MIRAMICHI STUDY – DATA COLLECTION FORM

ID Number: _____  
Year: _____

A. MATERNAL INFORMATION

Age at delivery: _____ Weight (kg): _____ Height (cm): _____  
Education: _____

Maternal Self-report:  
Alcohol: ☐  Drugs of Abuse: ☐  Smoking: ☐  
Notes: _____

B. URINE DRUG SCREEN

Current pregnancy: ☐  Previous pregnancy: ☐  Post-pregnancy: ☐

☐ Amphetamines  ☐ Opiates  ☐ Cocaine  
☐ Barbiturates  ☐ Oxycodone  ☐ THC (marijuana)  
☐ Benzodiazepines  ☐ PCP  ☐ Other:

Notes: _____

C. MATERNAL HEALTH CONDITIONS COMPLICATING PREGNANCY

☐ Arthritis  Details:  
☐ Asthma  Details:  
☐ Diabetes/gestational diabetes  Details:  
☐ Epilepsy  Details:  
☐ Hypertension  Details:  
☐ Hypothyroid  Details:  
☐ Learning Disabilities  Details:  
☐ Placental abruption  Details:  
☐ Psychiatric Disorders (e.g. depression, anxiety)  Details:  
☐ Strep B  Details:  
☐ UTI  Details:  
☐ Other  Details:  
☐ Other  Details:  
☐ Other  Details:  

D. NEONATAL INFORMATION
Sex: M □ F □
Gestational age at birth: _____ w _____ d
Birth Weight (g): _____ Length (cm): _____
Head circumference (cm): _____ Hospital Stay (days): _____
Apgar scores: 1 minute _____ 5 minutes _____ 10 minutes _____
Any complications at birth: Yes □ No □
Please specify (time of appearance, symptoms, medical interventions, duration in intensive care): _____
Birth Defects: Yes □ No □
If yes, what: _____
Notes: _____
**Appendix C. Questionnaire data collected from MIREC Study**

<table>
<thead>
<tr>
<th>Categories of Data</th>
<th>Timing of Collection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st Trimester</td>
</tr>
<tr>
<td>Sociodemographic Characteristics</td>
<td>X</td>
</tr>
<tr>
<td>Obstetrical History</td>
<td>X</td>
</tr>
<tr>
<td>Current Pregnancy</td>
<td>X</td>
</tr>
<tr>
<td>Evolution of Current Pregnancy</td>
<td>X</td>
</tr>
<tr>
<td>Family History of High Blood Pressure in Pregnancy</td>
<td>X</td>
</tr>
<tr>
<td>Non-Obstetrical History</td>
<td>X</td>
</tr>
<tr>
<td>Nutritional Supplements</td>
<td>X</td>
</tr>
<tr>
<td>Employment Status</td>
<td>X</td>
</tr>
<tr>
<td>Environmental Exposures</td>
<td>X</td>
</tr>
<tr>
<td>Smoking Status</td>
<td>X</td>
</tr>
<tr>
<td>Exposure to Second-Hand Smoke</td>
<td>X</td>
</tr>
<tr>
<td>Drinks and Alcohol Consumption</td>
<td>X</td>
</tr>
<tr>
<td>Residence Details (after 1st visit, collected only if there has been a move)</td>
<td>X</td>
</tr>
<tr>
<td>Activities</td>
<td>X</td>
</tr>
<tr>
<td>Diet</td>
<td>X</td>
</tr>
<tr>
<td>Sunlight Exposure</td>
<td>X</td>
</tr>
<tr>
<td>Anthropometric Measurements</td>
<td>X</td>
</tr>
<tr>
<td>Blood Pressure and Urine Collection</td>
<td>X</td>
</tr>
<tr>
<td>Nutrient Supplements</td>
<td>X</td>
</tr>
<tr>
<td>Gestational Age and Ultrasound Information</td>
<td>X</td>
</tr>
<tr>
<td>Evolution of Current Pregnancy</td>
<td>X</td>
</tr>
<tr>
<td>Current Medications</td>
<td>X</td>
</tr>
<tr>
<td>Clinical Tests</td>
<td>X</td>
</tr>
<tr>
<td>Food Frequency Questionnaire</td>
<td>X</td>
</tr>
<tr>
<td>Glucose Tolerance in Pregnancy</td>
<td>X</td>
</tr>
<tr>
<td>Corticosteroids in Pregnancy</td>
<td>X</td>
</tr>
<tr>
<td>Gestational Hypertension Prior to Admission</td>
<td>X</td>
</tr>
<tr>
<td>Blood Pressure and Urine Collection after Admission for Delivery</td>
<td>X</td>
</tr>
<tr>
<td>Maternal Conditions after Admission for Delivery</td>
<td>X</td>
</tr>
<tr>
<td>Labour and Delivery</td>
<td>X</td>
</tr>
<tr>
<td>Maternal Outcomes after Delivery</td>
<td>X</td>
</tr>
<tr>
<td>Neonatal Information</td>
<td>X</td>
</tr>
<tr>
<td>Breast milk Information</td>
<td>X</td>
</tr>
</tbody>
</table>

*Items **bolded** – Information provided by MIREC for prevalence of heavy prenatal ethanol exposure study*
Appendix D. GC-MS Parameters of FAEE Detection in Meconium Method

GC Method Parameters

- Set oven temperature program to 70°C for 2 minutes, at a rate of 20°C/min increase to 300°C and hold for 1 min.
- Set Injection Temp to 260°.
- Set injection mode to Splitless.
- Set Sampling Time: 2 min.
- Pressure: 61.5 kPa.
- Total Flow: 32.6 ml/min.
- Set Column Flow: 1.00 ml/min.
- Linear Velocity: 37.0 cm/sec.
- Set Purge Flow to 1.5 ml/min.
- Set Split Ratio to 30.0.

MS SCAN Method Parameters

- Set Ion Source Temp to 230°C.
- Interface Temp to 310°C
- Solvent Cut Time: 8:50 min
- Detector Mode: Relative
- Detector Gain: 0.30kV.
- Threshold: 100.
- Start Time: 9.87 min.
- End Time: 12.84 min.
- ACQ Mode: Scan.
- Event Time: 0.50 sec
- Scan Speed: 909.
- Start m/z: 80.
- End m/z: 500.

SPME Method Parameters

- Pre-incubation Time: 5 min.
- Incubation Temperature: 90°C.
- Pre-inc Agitator Speed: 250 rpm.
- Agitator On Time: 15 sec.
- Agitator Off Time: 10 sec.
- Vial Penetration: 25 mm.
- Extraction Time: 30 min.
- Injection Penetration: 45 mm.
- Desorption Time: 12 min.
- GC Runtime: 13:50 min.
Copyright Acknowledgements

The copyright of the two manuscripts used within this thesis is retained with the author and no further copyright permissions are required.